Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Effusol, a unique Juncus effusus phenanthrene, ameliorates amyloid β1-42-mediated neurotoxicity in mice

Haruna Tamano
University of Shizuoka
Daichi Murakami
University of Shizuoka
Toshiyuki Fukuda
Satoen CO., LTD
Yasuhito Akagi
Hagihara & CO., LTD
Shinji Ikeura
Hagihara & CO., LTD
Atsushi Takeda
takedaa@u-shizuoka-ken.ac.jp

University of Shizuoka

Research Article

Keywords: Effusol, Juncus effusus, Zn2+, reactive oxygen species, amyloid β1-42, Alzheimer's disease

Posted Date: April 29th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6492242/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Dehydroeffusol, a unique *Juncus effusus* phenanthrene, ameliorates amyloid β_{1-42} ($A\beta_{1-42}$)-mediated neurotoxicity. We tested the ameliorating effect of effusol, another unique *Juncus effusus* phenanthrene. Effusol (15 mg/kg) was orally delivered to mice once a day for 2 days and mice were subjected to intracerebroventricular (ICV) administration of $A\beta_{1-42}$ 1 day after the last deliver of effusol in the same procedure as dehydroeffusol. Fourteen days later, effusol ameliorated neurotoxicity in the dentate granule cell layer evaluated by propidium iodide. Effusol did not increase the synthesis of metallothioneins (MTs) for capturing toxic Zn^{2+} ferried by $A\beta_{1-42}$, unlike the effect of dehydroeffusol via MT synthesis. However, effusol reduced both intracellular increases in Zn^{2+} and reactive oxygen species (ROS) by $A\beta_{1-42}$. To test the effect of post-intake of effusol, mice were subjected to ICV administration of $A\beta_{1-42}$ and effusol was delivered to mice in the same manner. Effusol ameliorated $A\beta_{1-42}$-mediated neurotoxicity. These results first suggest that effusol ameliorates $A\beta_{1-42}$-mediated neurotoxicity in the dentate gyrus by reducing intracellular ROS generation, which is induced by Zn^{2+} dysregulation. A possible effect of effusol on $A\beta_{1-42}$ neurotoxicity is that effusol may reduce Aβ-induced synaptic hyperexcitation induced by glutamate exocytosis via activating $GABA_A$ receptors followed by ameliorating Zn^{2+} dysregulation.

1. Introduction

Amyloid-β (Aβ) peptides, which derived from amyloid precursor protein, a membrane protein, contribute to the Alzheimer disease (AD) pathogenesis [1, 2]. $A\beta_{1-40}$ and $A\beta_{1-42}$ are two major peptides. In the extracellular fluid, the concentration of $A\beta_{1-40}$ is much higher than that of $A\beta_{1-42}$, while aggregation of $A\beta_{1-42}$ is more facilitated than that of $A\beta_{1-40}$ followed by higher neurotoxicity of $A\beta_{1-42}$ [3, 4]. Furthermore, the aggregation of $A\beta_{1-42}$ is strongly facilitated in the presence of extracellular Zn^{2+} in vivo. When $A\beta_{1-42}$ is accumulated to approximately 500 pM in the extracellular fluid of rat brain, $A\beta_{1-42}$ readily captures Zn^{2+} and Zn-$A\beta_{1-42}$ complexes are preferentially passes through dentate gyrus neurons in the hippocampus. $A\beta_{1-42}$ uptake by dentate gyrus neurons is closely linked with more vulnerability to $A\beta_{1-42}$ neurotoxicity in the hippocampus followed by memory loss and cell death [5–8]. $A\beta_{1-42}$ neurotoxicity is avoided by either co-administrating CaEDTA for capturing extracellular Zn^{2+} or ZnAF-2DA for capturing intracellular Zn^{2+} [8]: Neuronal $A\beta_{1-42}$ accumulation is avoided by the conversion of CaEDTA into ZnEDTA, while intracellular ZnAF-2 captures Zn^{2+} released from intracellular Zn-$A\beta_{1-42}$ complexes followed by reducing $A\beta_{1-42}$ neurotoxicity. Zn^{2+} dysregulation induced by $A\beta_{1-42}$ serves as a trigger in the $A\beta_{1-42}$ pathogenesis [9].

Therefore, we paid attention to metallothioneins (MTs), intracellular Zn^{2+}-proteins, which capture excess (toxic) Zn^{2+} under physiological condition [9]. Memory loss and cell death by $A\beta_{1-42}$ are avoided by the pre-induction of MTs with dexamethasone, a blood-brain barrier-permeable steroid hormone, which

increases the capacity for capturing excess Zn^{2+} [8, 10]. Capturing excess Zn^{2+} ferried by $A\beta_{1-42}$ may contribute to the rescuing effect of dexamethasone-induced MTs on $A\beta_{1-42}$ neurotoxicity in vivo.

Traditional Chinese medicines contain active natural ingredients and have been recognized for their medication effects such as anti-cancer and anti-oxidative activities. Many researchers have paid attention to phenanthrene derivatives, one of the active ingredients in Chinese herbal medicines, e.g., *Juncaceae* [11]. Effusol and dehydroeffusol are unique and major phenanthrene derivatives isolated from *Juncus effusus*, a member of *Juncaceae* family. We reported that oral intake of dehydroeffusol blocks cell death in the dentate gyrus of mouse hippocampus after administering $A\beta_{1-42}$ into the lateral ventricle [12]. MT synthesis by dehydroeffusol is increased via activation of adrenergic β receptors and reduces excess Zn^{2+} ferried by $A\beta_{1-42}$ followed by the preventing effect against $A\beta_{1-42}$ neurotoxicity [13]. In contrast, Effusol concentration-dependently enhances $GABA_A$ receptor-mediated current in an in vitro system [14]. Effusol reduces intracellular Zn^{2+} dysregulation, which is induced by corticosterone-induced accumulation of extracellular glutamate, followed by ameliorating adverse events of corticosterone [15]. Therefore, it is possible that effusol increases MT synthesis and/or regulates glutamate excitotoxicity followed the rescuing effect against $A\beta_{1-42}$ neurotoxicity.

Biological half-life of MTs is 18–20 h [16]. In the case of dexamethasone-induced MTs, MTs reaches the maximum level 24 h after subcutaneous injection of dexamethasone once a day for two days followed by increase in capacity for capturing Zn^{2+} [10]. On the basis of evidence on the beneficial period of newly synthesized MTs, it is estimated that oral intake of dehydroeffusol prevents $A\beta_{1-42}$ neurotoxicity [13]. In the present study, effusol was orally delivered to mice once a day for 2 days and then mice were subjected to intracerebroventricular (ICV) administration of $A\beta_{1-42}$ 1 day after the last deliver of effusol according to the same procedure as dehydroeffusol [13].

2. Materials and method

2.1. Chemicals

Human $A\beta_{1-42}$ (ChinaPeptides, Shanghai, China) dissolved in saline was immediately used in every experiment. ZnAF-2DA (Sekisui Medical Co., LTD, Hachimantai, Japan) is plasma membrane-permeable and captures intracellular Zn^{2+}. ZnAF-2DA was dissolved in dimethyl sulfoxide and diluted with Ringer solution, which consisted of 119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4$, 1.0 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 26.2 mM $NaHCO_3$, and 11 mM D-glucose (pH 7.3). Aminophenyl Fluorescein (APF, Goryochemical, Sapporo, Japan) is plasma membrane-permeable and captures intracellular reactive oxygen species (ROS), i.e., $^{\bullet}OH$ and $ONOO^-$. APF dissolved in N, N-dimethylformamide was also used by diluting with Ringer solution.

2.2. Experimental animals

Mice (ddY male, 10 weeks of age, Japan SLC, Hamamatsu, Japan) was maintained under the standard conditions (a diurnal 12-h light cycle; room temperature, 23 ± 1°C; relative humidity, 55 ± 5%). A laboratory diet and water were freely given to mice. Mice were subcutaneously injected with chloral hydrate (30 mg/kg) and anesthetized prior to surgery for administering $A\beta_{1-42}$ and also decapitation for all experiments. The present animal experiments were done based on the Guidelines, which were made for the Care and Use of Laboratory Animals in the University of Shizuoka after permission by the Ethics Committee of Experimental Animals in the University of Shizuoka (Ethical approval no. 196418, 2019).

2.3. Isolation of effusol and administration

Effusol was purified from *Juncus effusus* (Rush, Igusa) obtained from HAGIHARA & CO., LTD. (Kurashiki, Japan). The structure of effusol was identified by spectroscopic methods including 1D and 2D NMR (^{1}H, ^{13}C, COSY, HMQC, and HMBC) and mass spectrometry (ESI-MS) as reported previously (Fig. 1) [15].

On the basis of the data on the minimum effective dose (15 mg/kg body weight in mice) of dehydroeffusol [17], effusol (15 mg/kg) was dissolved in saline containing 0.5% carboxymethyl cellulose sodium and delivered to mice orally once a day for 2 days in the same procedure as dehydroeffusol [13].

2.4. ICV administration of $A\beta_{1-42}$

To induce preferential neurodegeneration in the dentate granule cell layer by $A\beta_{1-42}$, vehicle (saline) and $A\beta_{1-42}$ (ChinaPeptides, Shanghai, China) in saline were administered into the mouse lateral ventricle for 40 min at the rate of 0.5 μL/min through a cannula (25 μM, 20 μL, 500 pmol/mouse) under anesthetization as reported previously [8, 12, 13].

2.5. Propidium iodide (PI) imaging

Two weeks after ICV administrating $A\beta_{1-42}$, PI fluorescence (Ex/Em: 535 nm/617 nm) of brain slices, which was used to assess neuronal damage, was observed in the dentate gyrus by using a Nikon A1 confocal microscopic system (Nikon Corp., Japan) [8, 12, 13].

2.6. MT immunostaining

One day after the last delivering effusol, MT immunostaining of brain slices was done by using anti-MT antibody [UC1MT] ab12228 (Abcam) and Alexa Fluor 488 goat anti-mouse secondary antibody (Thermo Fisher Scientific). MT images (Ex/Em: 495 nm/519 nm) were observed in the dentate gyrus [13].

2.7. Zn^{2+} imaging

One hour after ICV administering saline and $A\beta_{1-42}$ in saline, the fluorescence of brain slices with ZnAF-2DA, which was used for assess intracellular Zn^{2+} level, was observed in the dentate gyrus (Ex/Em: 488 nm/505–530 nm) [13].

2.8. Reactive oxygen species (ROS) imaging

One hour after ICV administration of saline containing APF (50 μM) and $A\beta_{1-42}$ in saline containing Aminophenyl Fluorescein (APF, 50 μM), the fluorescence of APF of brain slices, which was used for assess the level of intracellular ROS, i.e., $^{\bullet}OH$ and $ONOO^-$, was observed in the dentate gyrus (Ex/Em: 490 nm/500–550 nm).

2.9. Data analysis

Student's paired *t*-test was used for comparing the means of paired data (means ± standard error). One-way ANOVA followed by post hoc testing using the Tukey's test (the statistical software, GraphPad Prism 5) was used for multiple comparisons between treatments.

3. Results and discussion

Because phenanthrene derivatives, which are water-insoluble and lipophilic low-molecular weights, are blood-brain barrier-permeable as reported in morphine [18, 19], it is estimated that water-insoluble effusol passes through the blood-brain barrier and is transported into the brain extracellular fluid. On the basis of the data on the minimum effective dose of dehydroeffusol (15 mg/kg body weight in mice) [17], effusol (15 mg/kg) was delivered to mice orally once a day for 2 days in the same procedure as dehydroeffusol [13]. In the resent study, $A\beta_{1-42}$ was administered 1 day after the second delivering effusol. Effusol blocked $A\beta_{1-42}$ toxicity in the dentate granule cell layer by PI fluorescence, an index of dead cells (Fig. 1). However, effusol did not elevate the level of MT synthesis by MT immunostaining, unlike the case of dehydroeffusol [13] (Fig. 2).

When intracellular Zn^{2+} in the dentate granule cell layer was assessed by ZnAF-2 fluorescence, effusol reduced increase in intracellular Zn^{2+} induced by $A\beta_{1-42}$, which was observed 1 h after administering $A\beta_{1-42}$ (Fig. 3). Intracellular Zn^{2+} dysregulation induced by $A\beta_{1-42}$ may lead to the increase in ROS generation, as well as intracellular Zn^{2+} dysregulation induced by Zn^{2+} influx through GluR2-lacking α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors [9, 20]. It is likely that Zn^{2+} dysregulation leads to mitochondrial Zn^{2+} accumulation followed by mitochondrial ROS deneration.

Intracellular ROS assessed by APF was increased 1 h after administering $A\beta_{1-42}$, while the increase was also reduced by pre-oral intake of effusol (Fig. 4), suggesting that ROS generation induced by Zn^{2+} dysregulation is linked with $A\beta_{1-42}$ neurotoxicity and that decrease in ROS generation by effusol contributes to neuroprotection against $A\beta_{1-42}$ [9, 20]. Intracellular Zn^{2+} dysregulation by $A\beta_{1-42}$ induces Zn^{2+} accumulation and ROS generation in the mitochondria, followed by neurodegeneration [21]. Oxidative stress via ROS is associated with neurodegenerative diseases [22] and is also associated with the etiology of AD [23, 24]. Furthermore, the effect of post-oral effusol intake was tested 1 day after administering $A\beta_{1-42}$. Effusol also decreased $A\beta_{1-42}$ toxicity in the dentate granule cell layer by PI fluorescence (Fig. 5).

It has been reported that affected vesicular exocytosis for releasing glutamate, which is induced by soluble Aβ oligomers, contributes to pathological events in neurological diseases, which are also linked with AD pathophysiology: $A\beta_{1-42}$ oligomers preferentially leads to accumulating extracellular glutamate followed by synaptic dyshomeostasis of glutamate, and initially induces synaptic hyperactivity by inhibiting glutamate reuptake [25–27] or enhancing glutamate release probability [28]. Soluble $A\beta_{1-42}$ oligomers markedly increase vesicular storage of glutamate and then increase vesicular exocytosis for releasing glutamate by the action potential in addition to the spontaneous exocytosis [29]. These events may decrease vesicular concentration of glutamate and lead to reduced release probability. Astrocytes serve a key role for the clearance and metabolism of glutamate in the synaptic clefts, while Aβ also affects vesicular release of neurotransmitters in the astrocyte model system [30]. It is likely that $A\beta_{1-42}$-induced synaptic hyperexcitation accumulates glutamate in the brain extracellular fluid and that glutamate excitotoxicity leads to postsynaptic Zn^{2+} dysregulation via excess influx of extracellular Zn^{2+} through GluR2-lacking AMPA receptors, in addition to Zn^{2+} dysregulation via ferry of extracellular $A\beta_{1-42}$.

In contrast, γ-aminobutyric acid (GABA) is a major inhibitory neurotransmitter and GABAergic system plays a key role in regulating glutamate excitotoxicity in neurological diseases including AD [31, 32]. Effusol concentration-dependently enhance $GABA_A$ receptor-mediated current in an in vitro system [14], suggesting that $GABA_A$ receptor activation by effusol is involved in the effects on the traditional use of *Juncus effuses* in anxiety and insomnia. Effusol reduces glutamate-induced excitotoxicity in the hippocampus after exposure to corticosterone, perhaps via $GABA_A$ receptor activation followed by amelioration of Zn^{2+} dysregulation [15]. A possible effect of effusol on $A\beta_{1-42}$ neurotoxicity is that effusol may reduce Aβ-induced synaptic hyperexcitation induced by glutamate exocytosis via activating $GABA_A$ receptors followed by ameliorating Zn^{2+} dysregulation.

In conclusion, the present paper first suggests that effusol protects dentate gyrus neuron death via reducing ROS generation, which is induced by intracellular increase in Zn^{2+} after exposure to $A\beta_{1-42}$. It is likely that oral intake of effusol may protectively act on the $A\beta_{1-42}$ pathogenesis.

Declarations

CONFLICT OF INTEREST

Authors declare no conflicts of interest.

FUNDING DECLARATION

No Funding

Author Contribution

"A.T. wrote the main manuscript text and H.T. prepared figures 1-5. All authors reviewed the manuscript."

References

1. Perrin RJ, Fagan AM, Holtzman DM. Multimodal techniques for diagnosis and prognosis of Alzheimer's disease. Nature. 2009;461(7266):916–22.
2. Kepp KP. Alzheimer's disease due to loss of function: A new synthesis of the available data. Prog Neurobiol. 2016;143:36–60.
3. Mucke L, Masliah E, Yu GQ, Mallory M, Rockenstein EM, Tatsuno G. High-level neuronal expression of abeta 1–42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. J Neurosci. 2000;20(11):4050–8.
4. Yan Y, Wang C. Abeta42 is more rigid than Abeta40 at the C terminus: implications for Abeta aggregation and toxicity. J Mol Biol. 2006;364(5):853–62.
5. Takeda A, Nakamura N, Fujii H, Uematsu C, Minamino T, Adlard PA, Bush AI, Tamano H. Amyloid β-mediated Zn^{2+} influx into dentate granule cells transiently induces a short-term cognitive deficit. PLoS ONE. 2014;9(12):e115923.
6. Takeda A, Tamano H, Tempaku M, Sasaki M, Uematsu C, Sato S, Kanazawa H, Datki ZL, Adlard PA, Bush AI. Extracellular Zn^{2+} is essential for amyloid $\beta_{1\text{-}42}$-induced cognitive decline in the normal brain and its rescue. J Neurosci. 2017;37(30):7253–62.
7. Tamano H, Oneta N, Shioya A, Adlard PA, Bush AI, Takeda A. In vivo synaptic activity-independent co-uptakes of amyloid $\beta_{1\text{-}42}$ and Zn^{2+} into dentate granule cells in the normal brain. Sci Rep. 2019;9(1):6498.
8. Tamano H, Takiguchi M, Tanaka Y, Murakami T, Adlard PA, Bush AI, Takeda A. Preferential neurodegeneration in the dentate gyrus by amyloid $\beta_{1\text{-}42}$-induced intracellular Zn^{2+} dysregulation and its defense strategy. Mol Neurobiol. 2020;57(4):1875–88.
9. Takeda A, Tamano H. Insight into brain metallothioneins from bidirectional Zn^{2+} signaling in synaptic dynamics. Metallomics. 2024;16:mfae039.
10. Takeda A, Tamano H, Hashimoto W, Kobuchi S, Suzuki H, Murakami T, Tempaku M, Koike Y, Adlard PA, Bush AI. Novel defense by metallothionein induction against cognitive decline: from amyloid $\beta_{1\text{-}42}$-induced excess Zn^{2+} to functional Zn^{2+} deficiency. Mol Neurobiol. 2018;55(10):7775–88.
11. Li J, Feng W, Dai R, Li B. Recent Progress on the Identification of Phenanthrene Derivatives in Traditional Chinese Medicine and Their Biological Activities. Pharmacol Res - Mod Chin Med. 2022;3:100078.
12. Tamano H, Takiguchi M, Saeki N, Katahira M, Shioya A, Tanaka Y, Egawa M, Fukuda T, Ikeda H, Takeda A. Dehydroeffusol prevents amyloid $\beta_{1\text{-}42}$-mediated hippocampal neurodegeneration via

reducing intracellular Zn^{2+} toxicity. Mol Neurobiol. 2021;58(8):3603–13.

13. Tamano H, Takiguchi M, Murakami D, Kawano Y, Fukuda T, Ikeda H, Akagi Y, Ikeura S, Takeda A. Blockage of metallothionein synthesis via adrenaline β receptor activation invalidates dehydroeffusol-mediated prevention of amyloid $\beta_{1\text{-}42}$ toxicity. Neurosci Lett. 2024;825:137708.
14. Singhuber J, Baburin I, Khom S, Zehl M, Urban E, Hering S, Kopp B. GABA(A) receptor modulators from the Chinese herbal drug *Junci Medulla*—the pith of *Juncus effusus*. Planta Med. 2012;78(5):455–8.
15. Tamano H, Sato Y, Takiguchi M, Murakami T, Fukuda T, Kawagishi H, Suzuki M, Takeda A. CA1 LTP attenuated by corticosterone is canceled by effusol via rescuing intracellular Zn^{2+} dysregulation. Cell Mol Neurobiol. 2019;39(7):975–83.
16. Cousins RJ. Metallothionein synthesis and degradation: relationship to cadmium metabolism. Environ Health Perspect. 1979;28:131–6.
17. Fukuda T, Sato Y, Takiguchi M, Yamamoto T, Murasawa H, Pawlak A, Kobayashi H, Tamano H, Takeda A. Dehydroeffusol rescues amyloid $\beta_{25\text{-}35}$-induced spatial working memory deficit. Plant Foods Hum Nutr. 2020;75:279–82.
18. Bouw R, Ederoth P, Lundberg J, Ungerstedt U, Nordström CH, Hammarlund-Udenaes M. Increased blood-brain barrier permeability of morphine in a patient with severe brain lesions as determined by microdialysis. Acta Anaesthesiol Scand. 2001;45:390–2.
19. Ederoth P, Tunblad K, Bouw R, Lundberg CJ, Ungerstedt U, Nordström CH, Hammarlund-Udenaes M. Blood-brain barrier transport of morphine in patients with severe brain trauma. Br J Clin Pharmacol. 2004;57(4):427–35.
20. Haruna Tamura M, Sasaki S, Nakajima R, Nishio N, Saeki M, Katahira H, Tamano. Atsushi Takeda: Reactive oxygen species produced by Zn^{2+} influx after exposure to AMPA, but not NMDA and their capturing effect on nigral dopaminergic protection. Neurotoxicology. 2023;95:173–80.
21. Li X, Jiang LH. Multiple molecular mechanisms form a positive feedback loop driving amyloid β42 peptide-induced neurotoxicity via activation of the TRPM2 channel in hippocampal neurons. Cell Death Dis. 2018;9:195.
22. Halliwell B. Oxidative stress and neurodegeneration: where are we now? J Neurochem. 2006;97(6):1634–58.
23. Wang X, Wang W, Li L, Perry G, Lee HG, Zhu X. Oxidative stress and mitochondrial dysfunction in Alzheimer's disease. Biochim Biophys Acta. 2014;1842(8):1240–7.
24. Ionescu-Tucker A, Cotman CW. Emerging roles of oxidative stress in brain aging and Alzheimer's disease. Neurobiol Aging. 2021;107:86–95.
25. Li S, Hong S, Shepardson NE, Walsh DM, Shankar GM, Selkoe D. Soluble oligomers of amyloid beta protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. Neuron. 2009;62(6):788–801.

26. Talantova M, Sanz-Blasco S, Zhang X, Xia P, Akhtar MW, Okamoto S, Dziewczapolski G, Nakamura T, Cao G, Pratt AE, Kang YJ, Tu S, Molokanova E, McKercher SR, Hires SA, Sason H, Stouffer DG, Buczynski MW, Solomon JP, Michael S, Powers ET, Kelly JW, Roberts A, Tong G, Fang-Newmeyer T, Parker J, Holland EA, Zhang D, Nakanishi N, Chen HS, Wolosker H, Wang Y, Parsons LH, Ambasudhan R, Masliah E, Heinemann SF, Piña-Crespo JC, Lipton SA. Aβ induces astrocytic glutamate release, extrasynaptic NMDA receptor activation, and synaptic loss. Proc Natl Acad Sci USA. 2013;110(27):E2518–27.
27. Zott B, Simon MM, Hong W, Unger F, Chen-Engerer HJ, Frosch MP, Sakmann B, Walsh DM, Konnerth A. A vicious cycle of β amyloid-dependent neuronal hyperactivation. Science. 2019;365(6453):559–65.
28. Abramov E, Dolev I, Fogel H, Ciccotosto GD, Ruff E, Slutsky I. Amyloid-β as a positive endogenous regulator of release probability at hippocampal synapses. Nat Neurosci. 2009;12(12):1567–76.
29. Yang XK, Zhang FL, Jin XK, Jiao YT, Zhang XW, Liu YL, Amatore C, Huang WH. Nanoelectrochemistry reveals how soluble $A\beta_{42}$oligomers alter vesicular storage and release of glutamate. Proc Natl Acad Sci USA. 2023;120(19):e2219994120.
30. Pillai AG, Nadkarni S. Amyloid pathology disrupts gliotransmitter release in astrocytes. PLoS Comput Biol. 2022;18(8):e1010334.
31. Akyuz E, Celik BR, Aslan FS, Sahin H, Angelopoulou E. Exploring the Role of Neurotransmitters in Multiple Sclerosis: An Expanded Review. ACS Chem Neurosci. 2023;14:527–53.
32. Perovic M, Pavlovic D, Palmer Z, Udo MSB, Citadin CT, Rodgers KM, Wu CY, Zhang Q, Lin HW, Tesic V. Modulation of GABAergic system as a therapeutic option in stroke. Exp Neurol. 2025;384:115050.

Figures

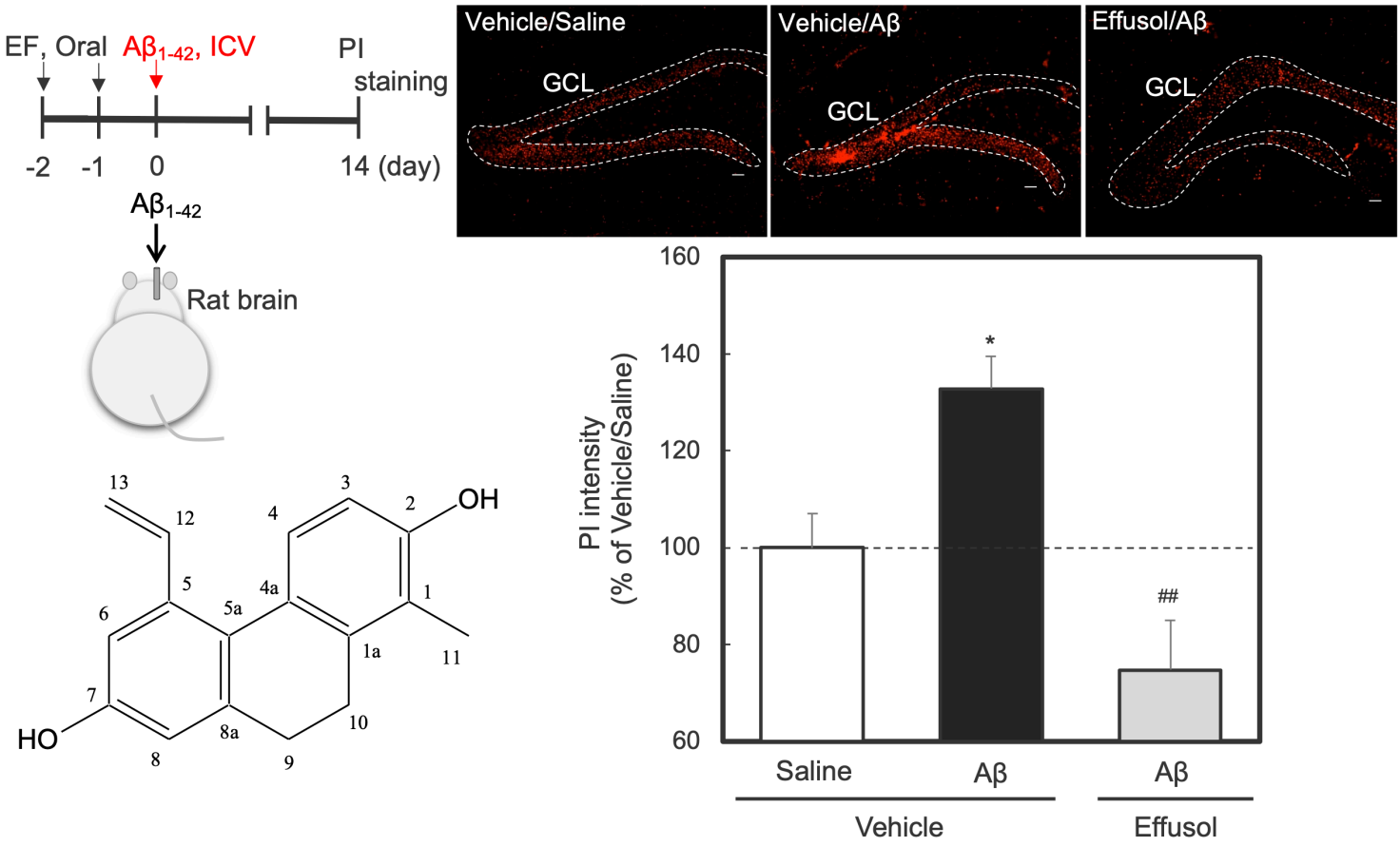


Figure 1

Effusol ameliorates $A\beta_{1\text{-}42}$-mediated neurotoxicity

$A\beta_{1\text{-}42}$ and vehicle (saline) were administered to mice 1 day after oral delivery of effusol (EF). Fourteen days later, $A\beta_{1\text{-}42}$-induced neurotoxicity by PI fluorescence was determined in the dentate granule cell layer (GCL) indicated by the dotted line (upper). Bar; 50 μm. Structure of effusol (lower light). The data showed the rate (%) of PI fluorescence of vehicle/$A\beta_{1\text{-}42}$ (n=12 mice) and effusol/$A\beta_{1\text{-}42}$ (n=11 mice) groups to that of vehicle/saline (n=17 mice) group showed as 100% (lower right). *, $p<0.05$, vs. vehicle/saline, $^{\#\#}$, $p<0.01$, vs. vehicle/Aβ (Tukey's test).

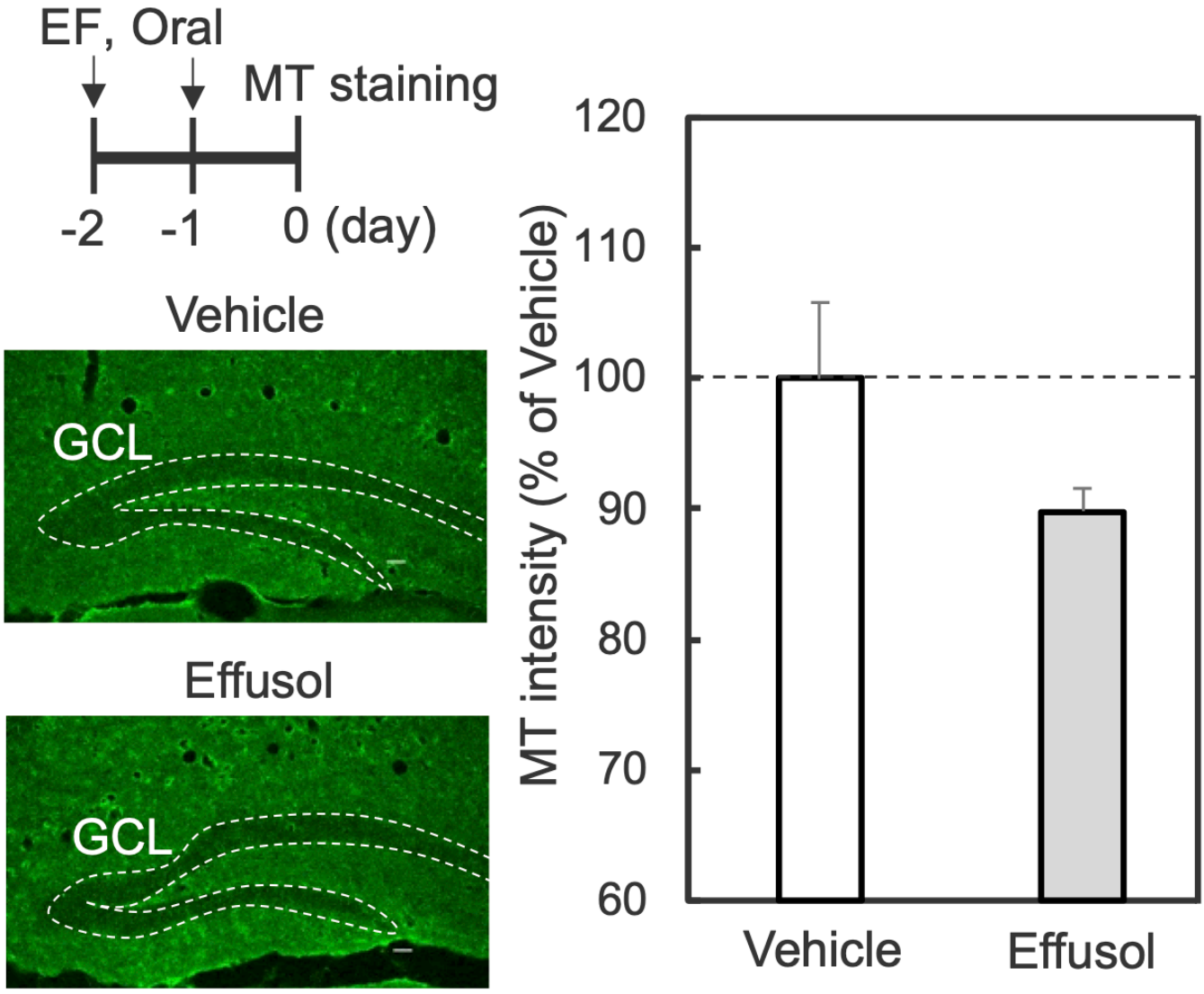


Figure 2

Effusol does not increase MT synthesis

MT immunostaining was determined in the GCL 1 day after oral delivery of effusol (EF) (left). Bar; 50 μm. The data showed the rate (%) of MT staining of effusol group (n=8 mice) to that of vehicle group showed as 100% (right) (n=12 mice).

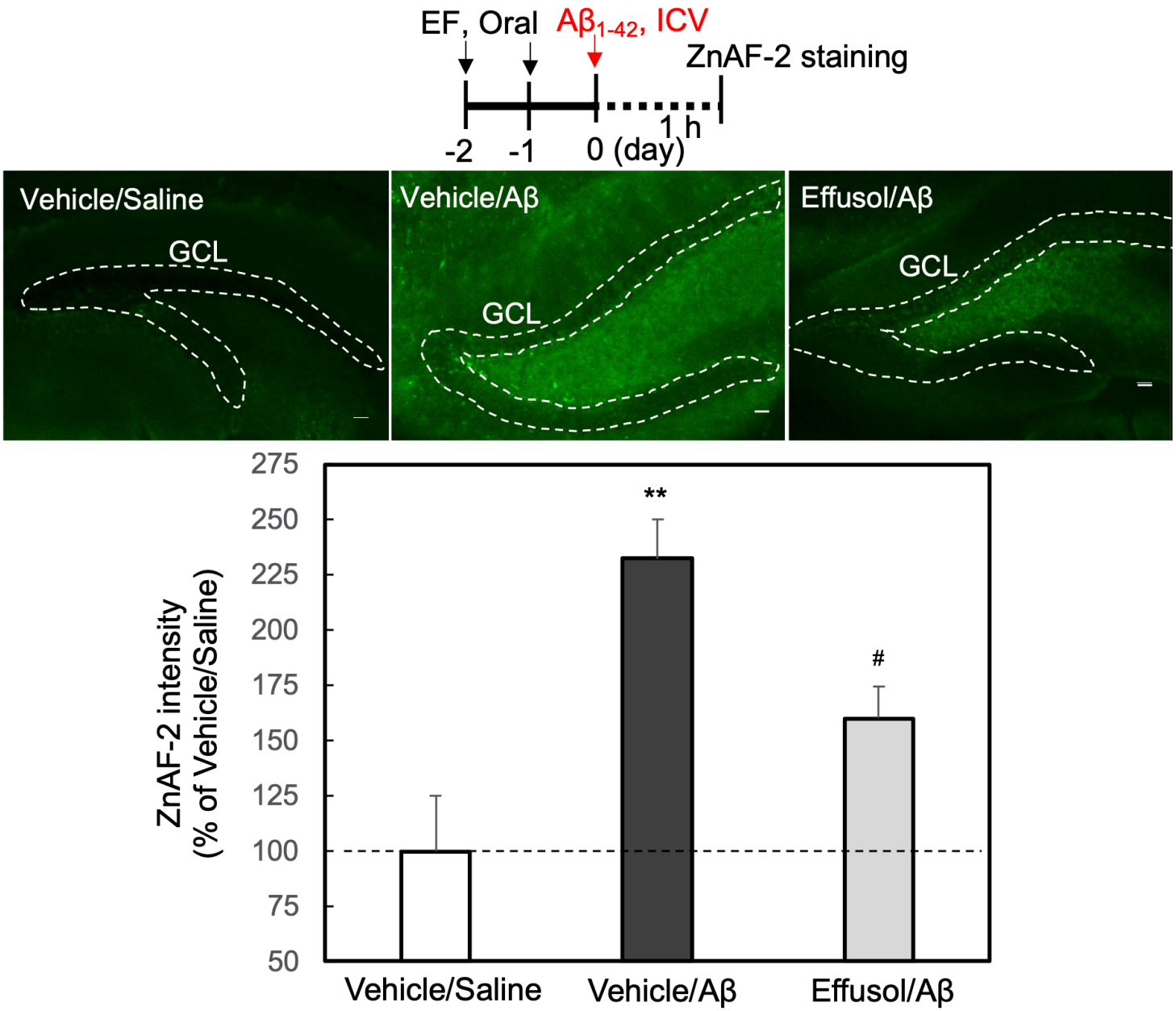


Figure 3

Effusol suppresses increase in intracellular Zn^{2+}

$A\beta_{1-42}$ and vehicle (saline) were administered to mice 1 day after oral delivery of effusol (EF). Intracellular Zn^{2+} level with ZnAF-2 fluorescence was determined in the GCL 1 h after adminstering $A\beta_{1-42}$ (Upper). Bar; 50 μm. The data showed the rate (%) of intracellular ZnAF-2 intensity of vehicle/$A\beta_{1-42}$ (n=12) and effusol/$A\beta_{1-42}$ (n=12 mice) groups to that of vehicle/saline (n=12 mice) group showed as 100% (lower). **, $p<0.01$, vs. vehicle/saline, $^{\#}$, $P<0.05$, vs. vehicle/Aβ (Tukey's test).

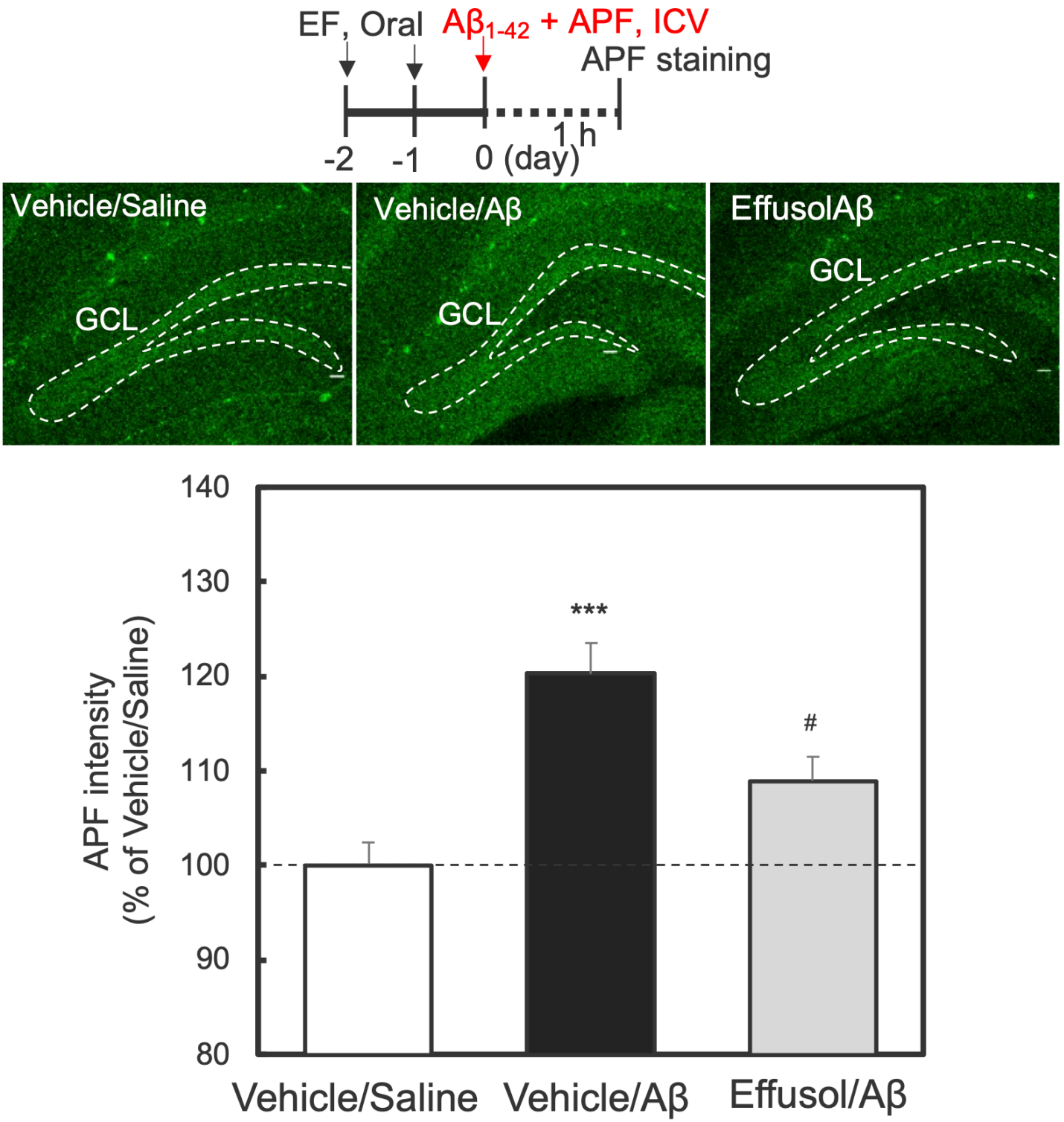


Figure 4

Effusol reduces increase in intracellular ROS

$A\beta_{1\text{-}42}$ and saline with APF were administered to mice 1 day after oral delivery of effusol (EF). Intracellular ROS with APF fluorescence was determined in the GCL 1 h after administering $A\beta_{1\text{-}42}$. (Upper). Bar; 50 μm. The data showed the rate (%) of intracellular APF intensity of vehicle/$A\beta_{1\text{-}42}$ (n=12

mice) and effusol/$A\beta_{1-42}$ (n=9 mice) groups to that of vehicle/saline (n=7 mice) group showed as 100% (lower). ***, p<0.01, vs. vehicle/saline; #, P<0.05, vs. vehicle/Aβ (Tukey's test).

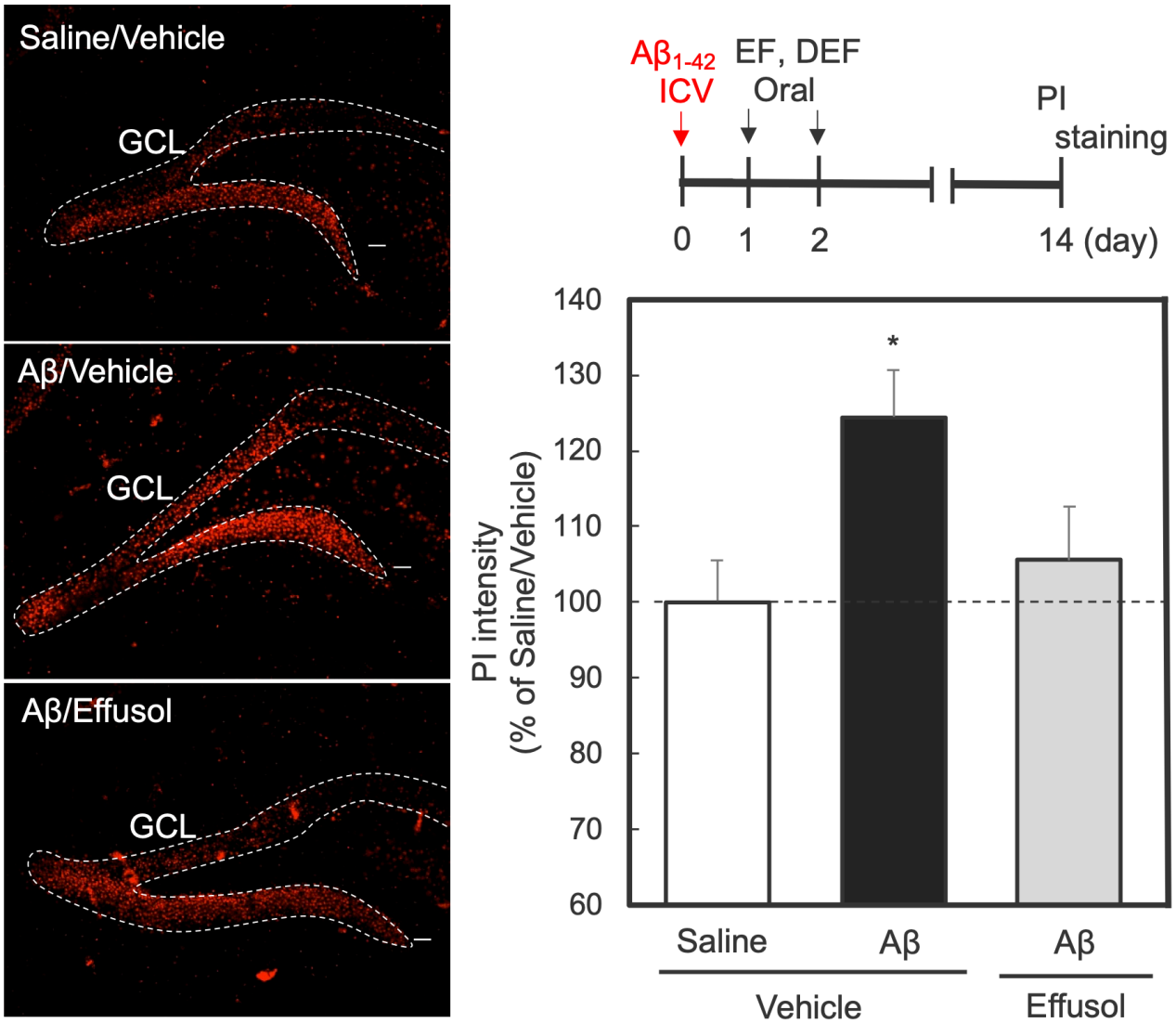


Figure 5

Post-oral intake of effusol decreases $A\beta_{1-42}$-induced neurotoxicity

One day after administering $A\beta_{1-42}$ and saline (vehicle), effusol (EF) and vehicle were orally delivered to mice. $A\beta_{1-42}$-induced neurotoxicity by PI fluorescence was determined in the GCL 14 day after administering $A\beta_{1-42}$ (left). Bar; 50 μm. The data showed the rate (%) of PI fluorescence of Aβ/vehicle (n=24 mice) and Aβ/effusol (n=12 mice) groups to that of saline/vehicle (n=28 mice) group showed as 100% (right). *, p<0.05, vs. saline/vehicle (Tukey's test).