Fosgonimeton Is Neuroprotective and Rescues Cognitive Performance in Models of Neuroinflammation

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CONCLUSIONS

1 Fosgonimeton protects against cognitive deficits in an LPS-induced mouse model of neuroinflammation

2 The effects of fosgonimeton on neuroinflammation may be driven by reduction of proinflammatory cytokine levels and protection against neurotoxicity

KEY TAKEAWAY

Fosgonimeton has procognitive and neuroprotective properties with therapeutic potential for neurodegenerative disorders, including AD, in which neuroinflammation is a known contributor to disease progression

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INTRODUCTION

- Neuroinflammation is a pathophysiological mechanism common to several neurodegenerative diseases, including AD[1]
- HGF signaling through the MET receptor is neurotrophic and induces neuroprotective, prosurvival, and anti-inflammatory mechanisms[2]
- Fosgonimeton, a small-molecule positive modulator of HGF/MET,[3,4] has the potential to protect against several components of neurodegeneration, including neuroinflammation
- LPS is a potent activator of TLR4 that can initiate strong release of TNF-α, IL-6, and IL-1β, all of which are proinflammatory mediators associated with neurodegeneration[5,6]
  - LPS administration is widely used to model neuroinflammation in vitro and in vivo[6,7]

Figure 1. Fosgonimeton positively modulates the neuroprotective and neurotrophic HGF/MET pathway
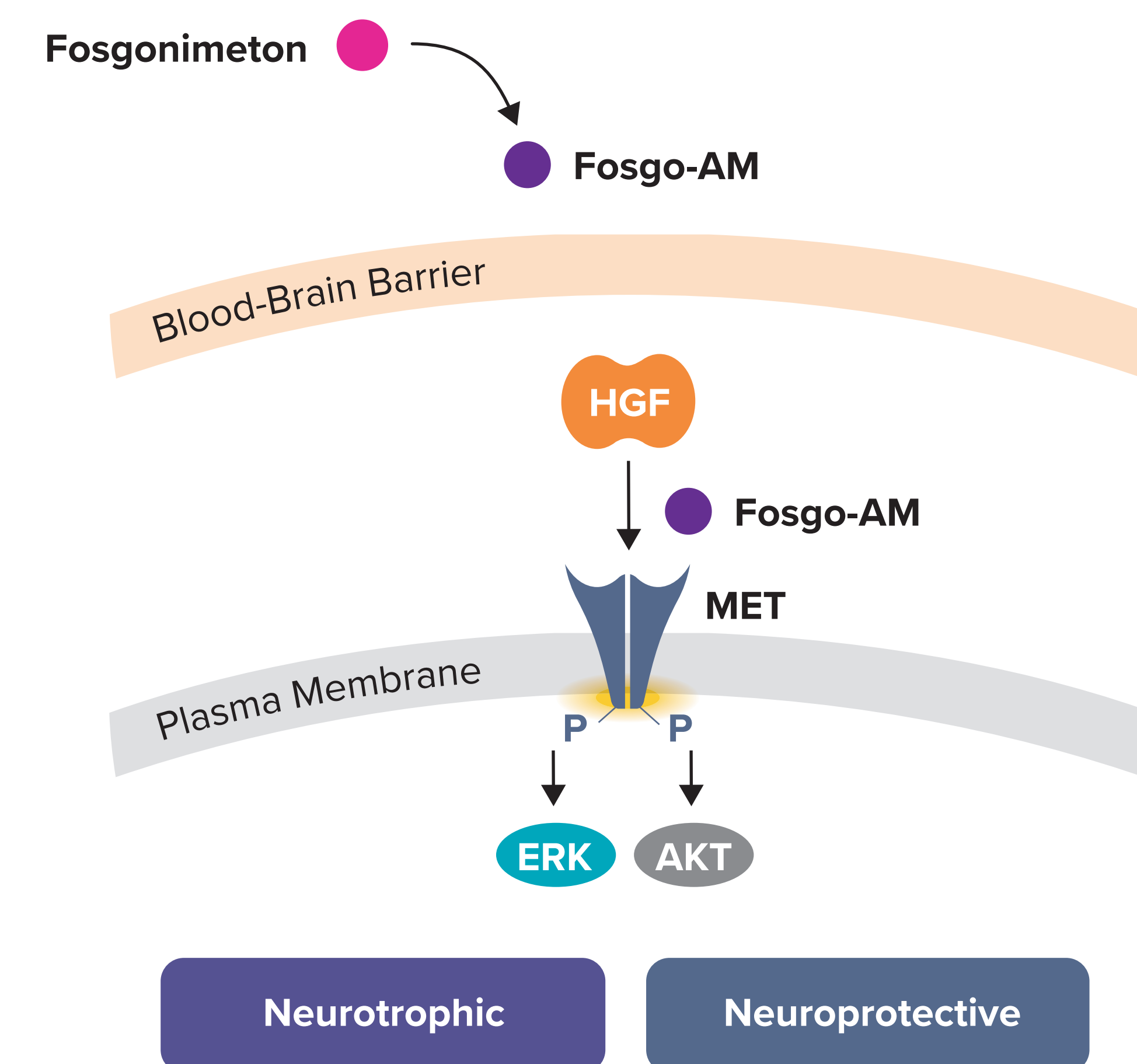
Fosgonimeton is converted in the blood to fosgo-AM, a brain-penetrant small molecule that promotes HGF/MET-driven neurotrophic and neuroprotective signaling cascades in the brain.

OBJECTIVE

To assess the ability of fosgonimeton to protect against cognitive deficits in an LPS-induced neuroinflammatory model of cognitive impairment, as well as LPS-induced cytokine release and neurotoxicity in vitro

METHODS

In vivo LPS-induced cognitive impairment assay

- Four- to five-week-old male CD-1 mice received a single IP injection of LPS (0.25 mg/kg) to induce neuroinflammation, followed by daily treatment with fosgonimeton (0.125, 0.25, 0.5, 1, or 1.25 mg/kg SC), vehicle (normal saline SC and IP), or the positive control memantine (0.1 mg/kg IP) for 14 days
- One hour after treatment on day 14, mice were evaluated in the T-maze spontaneous alternation test to assess the effects of fosgonimeton on LPS-induced cognitive impairment

In vitro LPS-induced proinflammatory cytokine release assay

- THP-1–differentiated macrophages were pretreated for 20 minutes with fosgo-AM, the active metabolite of fosgonimeton, 0.01 pM, 1 pM, 100 pM, 10 nM, or 1 μM and then challenged with LPS 50 ng/mL for 24 hours
- Proinflammatory cytokines IL-1β and IL-6 were measured via HTRF, and TNF-α was measured via ELISA

In vitro neuroprotection assay

- Cortical neurons from rats (1-3 days postnatal) were cultured to maturity (35-40 days), pretreated for 15 minutes with fosgo-AM 1 nM, 10 nM, 100 nM, or 1 μM and then subjected to LPS 1 μM for 24 hours
- The CellTiter-Glo Luminescent Cell Viability Assay (Promega), which measures ATP as a marker of living cells, was used to assess neuron viability after LPS injury

Figure 2. Cognitive impairment of spatial working memory assessed by performance in the T-maze spontaneous alternation test
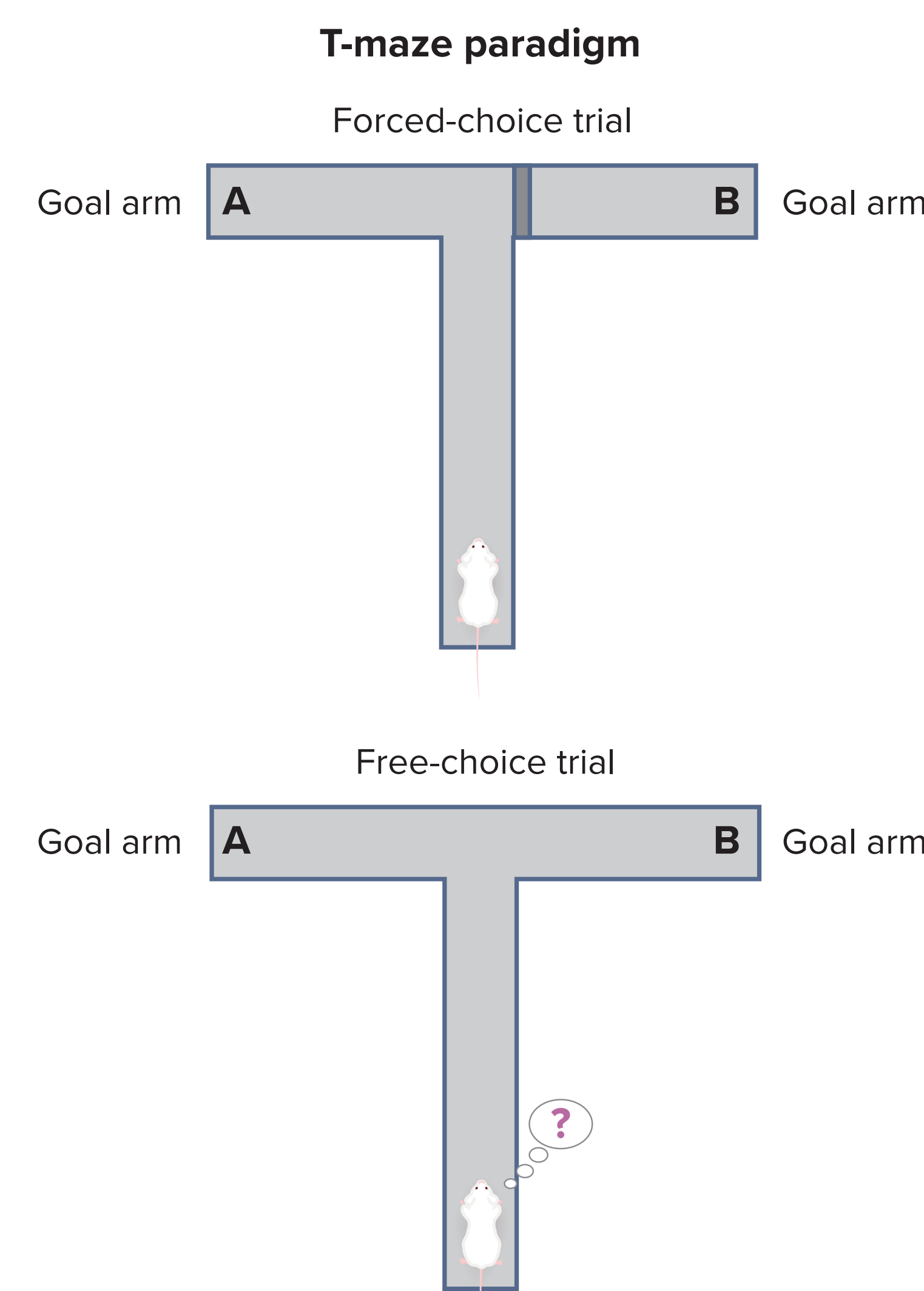
T-maze performance was used to evaluate cognitive impairment. Mice were subjected to one forced-choice trial, in which a door closed off one of the two arms, followed by 14 free-choice trials. The rate at which mice chose the alternate goal arm (ie, the one they did not choose during the previous trial) served as an index of working memory performance.

RESULTS

### Figure 3. Fosgonimeton attenuates LPS-induced cognitive impairment
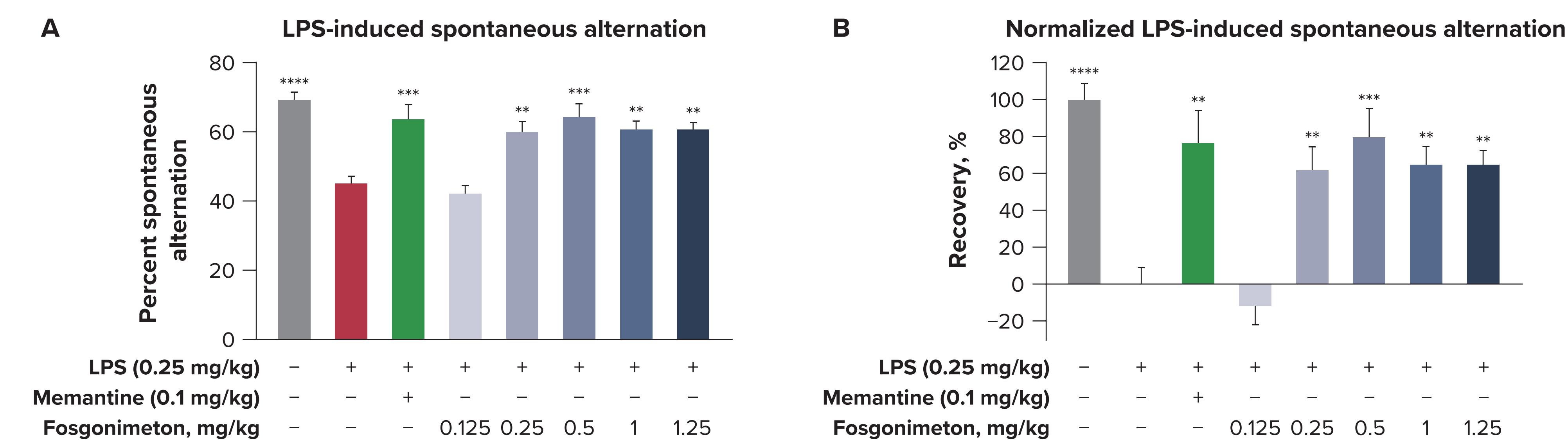
(A) Percent spontaneous alternation was calculated as the proportion of the 14 free-choice trials in which mice went in an alternate goal arm than they did in the trial immediately before. Spontaneous alternation was reduced in LPS-exposed mice (red bar) compared with controls (gray bar), indicating cognitive impairment, but this reduction was significantly ameliorated in LPS-exposed mice treated with fosgonimeton (at all but the lowest tested dose, blue bars), or with those treated with the positive control memantine (green bar). (B) Percentage recovery was calculated by normalizing the control group to 100% (gray bar) and the LPS group to 0%, then calculating the relative response for each animal. The percentage recovery relative to the vehicle-treated LPS mice was 61.8% with fosgonimeton 0.25 mg/kg, 79.4% with 0.5 mg/kg, 64.7% with 1 mg/kg, and 64.7% with 1.25 mg/kg (blue bars). Treatment with memantine resulted in 76.4% recovery (green bar).
All data displayed as mean + SEM. One-way ANOVA with Dunnett's test for multiple comparisons vs LPS control group (percent spontaneous alternation), or the Kruskal-Wallis test with Dunn's test for multiple comparisons vs LPS control group (% recovery) (Prism, version 9.5.0; GraphPad).
**p < 0.01; ***p < 0.001; ****p < 0.0001.

Figure 4. Fosgo-AM significantly reduces LPS-induced proinflammatory cytokine release in vitro
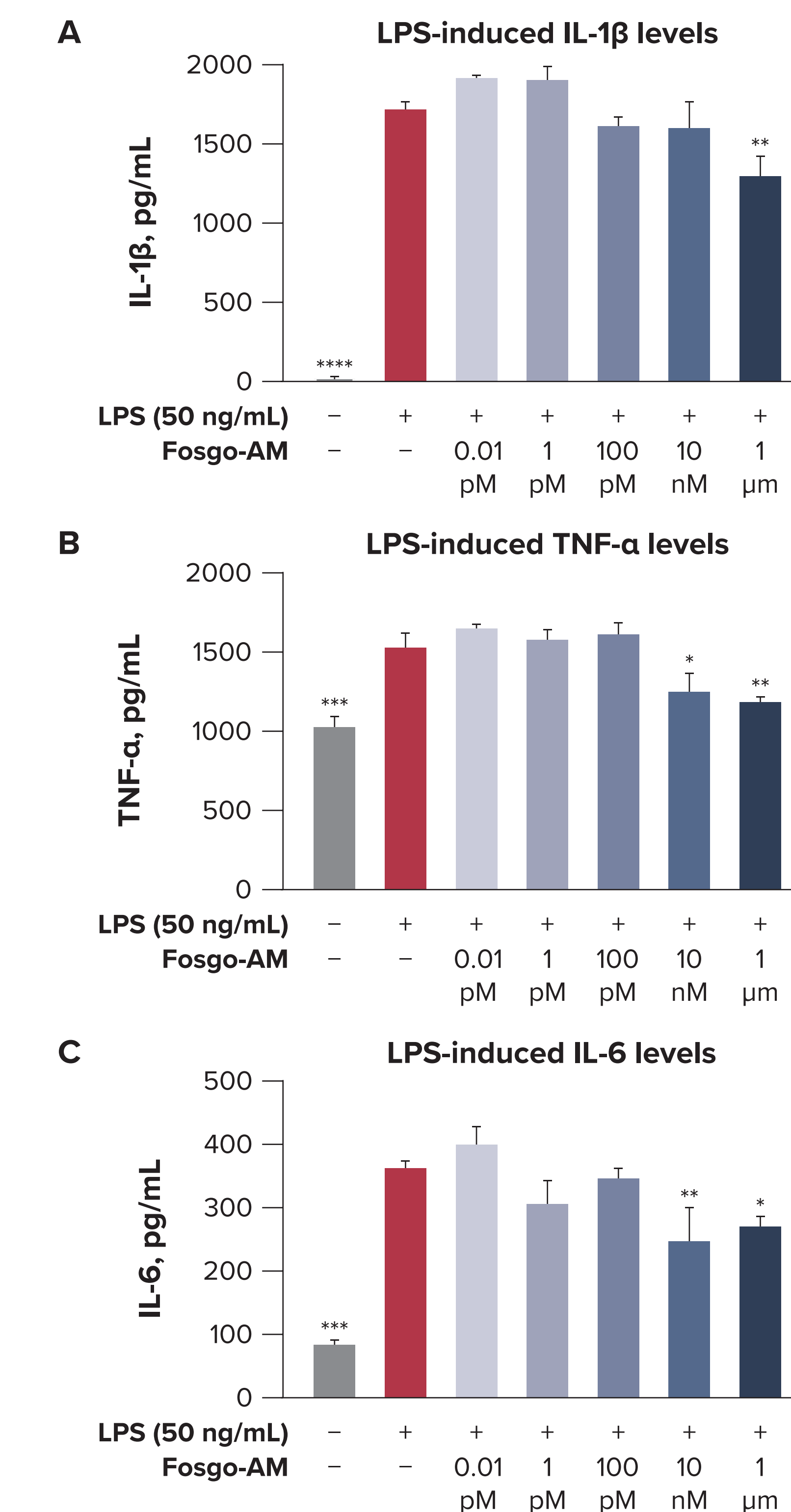
THP-1–differentiated macrophages pretreated with fosgo-AM had significantly decreased levels of (A) IL-1β release, (B) TNF-α release, and (C) IL-6 release after LPS challenge compared with vehicle.
All data displayed as mean + SEM. One-way ANOVA with Dunnett's test for multiple comparisons vs LPS control group (Prism, version 9.5.0; GraphPad).
*p < 0.05; **p < 0.01; ***p < 0.001; ****p <0.0001.

Figure 5. Fosgo-AM protects cortical neurons from LPS-induced cytotoxicity
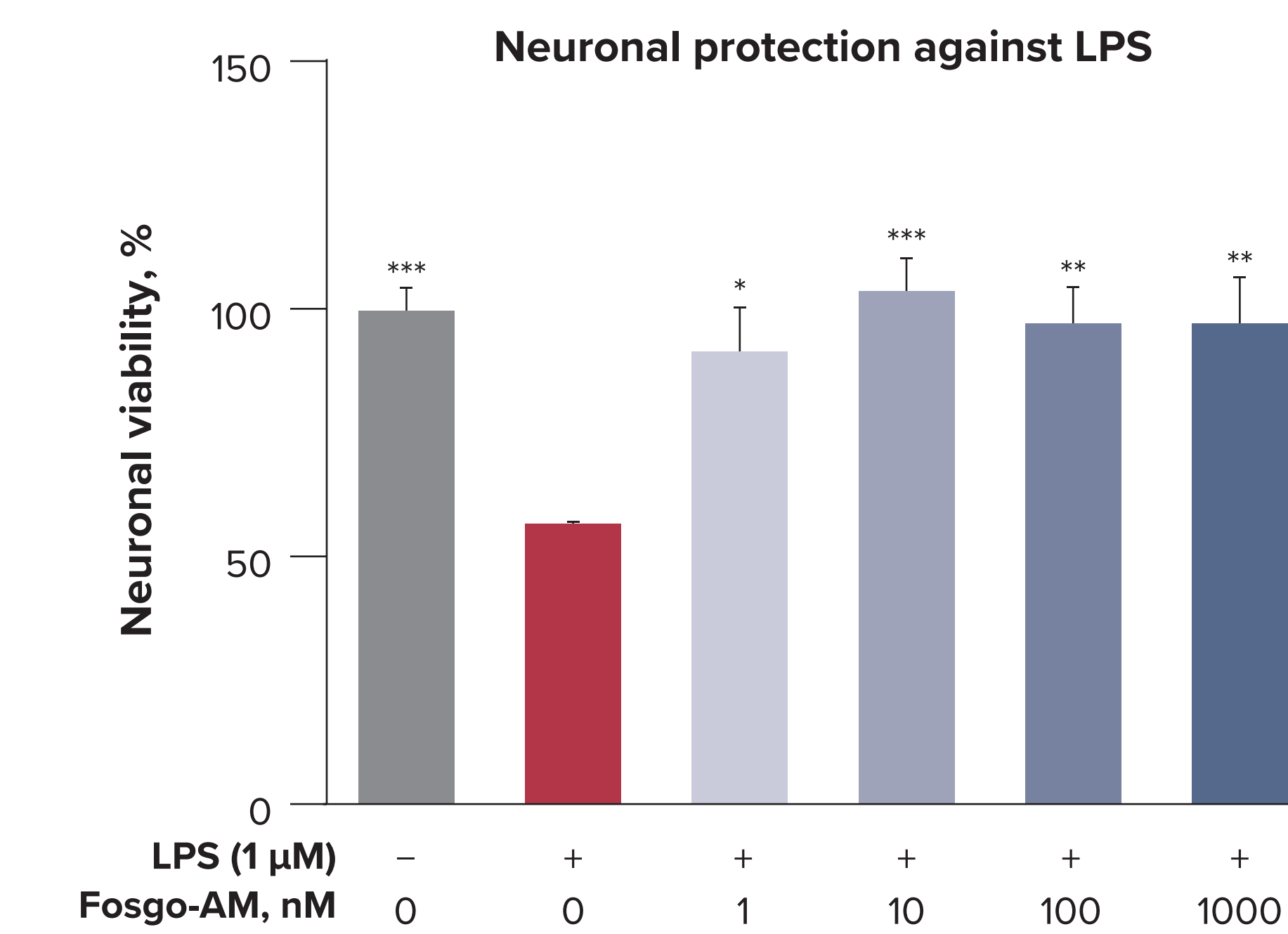
Fosgo-AM pretreatment significantly improved viability of cultured cortical neurons against inflammatory damage induced by LPS.
All data displayed as mean + SEM. One-way ANOVA with Tukey's test (Prism, version 9.5.0; GraphPad).
*p < 0.05; **p < 0.01; ***p < 0.001.

Abbreviations AD, Alzheimer's disease; AKT, protein kinase B; ANOVA, analysis of variance; ATP, adenosine triphosphate; ELISA, enzyme-linked immunosorbent assay; ERK, extracellular signal-regulated kinase; fosgo-AM, active metabolite of fosgonimeton; HGF, hepatocyte growth factor; HTRF, homogeneous time-resolved fluorescence; IL-6, interleukin 6; IL-1β, interleukin 1β; IP, intraperitoneal; LPS, lipopolysaccharide; P, phosphorylation; SC, subcutaneous; SEM, standard error of the mean; THP-1, human monocyclic cell line 1; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor α.

References 1. Jellinger KA et al. *J Cell Mol Med*. 2010;14:457-487. **2.** Desole C et al. *Front Cell Dev Biol*. 2021;9:683609. **3.** Hua X et al. *J Alzheimer's Dis*. 2022;86:1399-1413. **4.** Johnston JL et al. *Neurotherapeutics*. 2022;10.1007/s13311-022-01325-5. **5.** Batista C et al. *Int J Mol Sci*. 2019;20:2993. **6.** Zhao J et al. *Sci Rep*. 2019;9:5790. **7.** Skrzypczak-Wiercioch A et al. *Molecules*. 2022;27(17):5481.