Positive Modulation of Hepatocyte Growth Factor/MET by a Novel Small Molecule Induces Neurotrophic and Procognitive Effects

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Disclosures

- Jewel Johnston, Robert Taylor, Sherif Reda and Kevin Church are all employees and stockholders of Athira Pharma, Inc.

Alzheimer's and Parkinson's Disease Dementia: Critical Unmet Need

Alzheimer's Disease

55 million
people living with Alzheimer's dementia today[1]

Over 100 million globally by 2050
~900,000 new patients diagnosed annually in the US alone[1,2]

Parkinson's Disease Dementia

Nearly 1 million
people in the US, and more than 10 million people globally, are living with Parkinson's disease[3]

~50%
of patients with Parkinson's disease experience dementia symptoms[4]

Currently available therapies for both conditions have limited efficacy that wanes over time

Critical unmet treatment need for these patient populations, particularly for therapies focused on restoring neuronal health and function[2]

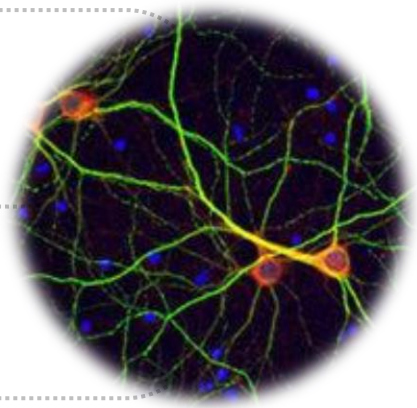
1. https://www.alzint.org/about/dementia-facts-figures/. 2. https://www.alz.org/media/documents/alzheimers-facts-and-figures.pdf. 3. https://www.parkinson.org/understanding-parkinsons. 4. https://www.alz.org/alzheimers-dementia/what-is-dementia/types-of-dementia/parkinson-s-disease-dementia.

Alzheimer's Disease Pathology

Multifactorial and Complex Pathologies Ultimately Lead to Neurodegeneration

Alzheimer's disease

- **Nervous system** → Protein misfolding, Oxidative stress, synaptic dysfunction
- **Vasculature** → Reduced cerebral blood flow
- **Immune system** → Inflammation
- **Neurotransmitters** → Neurotransmitter system dysfunction



Neurodegeneration synaptic loss

Loss of network connectivity

Symptom onset

Disease progression

https://www.alz.org/media/documents/alzheimers-facts-and-figures.pdf

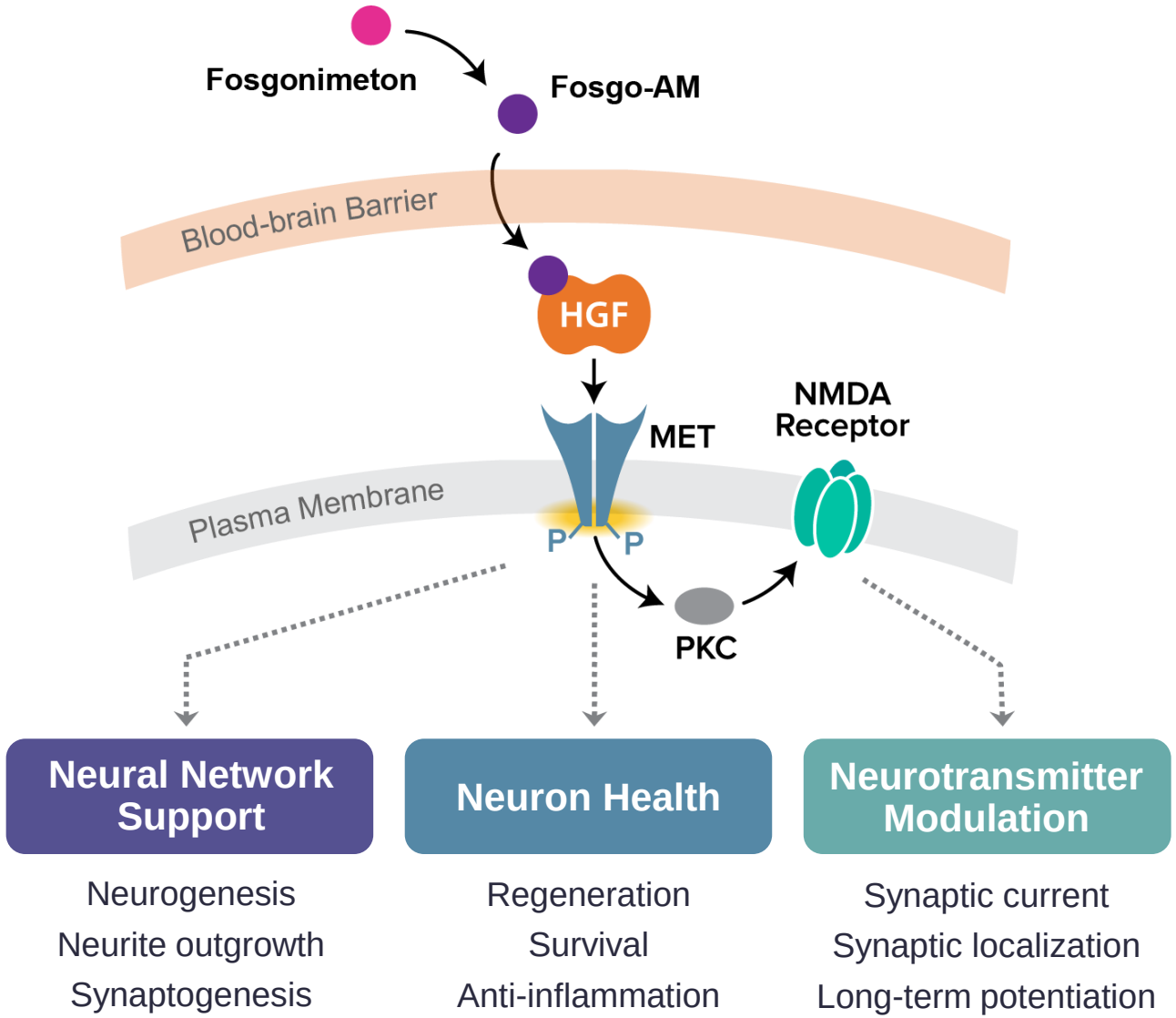
Fosgonimeton: A Positive Modulator of the HGF/MET Neurotrophic System

Multimodal, Protective, and Regenerative

Fosgonimeton (ATH-1017):

Small molecule prodrug that is immediately converted to its active metabolite, fosgo-AM (ATH-1001)

- Crosses the blood-brain barrier
- Positively modulates HGF/MET



HGF, hepatocyte growth factor; NMDA, N-methyl-D-aspartate; PKC, protein kinase C.
HGF/MET signaling and downstream effects described in: Desole et al. *Frontiers in Cell and Dev Bio.* 2021. Funakoshi and Nakamura. *Curr Signal Transduc Ther.* 2011.

Objective

To evaluate the neurotrophic effects of fosgo-AM, the active metabolite of fosgonimeton, in several preclinical models

- In vitro assessment of HGF/MET pathway signaling activation in HEK293 cells
- Promotion of synaptogenesis and neurite outgrowth in in vitro rat primary hippocampal neurons
- Reversal of memory deficits in vivo using the Morris water maze in the scopolamine amnesia rat model

HEK, human embryonic kidney; HGF, hepatocyte growth factor.

First Step: In Vitro Assessment of HGF/MET Signaling Pathway Activation

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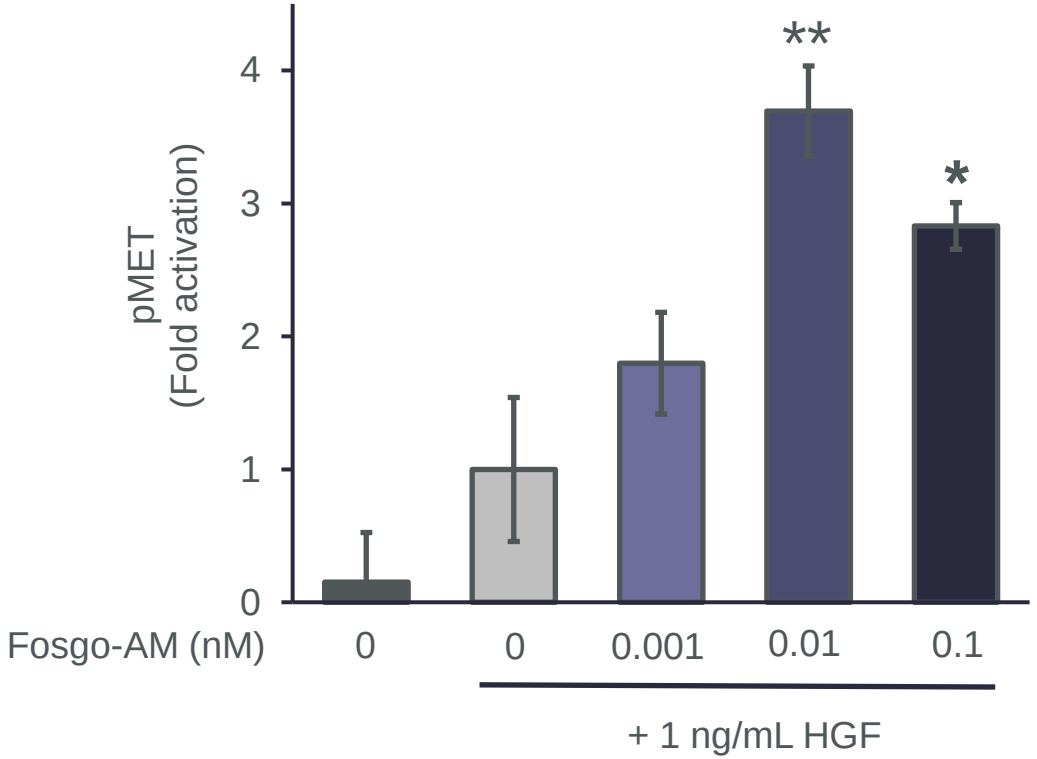
Positive Modulation of MET Receptor by Fosgo-AM in HEK293 Cells in the Presence of HGF

- Fosgo-AM enhancement of the HGF/MET pathway was assessed in vitro in HEK293 cells
  - Phosphorylated MET (pMET) were evaluated via ELISA in the presence of 1 ng/mL of HGF

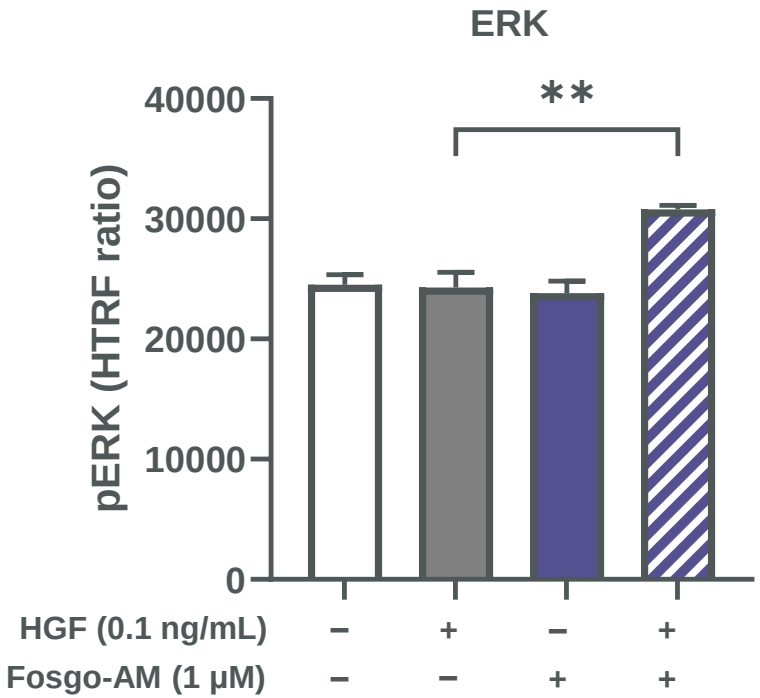


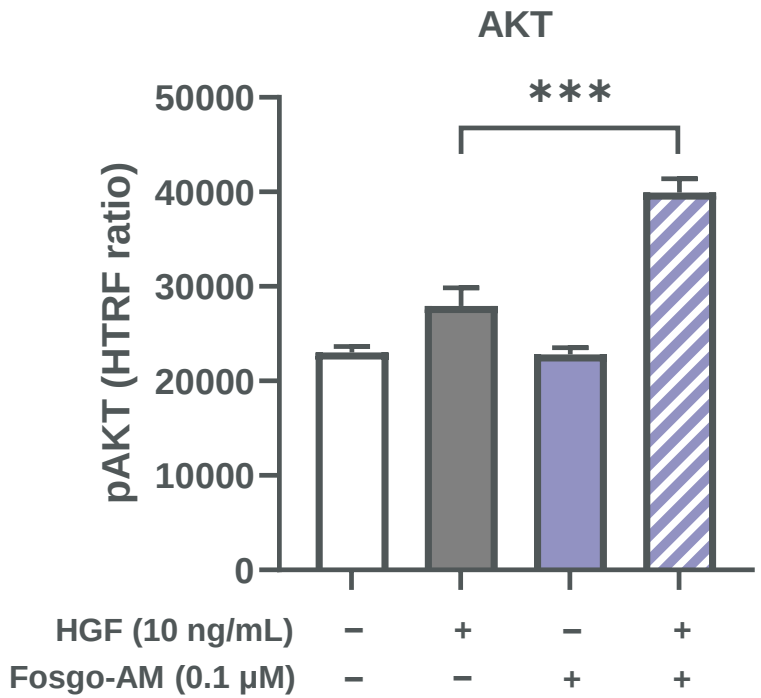
Fosgo-AM positively modulates the HGF/MET system in vitro

Data presented are mean ± SEM. Statistical difference determined by 1-way ANOVA with Dunnett's post test.
*P<0.05, **P<0.01 vs HGF alone.
ELISA, enzyme-linked immunosorbent assay; HEK, human embryonic kidney; HGF, hepatocyte growth factor; SEM, standard error of the mean.

Phosphoactivation of ERK and AKT by Fosgo-AM in HEK293 Cells

- Phosphoactivation of intracellular signaling molecules downstream of HGF/MET were evaluated via HTRF





ERK and AKT phosphorylation were significantly enhanced by treatment with fosgo-AM

Data for each group is presented as mean ± SEM. Statistical significance was determined by 1-way ANOVA with Tukey's multiple comparison post-test.
***P*<0.01, ****P*<0.001 vs HGF alone.
ERK, extracellular signal-regulated kinase; HEK, human embryonic kidney; HGF, hepatocyte growth factor; HTRF, homogenous time-resolved fluorescence; SEM, standard error of the mean.

Next Step: Assess Effects of Fosgo-AM on Primary Rodent Neuronal Cultures

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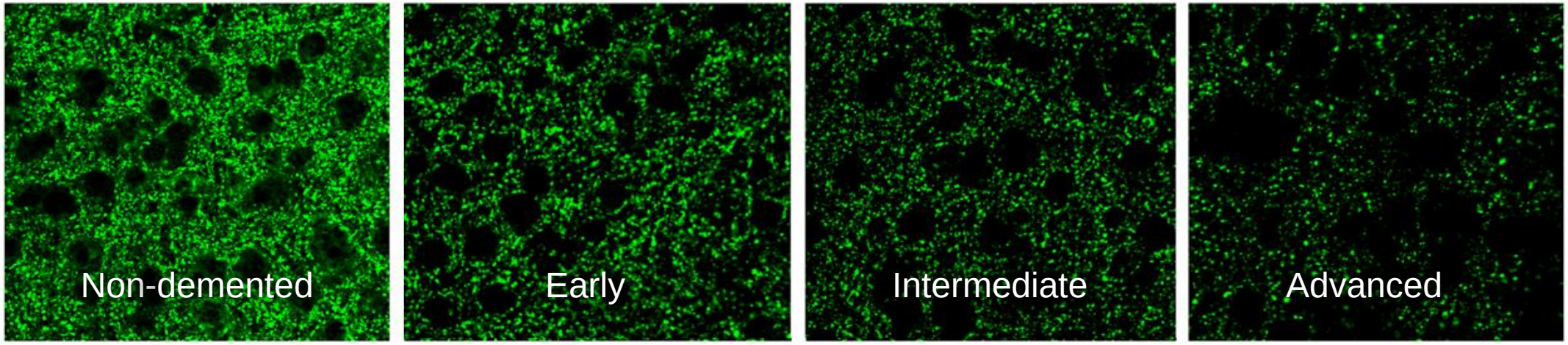
Synapse Loss in Alzheimer's Disease

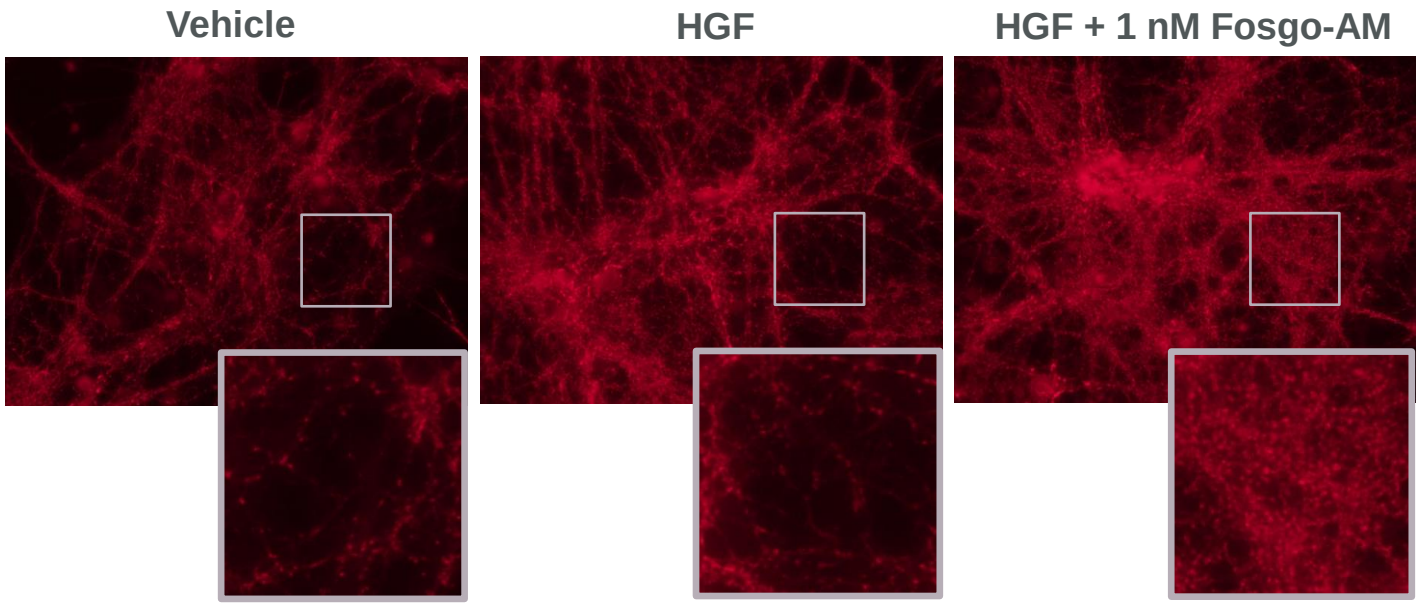
- In Alzheimer's disease, **25% to 36% of synapses are lost**[1]
- **Synapse loss is an early event in disease progression** that impacts several brain regions, including the hippocampus and frontal cortex, which are important for learning and memory[2]

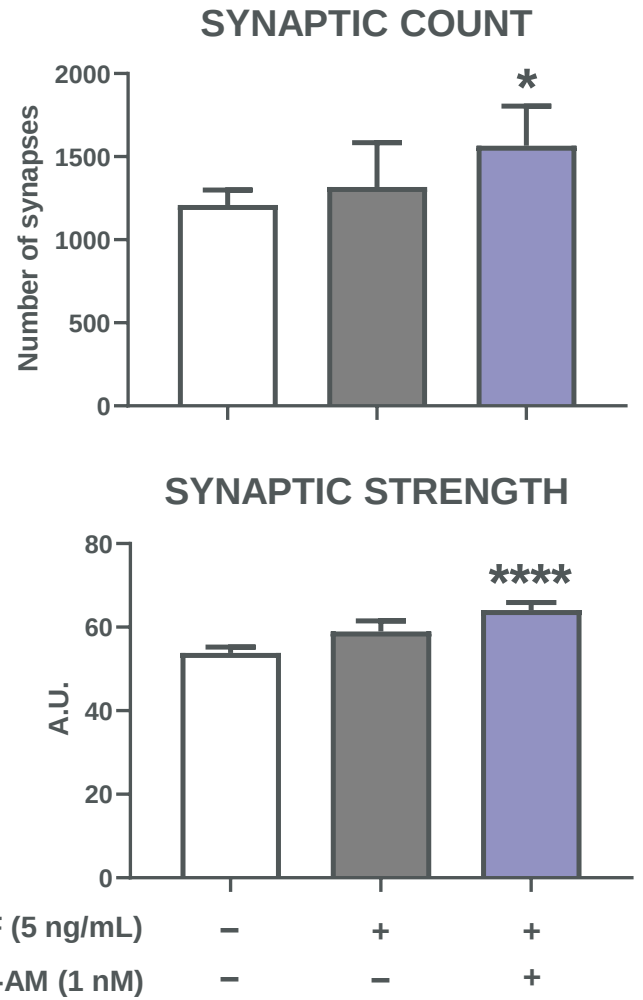


1. Davies et al. *J Neurol Sci.* 1987. 2. Masliah et al. *Neurosci Letters.* 1989..
Slide adapted from Olichney, as presented by Olichney, in Athira P300 KOL Webinar – November 2021.

Fosgo-AM Enhances Synaptogenesis

- In primary rat hippocampal neuron cultures, synaptic count (number of synapses) and synaptic strength (relative abundance of presynaptic vesicles per synapse) were calculated per cell



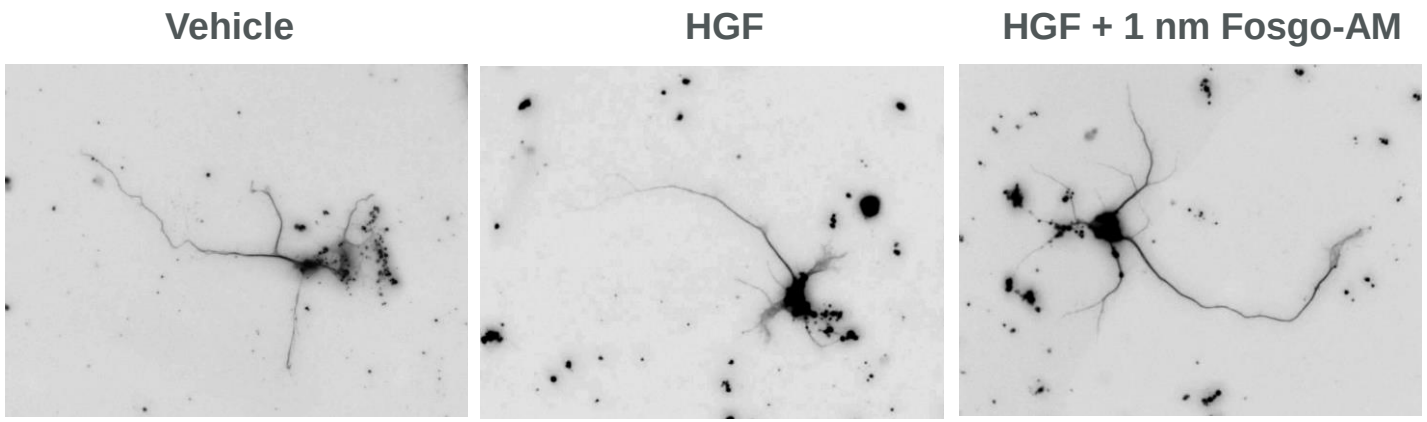


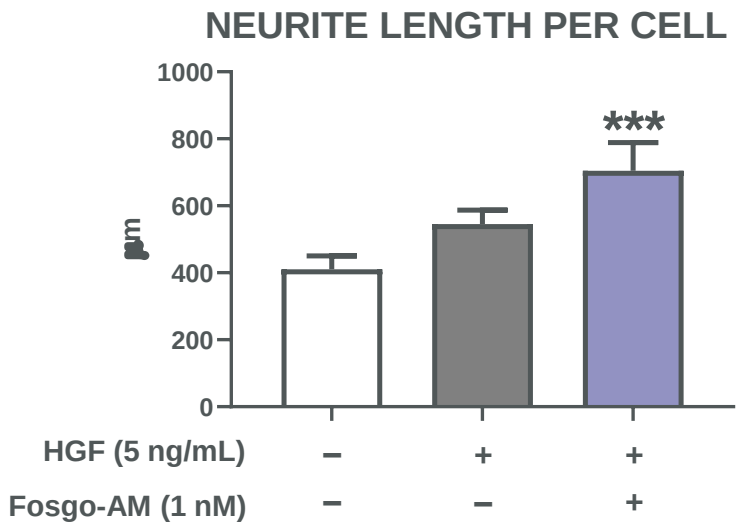
Co-treatment with HGF and fosgo-AM significantly enhances synaptogenesis

Data presented are mean ± SEM.
*P<0.05, ****P<0.0001 vs vehicle.
HGF, hepatocyte growth factor; SEM, standard error of the mean.

Fosgo-AM Enhances Neurite Outgrowth

- Neurite outgrowth was assessed by measuring neurite length in primary rat hippocampal neuron cultures



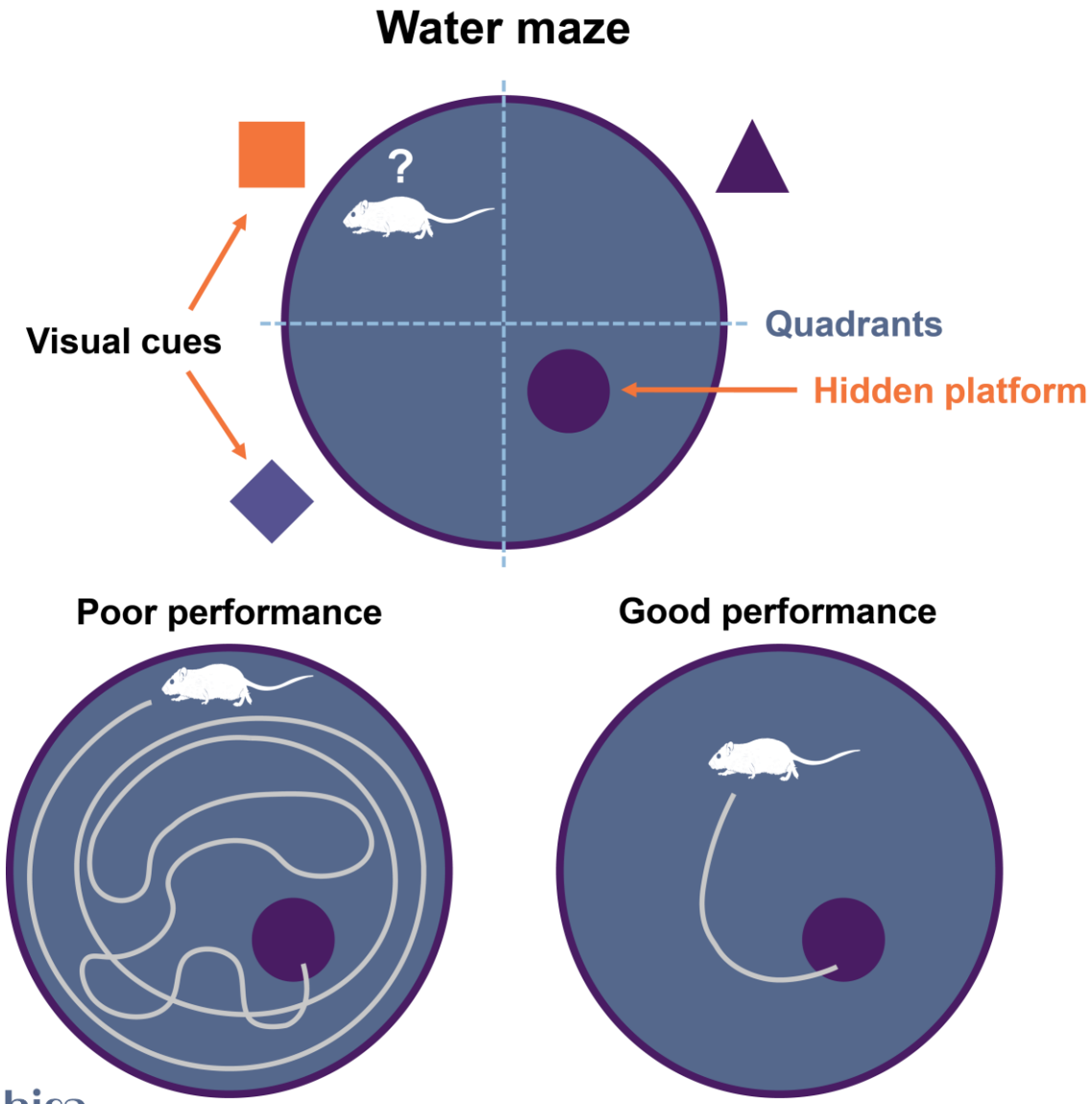


Co-treatment with HGF and fosgo-AM significantly enhances neurite outgrowth

Data presented are mean ± SEM.
***P<0.001 vs vehicle.
HGF, hepatocyte growth factor; SEM, standard error of the mean.

Next Step: Assess Effects of Fosgo-AM in an In Vivo Model of Cognition

Morris Water Maze Training Paradigm



Maze set-up

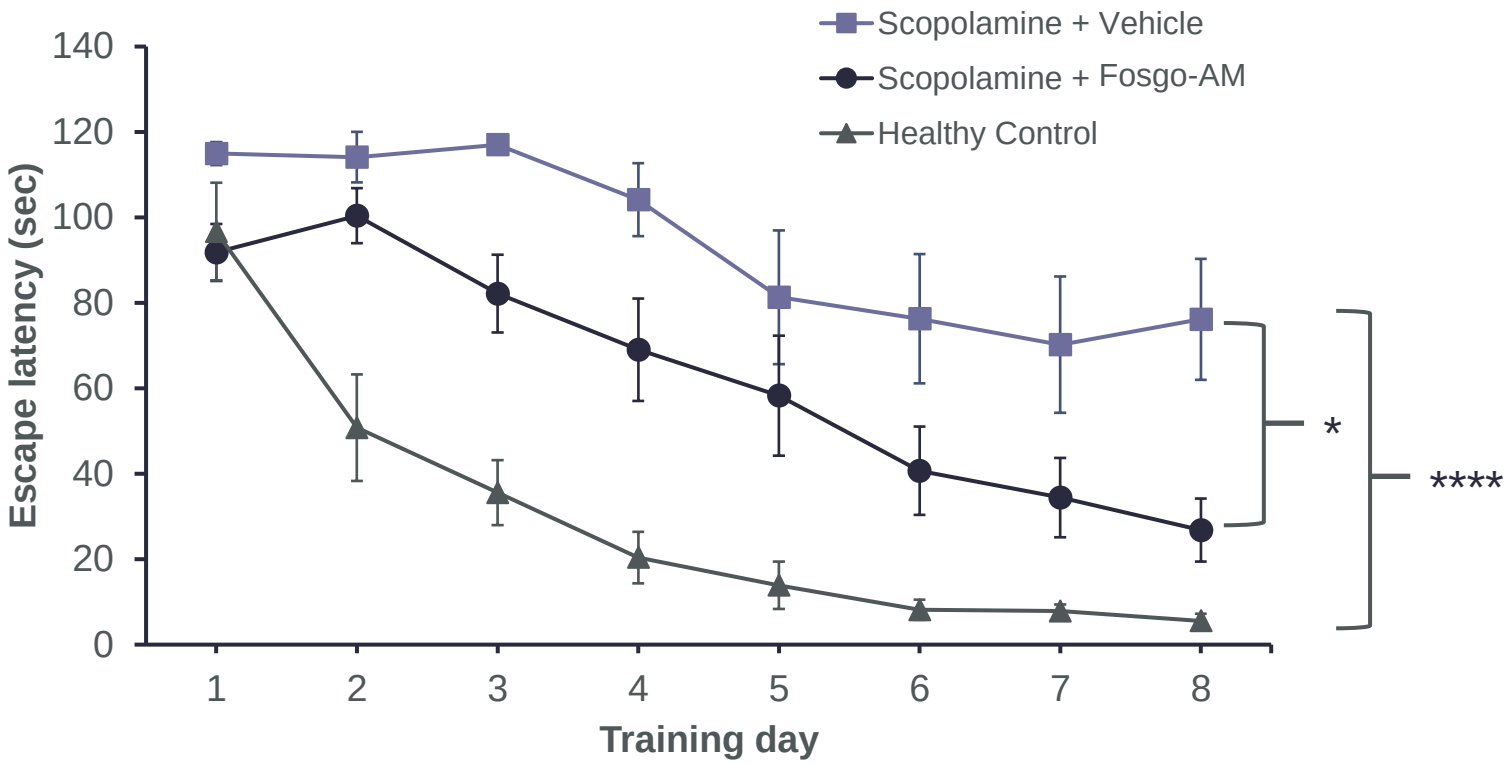
- Maze is artificially divided into 4 quadrants with a hidden platform centered in 1 quadrant
- 8 visual cues randomly placed on the maze wall, which remained consistent during trials

Trial days 1–8

- Animals placed on platform for 30 sec prior to first trial
- Animals then randomly placed in a quadrant of the maze and given 120 sec to find the platform
- Trials repeated 5 times for 8 days

Fosgo-AM Improves Escape Latency of Animals With Scopolamine-induced Amnesia in the Morris Water Maze

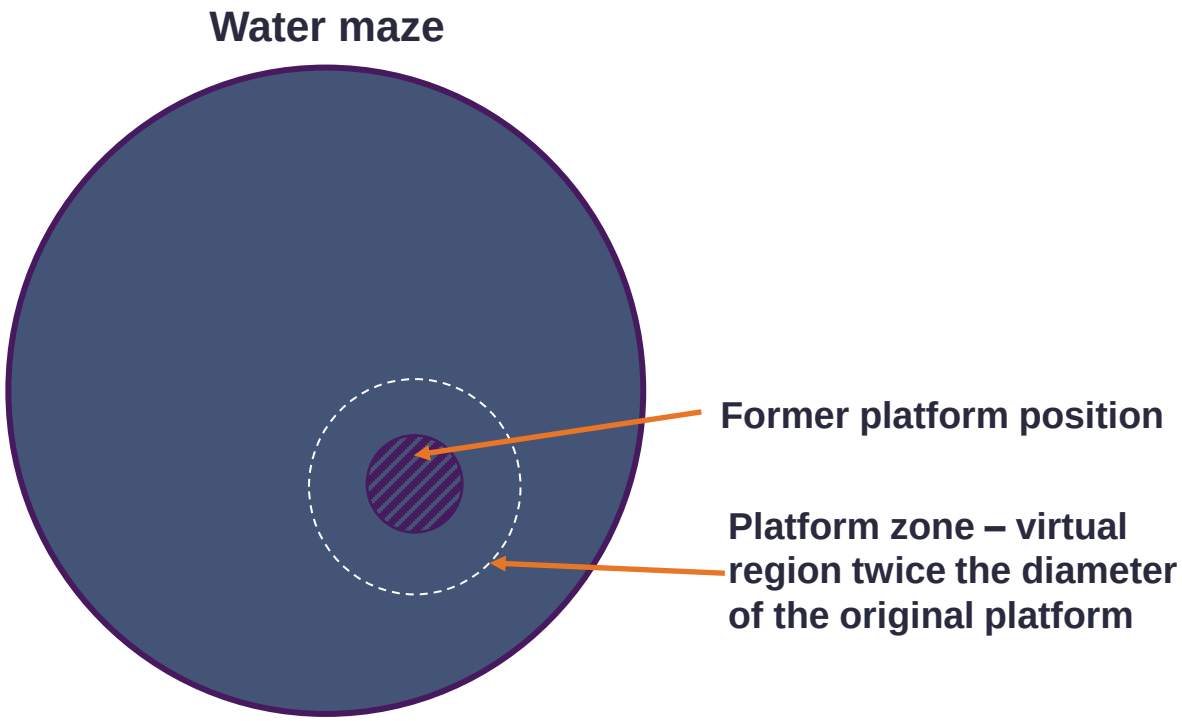
- Healthy control animals quickly learn to locate a hidden platform
- Treatment with cholinergic antagonist, scopolamine, leads to spatial memory deficits
- Treatment with fosgo-AM reverses this memory deficit



Fosgo-AM significantly reduced escape latency vs vehicle in Morris water maze

Data presented are mean ± SEM.
*P<0.05, ****P<0.0001 vs scopolamine + vehicle.
SEM, standard error of the mean.

Morris Water Maze Probe Trials



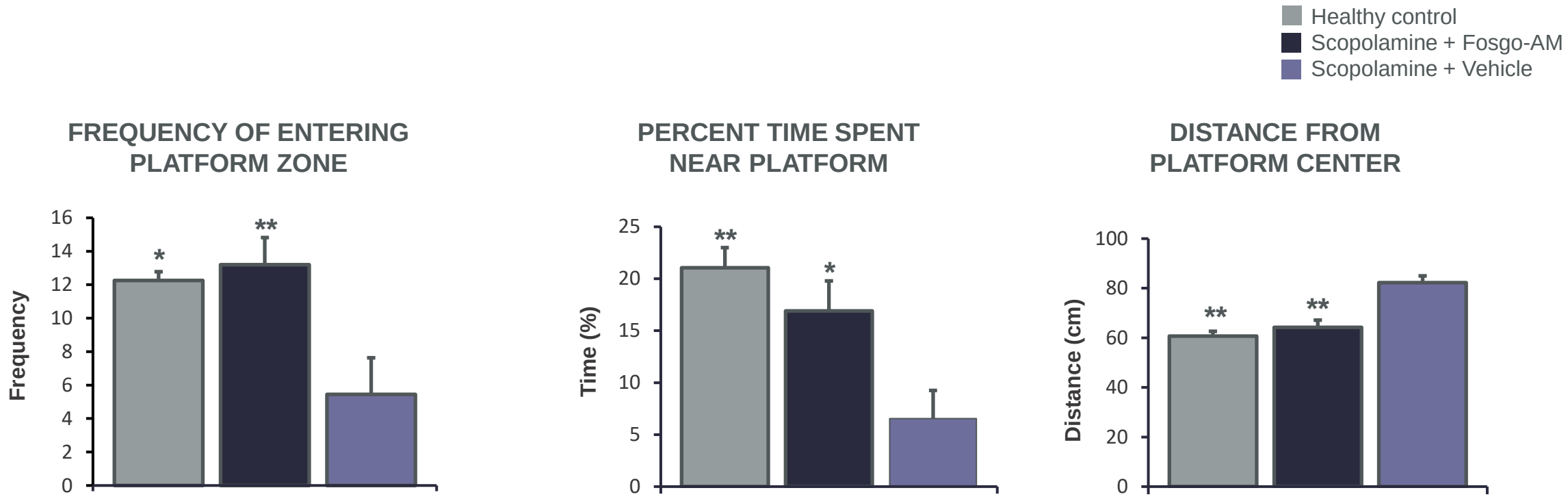
Probe trial

- 24 Hours after training
- Platform was removed from the maze
- Animals were allowed to swim the maze for 120 sec

3 Recorded behaviors

- Frequency of platform zone entries
- Percent of time spent in platform zone
- Mean distance from the former platform center

Fosgo-AM Restored Measures of Learning Persistence in Animals With Scopolamine-induced Amnesia in the Morris Water Maze



Treatment with fosgo-AM significantly restored all measures of learning persistence compared with the scopolamine + vehicle group

Data presented are mean ± SEM. Data collected during the probe trials were analyzed using 1-way ANOVAs with Bonferroni post hoc tests compared to scopolamine + vehicle controls, with significance set at $P \leq 0.05$.
*$P<0.05$, **$P<0.01$, ****$P<0.0001$ vs scopolamine + vehicle.
SEM, standard error of the mean.

Summary of Findings

In vitro findings validate MOA and supports neurotrophic potential of fosgonimeton

- Fosgo-AM treatment significantly increased MET activation levels in vitro
- Fosgo-AM demonstrated neurotrophic effects in cultured hippocampal neurons
  - Induced neurite outgrowth
  - Promoted synaptogenesis

In vivo findings support the procognitive potential of fosgonimeton

- Treatment with fosgo-AM reversed scopolamine-induced amnesia, restored learning persistence in the Morris water maze, and demonstrated pro-cognitive activity

Taken together, these results support the therapeutic potential of fosgonimeton, acting through the active metabolite (fosgo-AM), for the treatment of neurodegenerative disorders, including Alzheimer's and Parkinson's disease dementia

FST, forced swim test; MMN, mismatch negativity; MOA, mechanism of action.

Thank You

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