Presentation 511.06

Fosgonimeton, a Small-Molecule Positive Modulator of HGF/MET, Protects Against Neuronal Damage and Motor Deficits in Preclinical Models of Parkinson's Disease

Sharay Setti, Andrée-Anne Berthiaume, Sherif Reda, Robert Taylor, Maya Kneip, Jewel Johnston, **Kevin Church**

Disclosures: All authors are employees of Athira Pharma, Inc. Bothell, WA, USA.

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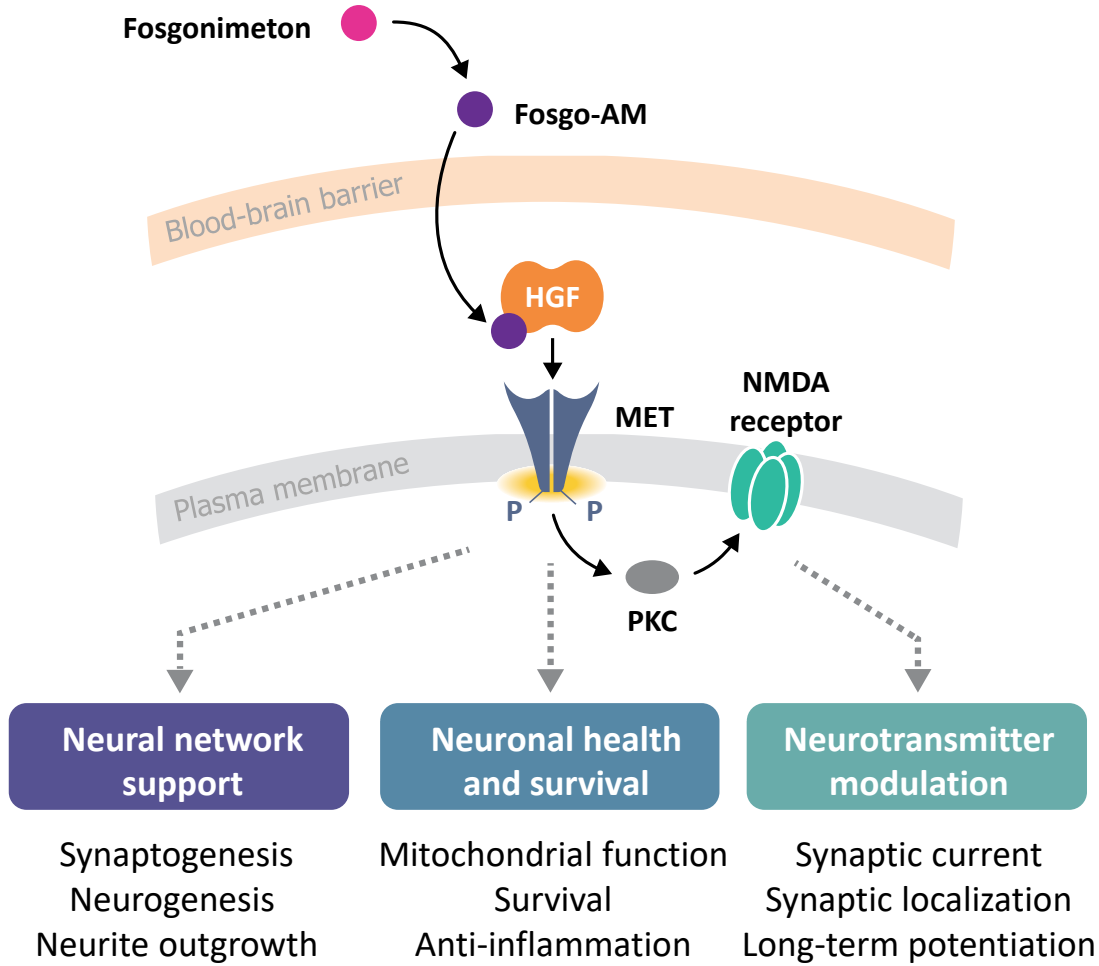
Positive modulation of HGF/MET signaling may be therapeutic in Parkinson's disease (PD)

- Loss of dopaminergic neurons leads to the motor and psychiatric symptoms of PD
- MET receptors are expressed in multiple nervous system cell types, including dopaminergic neurons[1]
- Activation of the HGF/MET pathway induces neuroprotective effects that counteract neurotoxicity, neuroinflammation, and oxidative stress[2-5]
- Promotion of HGF/MET signaling has been shown to be beneficial in models of PD[6,7]

Modulating the HGF/MET system has the potential to alleviate key components of PD based on its neurotrophic and neuroprotective functions



Fosgo-AM, active metabolite of fosgonimeton; HGF, hepatocyte growth factor; NMDA, *N*-methyl-D-aspartate; P, phosphorylation; PD, Parkinson's disease; PKC, protein kinase C.

1. Funakoshi H, Nakamura T. *Curr Signal Transduct Ther.* 2011;6:156-167. 2. Matsumoto K et al. *Biomedicines*. 2014;2(4):275-300. 3. Maina F, Klein R. *Nat Neurosci*. 1999;2(3):213-217.
4. Kitamura K et al. *Int J Mol Sci*. 2019;20(5):1054. 5. Reda S et al. Poster presented at: Alzheimer's Association International Conference; July 31-August 3, 2022; San Diego, CA.
6. Koike H et al. *Gene Ther*. 2006;13:1639-1644. 7. Liu XS et al. *Biomed Res Int*. 2014;2014:909657.

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Objective: Evaluate the effects of fosgonimeton, a positive modulator of HGF/MET, in preclinical models of PD

In vitro models

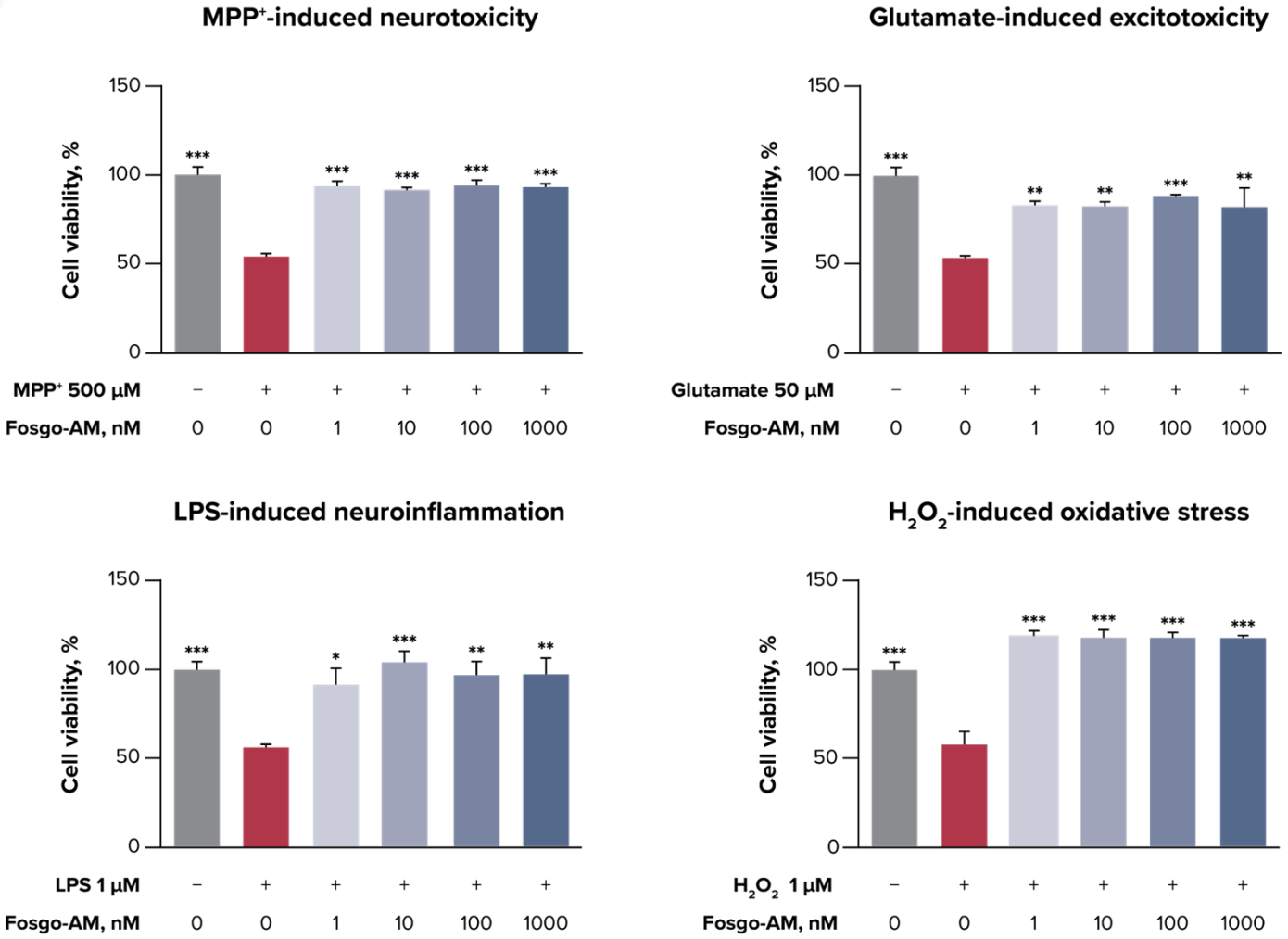
Neurotoxicity panel in rat primary cortical neuron culture
Rotenone treatment in rat primary dopaminergic neuron culture
6-OHDA administration in rat primary dopaminergic neuron culture

In vivo model

Unilateral 6-OHDA–induced striatal lesion in rats

6-OHDA, 6-hydroxydopamine.

Fosgonimeton promotes survival of cortical neurons challenged with neurotoxic insults[1]



ANOVA, analysis of variance; H_2O_2, hydrogen peroxide; MPP+, 1-methyl-4-phenylpyridinium; LPS, lipopolysaccharide.
Cell viability was measured by Cell-Titer Glo (Promega). Statistics applied: 1-way ANOVA with Tukey test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus insult control. Data presented as mean ± SEM.

1. Reda S et al. Poster presented at: Alzheimer's Association International Conference; July 31-August 3, 2022; San Diego, CA.

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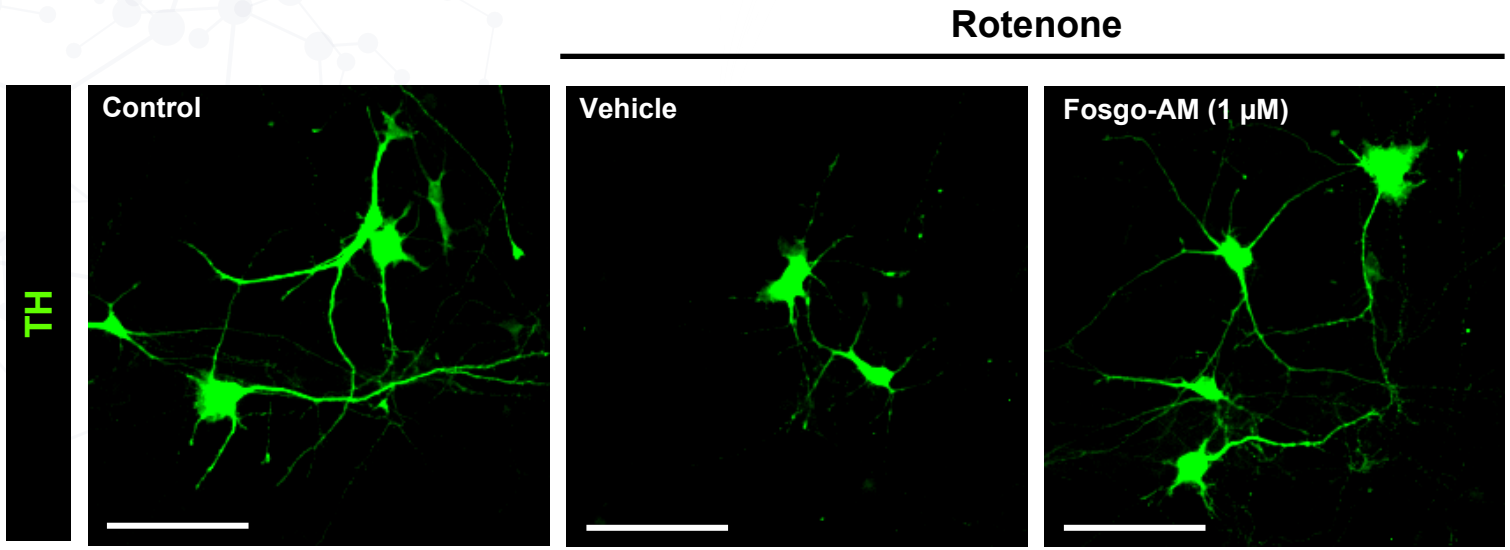
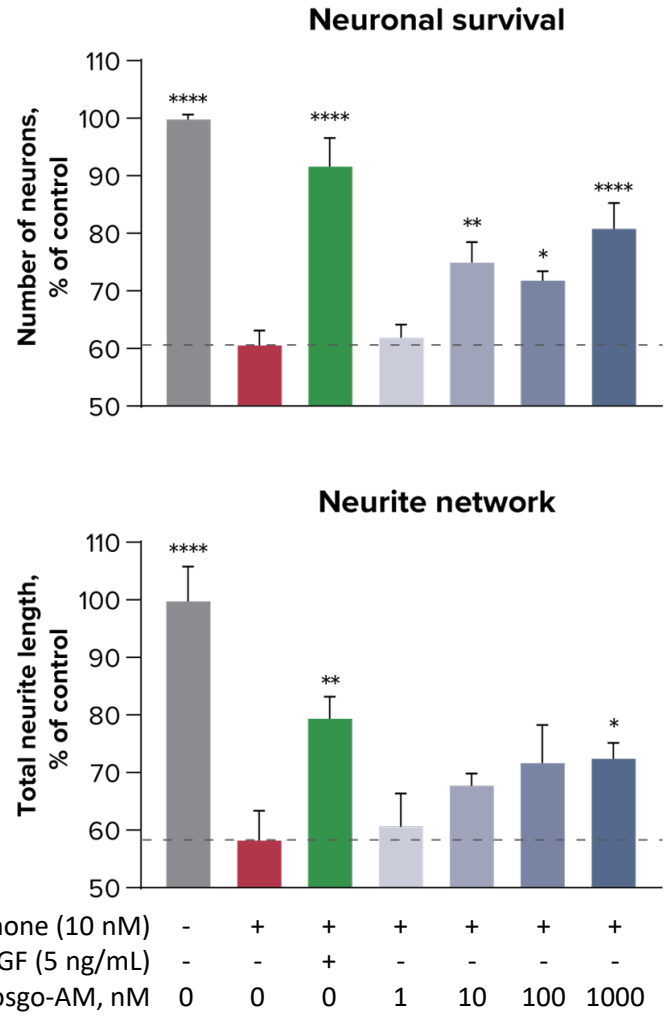
Fosgonimeton promotes dopaminergic neuron health





Fosgo-AM attenuated rotenone-induced neuron degeneration

TH, tyrosine hydroxylase.

On day 6 in culture, rat midbrain dopamine neurons were pretreated for 20 minutes with fosgo-AM or HGF, followed by a 48-hour incubation with rotenone. The total number of TH neurons, total neurite network of TH positive neurons, and α-synuclein aggregation were automatically quantified with ImageXpress acquisition and MetaXpress analysis software (Molecular Devices). Scale bar: 100 μm. Statistics applied: 1-way ANOVA with Fisher least significant difference test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ versus disease control. Data presented as mean ± SEM.

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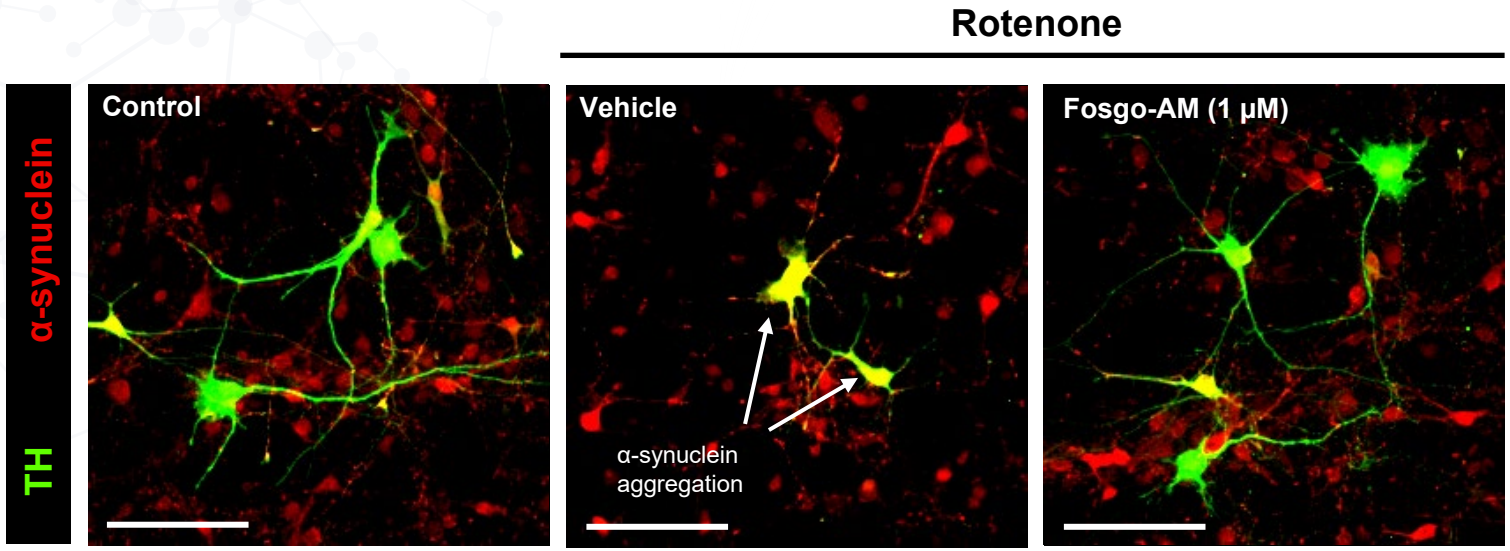
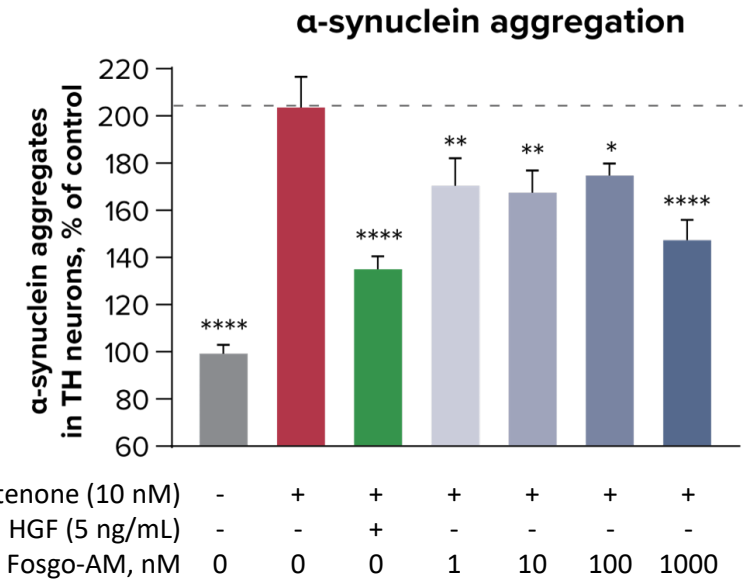
Fosgonimeton reduces protein pathology in dopaminergic neurons





Fosgo-AM reduced rotenone-induced α-synuclein aggregation in TH+ neurons

On day 6 in culture, rat midbrain dopamine neurons were pretreated for 20 minutes with fosgo-AM or HGF, followed by a 48-hour incubation with rotenone. The total number of TH neurons, total neurite network of TH positive neurons, and α-synuclein aggregation were automatically quantified with ImageXpress acquisition and MetaXpress analysis software (Molecular Devices). Scale bar: 100 µm. Statistics applied: 1-way ANOVA with Fisher least significant difference test. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 versus disease control. Data presented as mean ± SEM.

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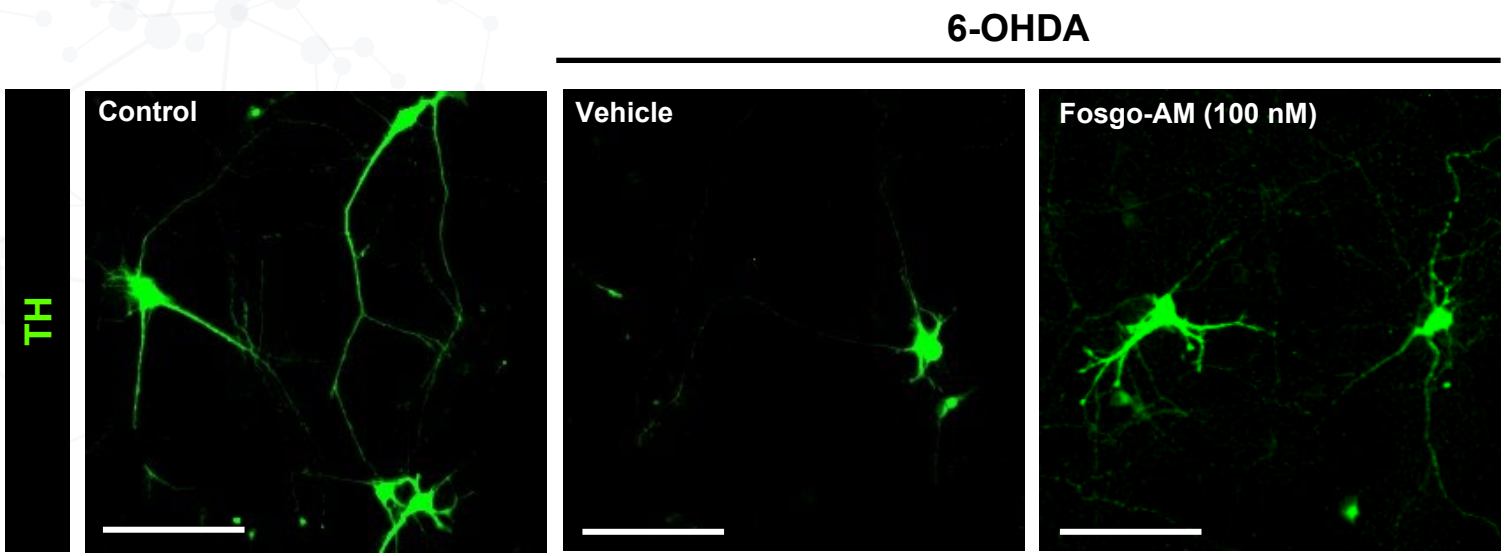
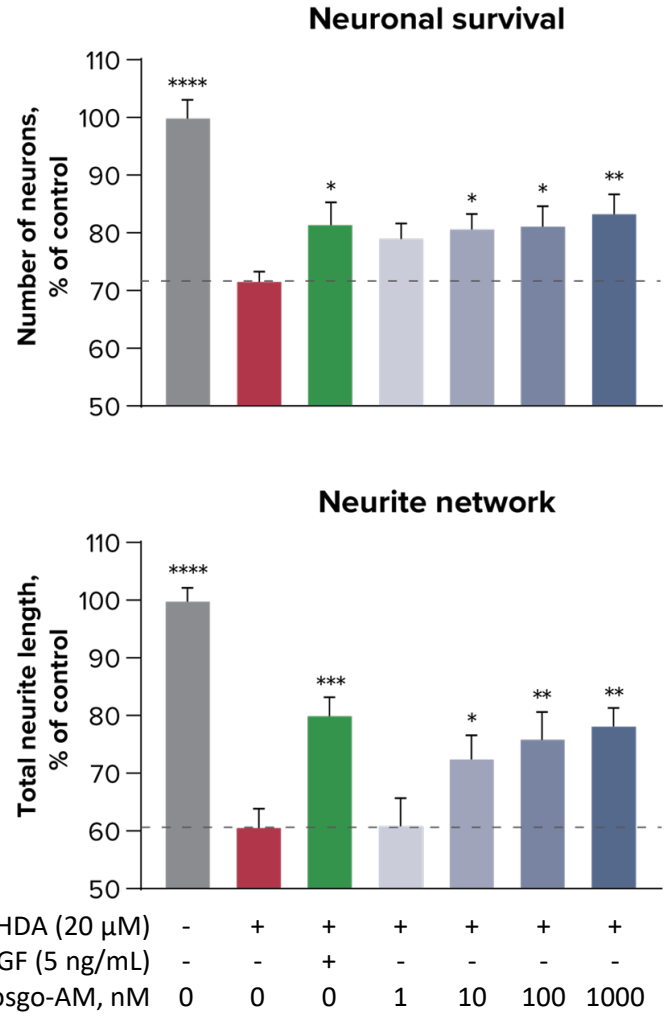
Fosgonimeton promotes dopaminergic neuron health





Fosgo-AM attenuated 6-OHDA-induced neuron degeneration

On day 6 in culture, rat midbrain dopamine neurons were pretreated for 20 minutes with fosgo-AM or HGF, followed by a 24-hour incubation with 20-µM 6-OHDA. The total number of TH neurons, total neurite network of TH positive neurons, and α-synuclein aggregation were automatically quantified with ImageXpress acquisition and MetaXpress analysis software (Molecular Devices). Scale bar: 100 µm. Statistics applied: 1-way ANOVA with Fisher least significant difference test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ versus disease control. Data presented as mean ± SEM.

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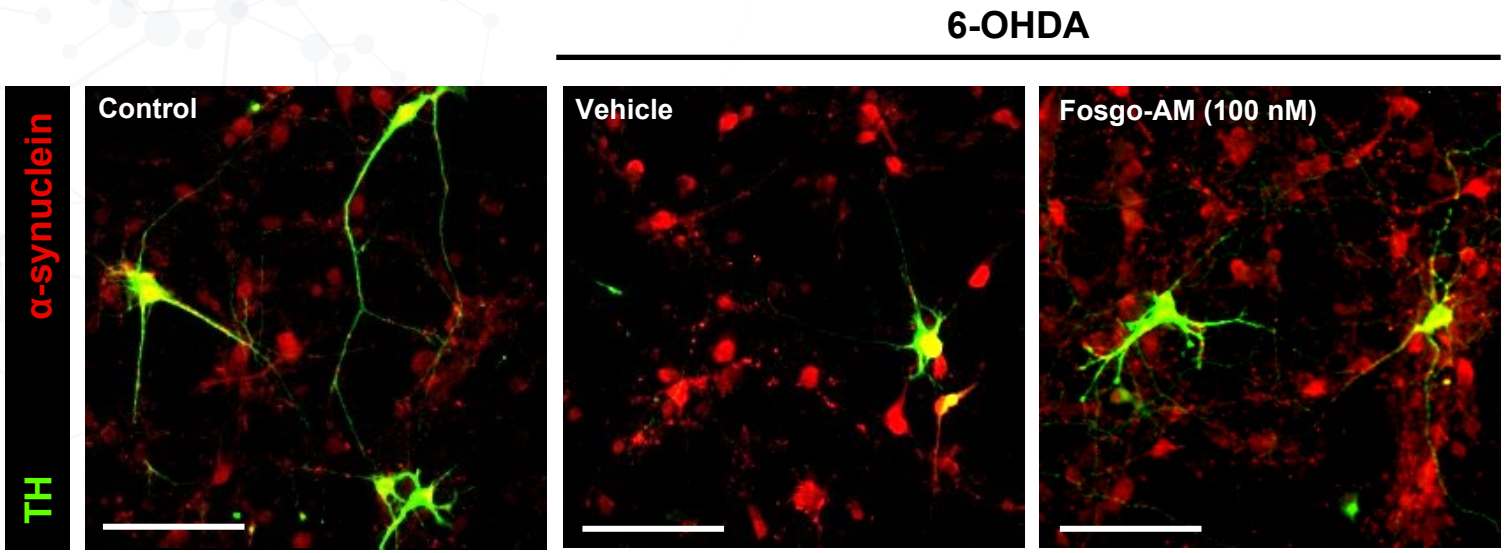
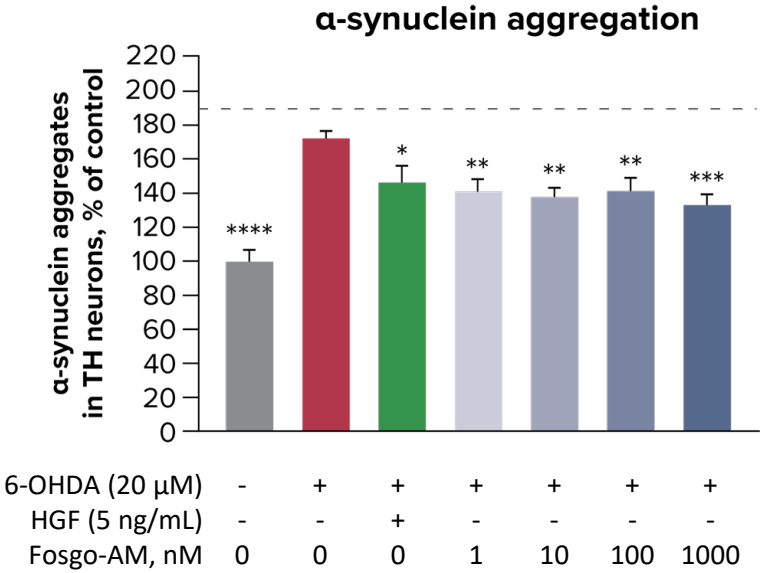
Fosgonimeton reduces protein pathology in dopaminergic neurons





Fosgo-AM reduced 6-OHDA-induced α-synuclein aggregation in TH+ neurons

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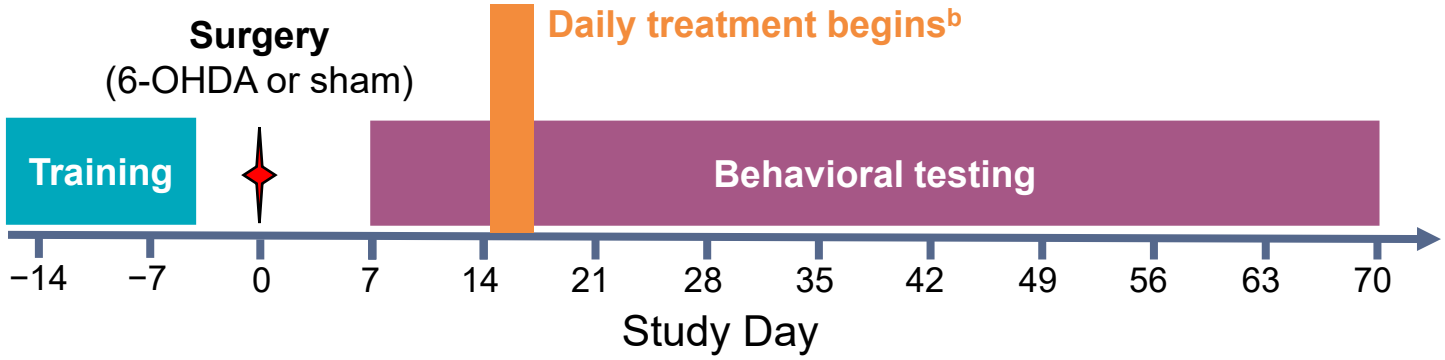
In vivo study design: 6-OHDA rat model of PD

- 6-OHDA induces dopaminergic neurotoxicity and can be used to model PD
  - When injected into the striatum[a] of rats, it results in PD-like motor symptoms
  - Unilateral injections cause motor impairment on the side of the body contralateral to the lesion



Beginning ~2 weeks after 6-OHDA administration, rats were given fosgonimeton SC daily for 8 weeks

AP, anterior-posterior; DV, dorsal-ventral; ML, medial-lateral SC, subcutaneous.

[a]Unilateral 6-OHDA or vehicle injections were targeted to the right caudate nucleus at stereotaxic coordinates AP, −0.21; ML, −3.0; DV, −7.0.
[b]To accommodate surgery and behavioral testing schedules for all 90 animals, treatment started on day 15 or 16 for each animal. To stratify rats based on lesion severity, animals were randomly assigned to treatment after an apomorphine-induced rotation test.

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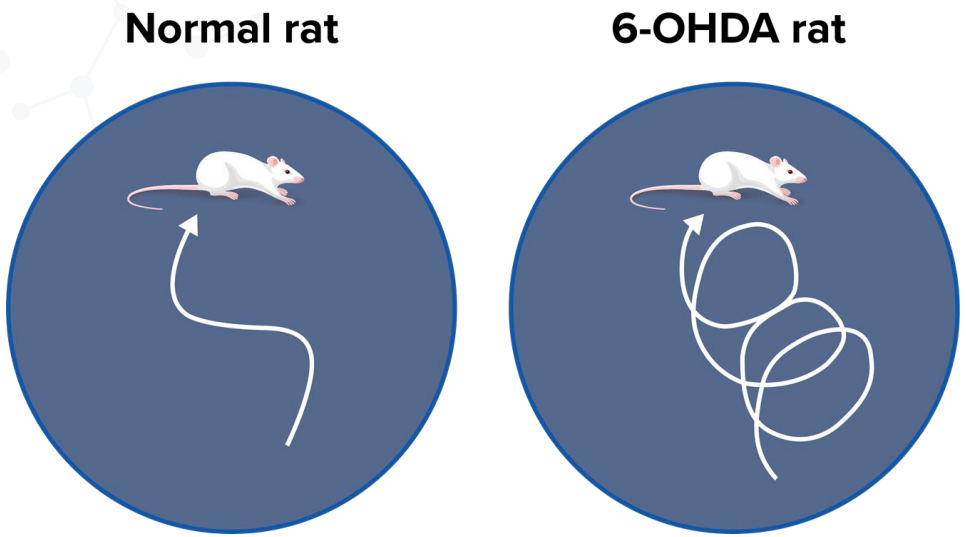
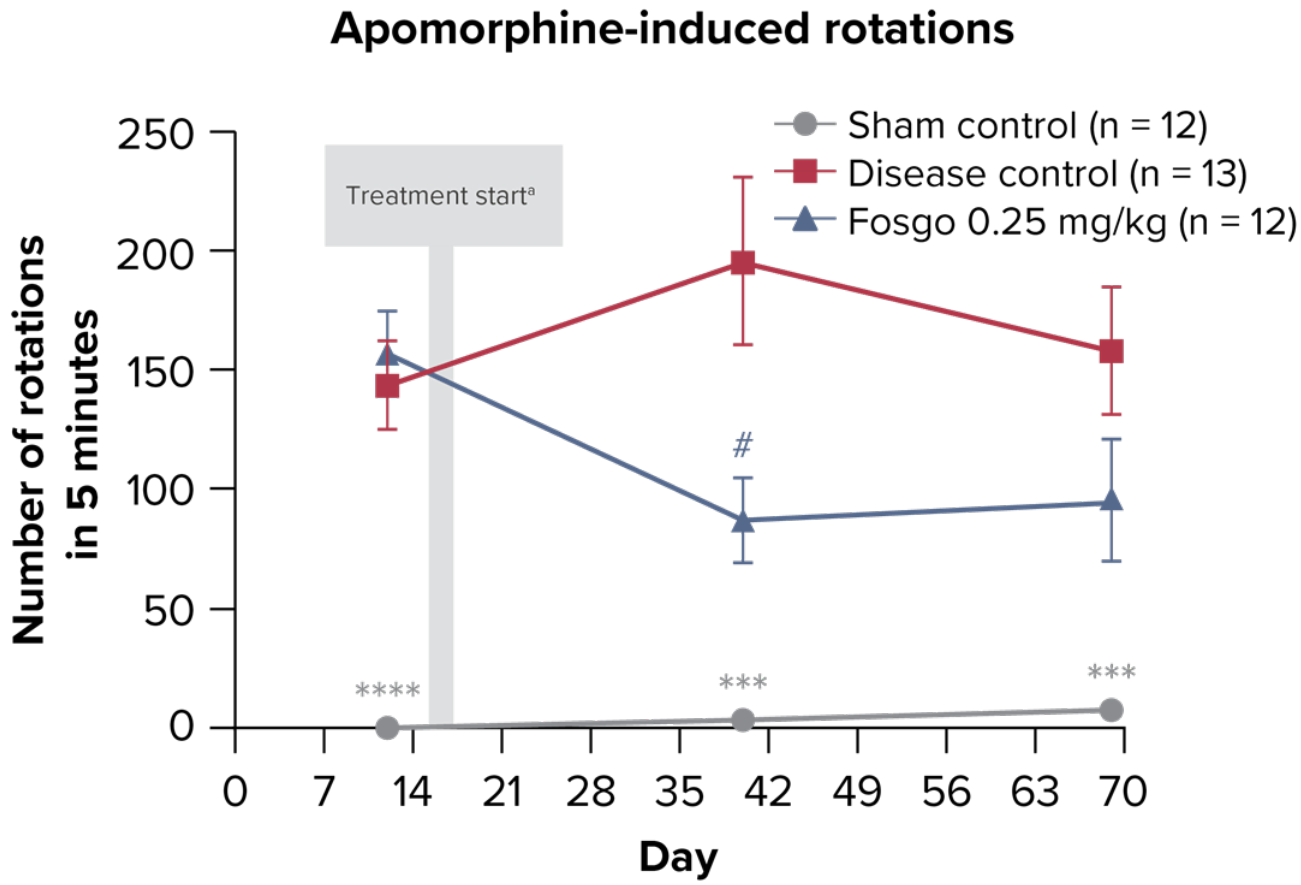
Fosgonimeton reduces apomorphine-induced rotations in a rat model of PD

- Treatment with the stimulant apomorphine causes rats with unilateral motor deficits to rotate in circles
  - The number of rotations correlates with severity of the 6-OHDA lesion[1]





Statistics applied: 2-way ANOVA with Dunnett test. Statistical significance indicated with * represent sham control versus disease control; # represent fosgo versus disease control. The following applies to all symbols: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$ versus disease control.

[a]Animals were randomly assigned to treatment groups so that each had similar degrees of deficit on the apomorphine-induced rotation test.

1. Grealish S et al. *Eur J Neurosci.* 2010;31:2266-2278.

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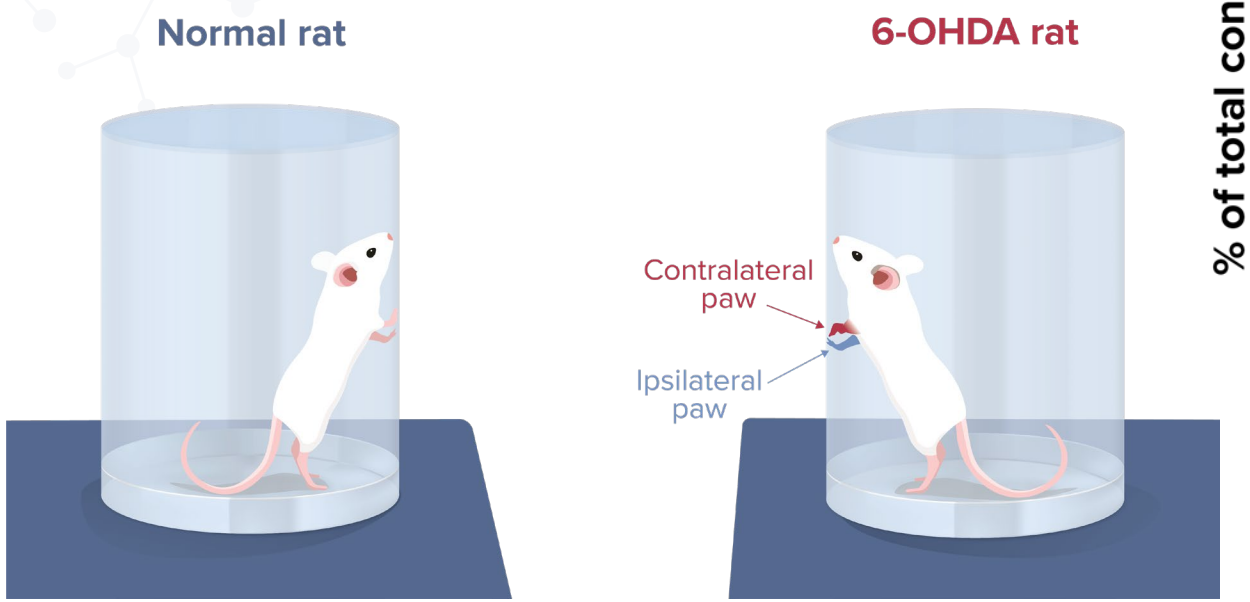
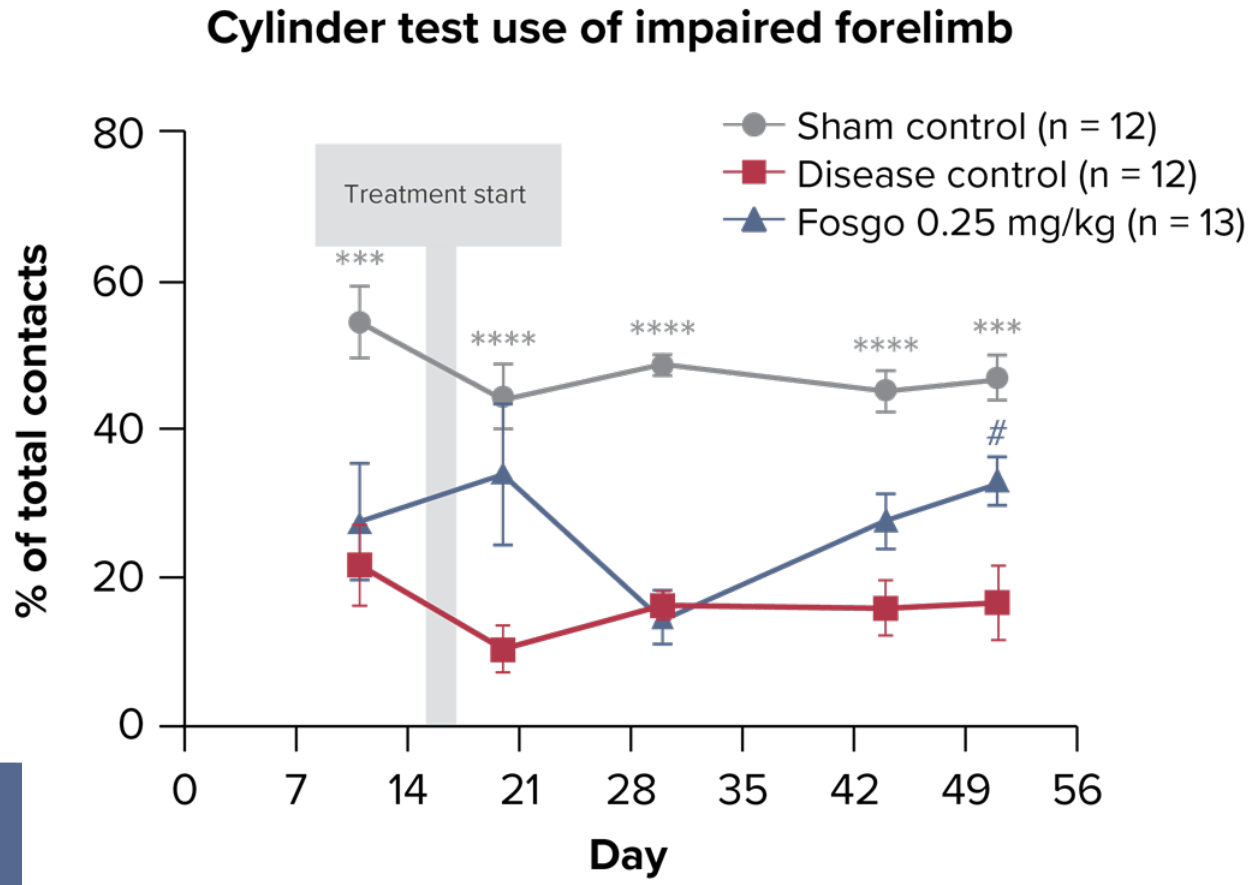
Fosgonimeton increases use of impaired forelimb in a rat model of PD

- During vertical exploration, rats with unilateral 6-OHDA lesions use their impaired forelimb less frequently than their intact forelimb
  - The impaired limb is contralateral to the lesioned hemisphere





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[a]Trials in which rats did not touch the cylinder walls at least 5 times were excluded from calculations of percentage of total contacts.

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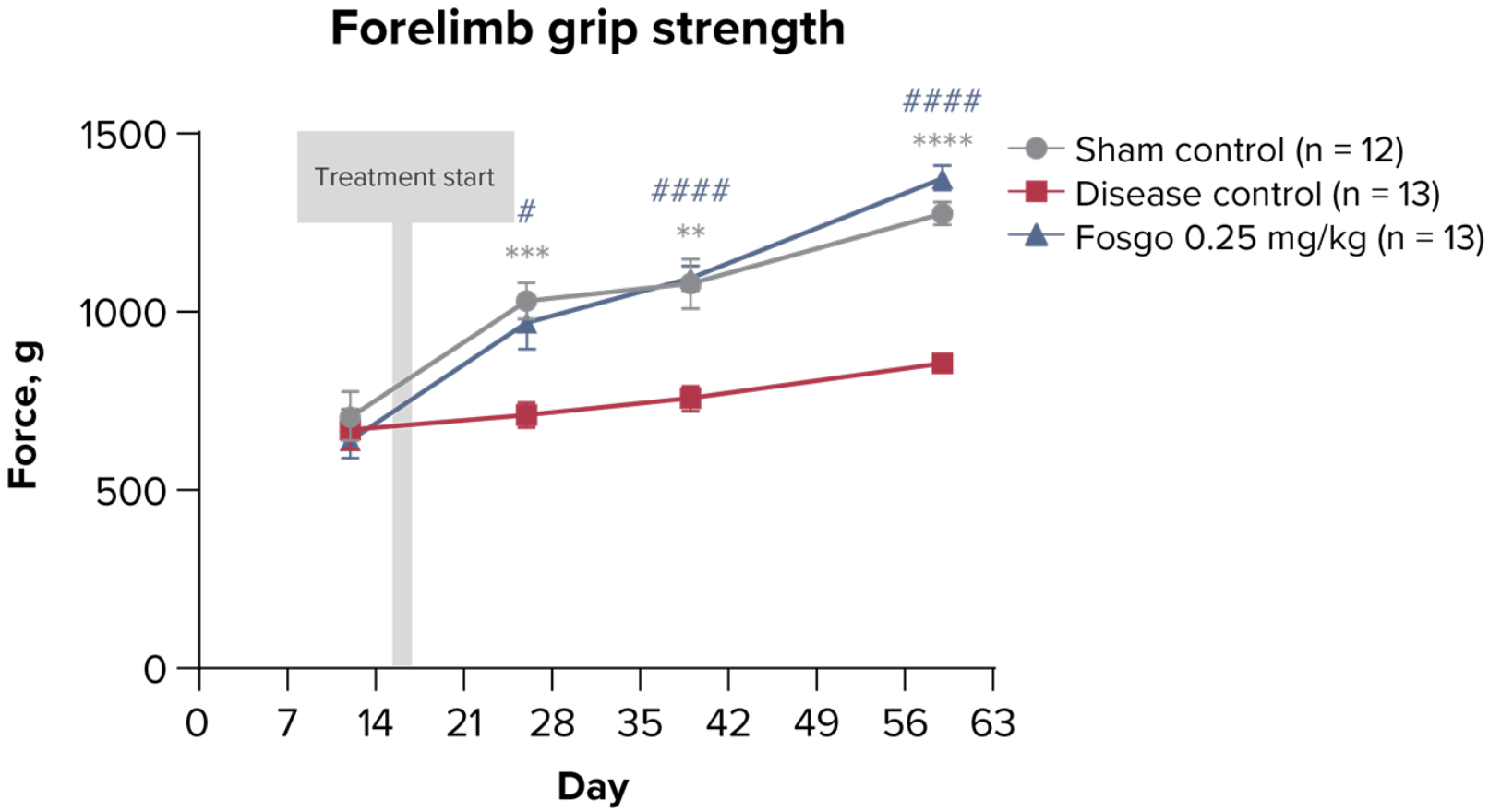
Fosgonimeton rescues grip strength in a rat model of PD

- Rats have consistently weaker grip strength (ie, exert less pull force) following dopamine depleting surgery[1]
- After surgery, sham control animals initially had decreased grip strength, which recovered over time



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1. Tiwari P et al. *Annals Neurosci.* 2021;28:137-149.

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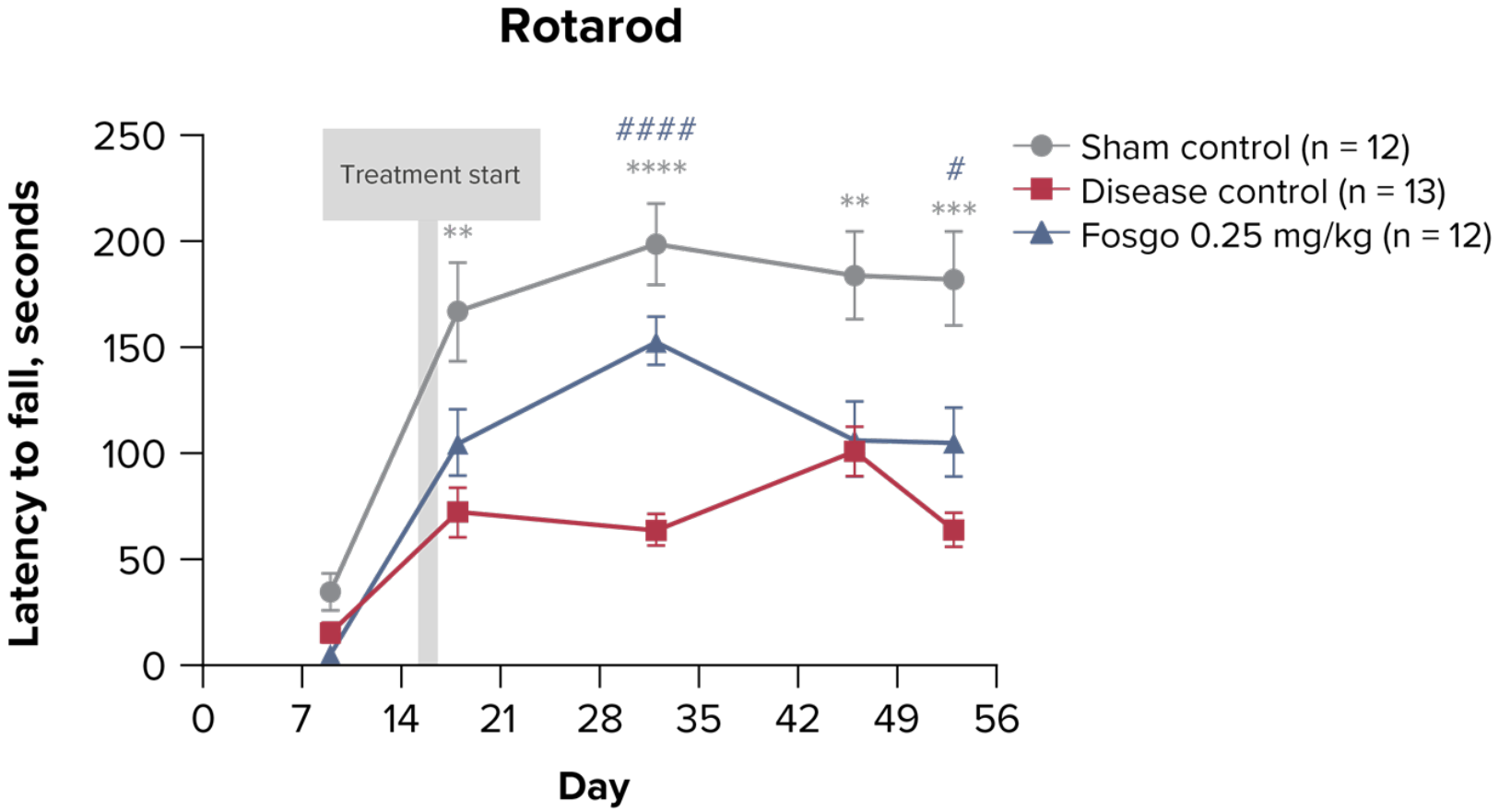
Fosgonimeton prolongs latency to fall from rotarod in a rat model of PD

- 6-OHDA-lesioned rats have a shorter latency to fall from a rotating rod than normal healthy rats
  - The rotarod test involves strength and coordination



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Summary and conclusions

In vitro fosgo-AM treatment

1. Protected cortical neurons from neurological insults central to neurodegeneration
2. Promoted neuronal survival and preserved neurite network of dopaminergic neurons challenged with rotenone and 6-OHDA
3. Reduced rotenone- and 6-OHDA–induced α-synuclein aggregation in dopaminergic neurons

In vivo fosgonimeton treatment

1. Improved symptoms in a unilateral 6-OHDA model of PD, as evidenced by
   - Reduced number of apomorphine-induced rotations
   - Improved motor function and coordination, based on 3 distinct behavioral assays

These data suggest fosgonimeton may have therapeutic potential in Parkinson's disease

Acknowledgments

- Experimental procedures were conducted by Neuro-Sys, Sai Life Sciences, and Syngene International
- Medical writing and editorial support was provided by ApotheCom
- This study was sponsored by Athira Pharma

For more information:

https://bit.ly/3zphDAI

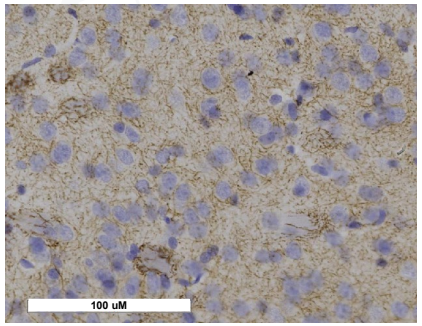
Appendix

Confirmation of 6-OHDA-induced dopaminergic lesions

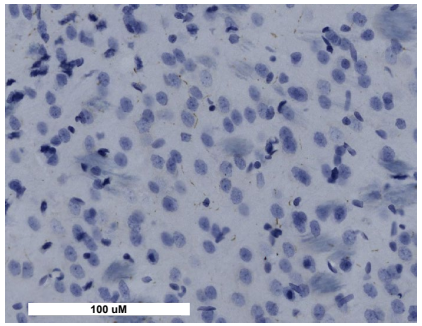
- At study termination, immunohistochemical staining was performed to identify TH-positive neurons in the substantia nigra and TH-positive nerve terminals in the striatum to confirm dopamine depletion

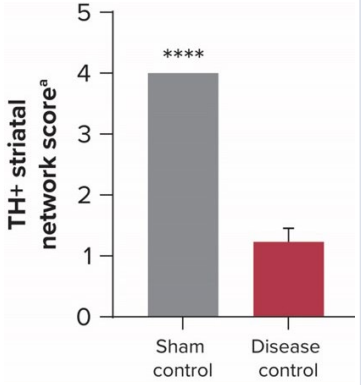
Striatal TH immunostaining

Sham surgery



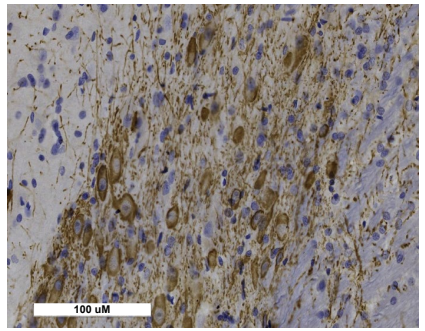
6-OHDA lesion surgery





Substantia nigra TH immunostaining

Sham surgery



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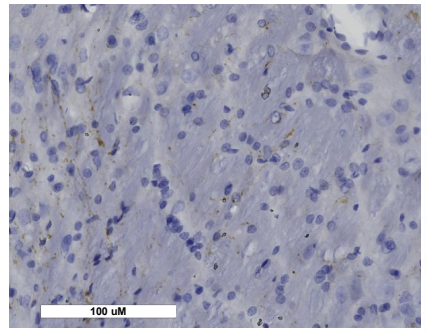

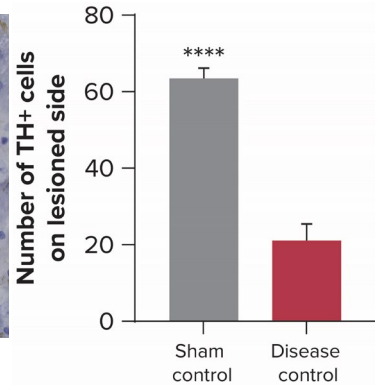

Unilateral 6-OHDA or vehicle injections were targeted to the right caudate nucleus at stereotaxic coordinates AP, −0.21; ML, −3.0; DV, −7.0.
Statistics applied: for positive striatal network score: Mann-Whitney Test; for number of of TH+ cells in substantia nigra: Welch *t* test. ****P <0.0001.
[a]Striatal networks scores were determined by a trained pathologist who was blinded to treatment conditions; each individual striatal image was assigned a score from 1 to 4, with 4 being no depletion of TH-immunoreactive cells and 1 being total depletion.

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