

Tabex™: An inexpensive, plaque halting, synapse protecting treatment for Alzheimer's disease

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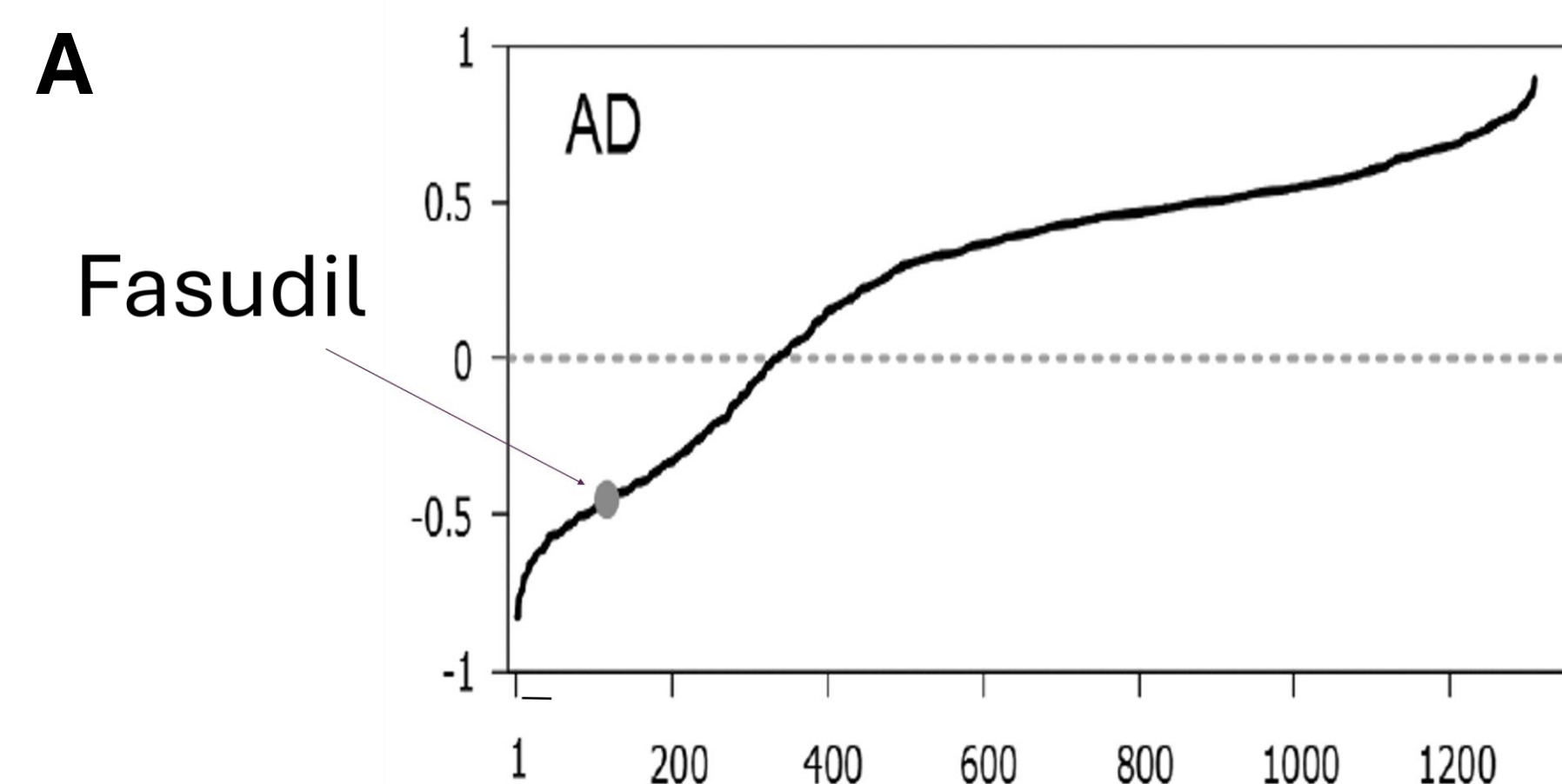
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Introduction

We previously reported that approved ROCK antagonist, fasudil, protects synapses¹ and cognition² against amyloid- β (A β) and substantially reduces amyloid deposition in brains of 3xTg-AD mice².

We performed bioinformatic pathway analyses using transcriptomic data generated from frontal cortex of the fasudil-treated 3xTg-AD mice and large data sets obtained from Alzheimer's disease (AD) brain. This showed that fasudil also reverses pathways in brain associated with disease in AD³.

To determine how this ability of fasudil relates to that of other approved drugs for which full transcriptomic data sets are available, we downloaded transcriptomic data for 1309 approved drugs available at the Broad Institute Connectivity MAP (CMAP) database. We then plotted the ability of each drug to anti-correlate (reverse) or correlate (promote) with the expression pattern of genes comprising the pathways associated with disease in AD brain. Doing this, fasudil sat within the top 9% of anti-correlating drugs³ (**Fig. A**). The top 30 drugs with better anti-correlating profiles are shown in **Table B**. Top of this list is cytosine, the active component of Tabex™, a long-established smoking cessation aid approved and marketed in Central and Eastern Europe.



B Top 30 AD signature-reversing drugs

Anticorrelation ranking	Name(s)	Use
1	cytisine: Tabex™	Nicotinic agonist, smoking cessation aid
2	diclofenamide	Carbonic anhydrase inhibitor
3	neostigmine	Nicotinic and Muscarinic agonist
4	pivmecillinam	Antibiotic - penicilin derivative
5	Prestwick-675	-
6	sulfaquinoxaline	Synthetic bacteriostatic antibiotic
7	bretylium tosilate	Antiarrhythmic, blocks noradrenaline release.
8	lansoprazole	Proton pump inhibitor (PPI) reduces acid production in the stomach
9	amrinone	PDE3 inhibitor
10	sulindac sulfide	Active metabolite of the nonsteroidal anti-inflammatory drug (NSAID) sulindac
11	indoprofen	Nonsteroidal anti-inflammatory drug (NSAID) Withdrawn.
12	ergocalciferol	Vitamin D2 or calciferol
13	tomelukast	leukotriene receptor antagonist
14	tocainide	Sodium channel blocker antiarrhythmic
15	trapidil	Platelet-derived growth factor antagonist
16	mimosine	Toxic, non-protein amino acid, used to synchronize cells in G1 phase.
17	Prestwick-972	-
18	CP-863187	inhibitor of the p38α
19	metrizamide	iodine-based contrast agent used for neuroradiological imaging
20	ceforanide	cephalosporin antibiotic
21	bethanechol	Direct muscarinic agonist used to promote urine flow
22	BCB000040	-
23	benzbromarone	Treatment of high serum uric acid levels
24	5186324	
25	ciprofibrate	Treatment of severe hypertriglyceridaemia
26	enalapril	Angiotensin-converting enzyme inhibitors
27	melatonin	Short-term sleep aid
28	Prestwick-857	-
29	chloropyrazine	Organic compound used as a building block in the synthesis of pharmaceuticals
30	citalopram	Selective serotonin uptake inhibitor, antidepressive

Figure A, B. Transcriptional profiles of 1309 drugs on CMAP with full genome data were compared to an AD transcriptional signature derived from large AD brain transcriptomic data sets and ranked according to their ability to mimic or reverse the AD signature. Graph, A, shows the position of fasudil. Table, B, lists the top 30 best anti-correlating compounds. The drug with the best anti-correlation score is cytosine.

Methods

We tested cytosine in assays of A β synaptotoxicity (DIV 28 rat primary cortical cultures or human iPSC-derived glutamatergic neurons exposed to 3 μ M oligomeric A β_{1-42} for 6 h) finding that at 20 μ M it was able to fully protect dendritic spines and synaptic markers from this toxic dose of A β (**Figs. C–G**).

We then gave cytosine (20 mg/kg/day) to mixed sex 5xFAD transgenic mice for 8 weeks in their drinking water. A second group received water only (n=12/group). All mice were then subject to manganese enhanced MRI (MEMRI) before and after drug to image amyloid deposits in the living animal brain. Remarkably, cytosine prevented the increase in amyloid deposition that occurred over the 8 weeks in the water only group (**Fig. H**). Live MEMRI data were confirmed by postmortem IHC, which showed 25% fewer amyloid plaques in the cytosine group compared to the water controls (p <0.001). These data show that in addition to reversing AD-associated pathways, cytosine also protects synapses from toxic A β species, the principal driver of synaptic failure in AD, and reduces brain amyloid burden, a central therapeutic objective of current immunotherapies. Given cytosine has been used safely for many decades, shows high CNS availability, is globally accessible and available over the counter at very low cost, makes it a uniquely strong candidate for rapid repurposing as a disease-modifying treatment for AD.

Results

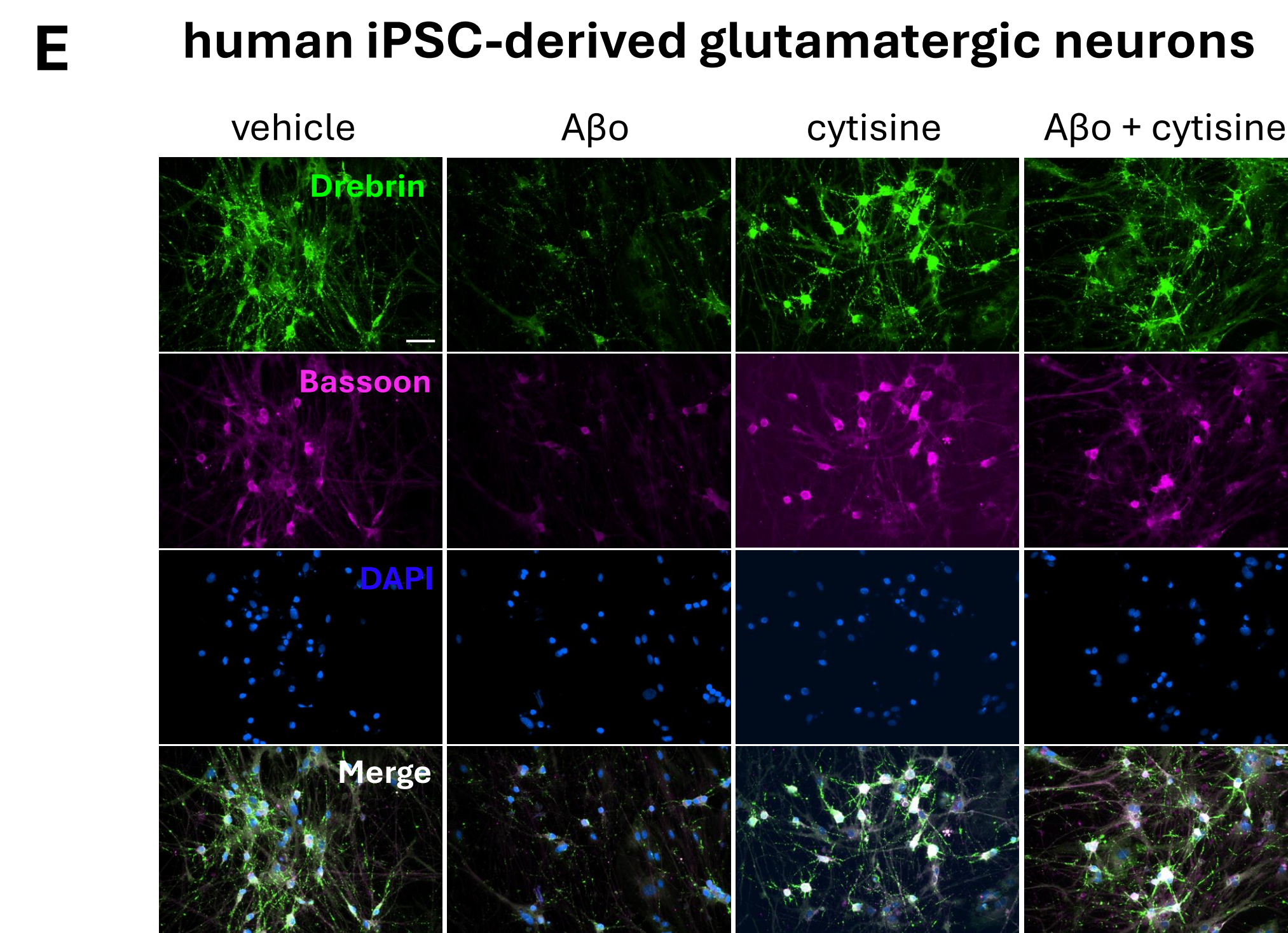
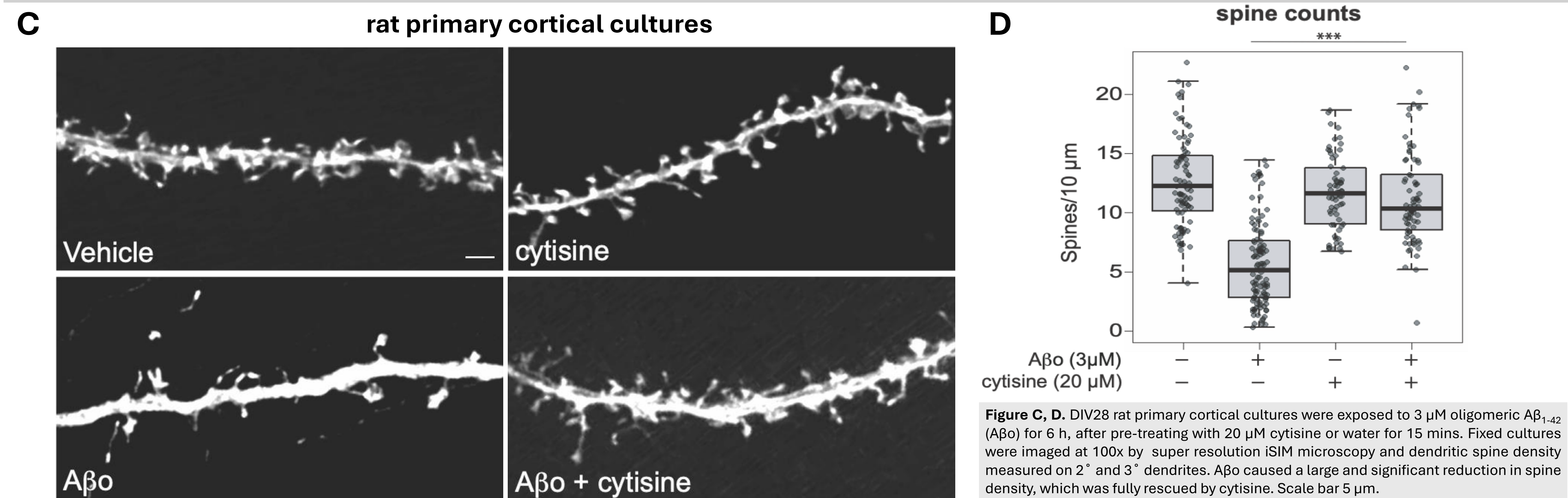


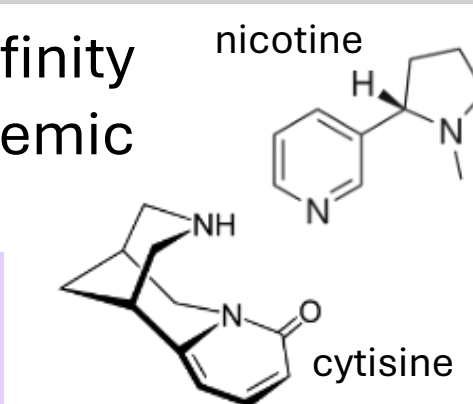
Figure E-G. Human iPSC-derived glutamatergic neurons were cultured with mixed rat glia until DIV28 and then exposed to 3 μ M oligomeric $A\beta_{1-42}$ ($A\beta$) for 6 h, after pre-treating with 20 μ M cytosine or water for 30 mins. Fixed cultures were stained for pre- and post-synaptic markers Bassoon and Drebrin, respectively, imaged at 20x by confocal microscopy and puncta were measured. $A\beta$ caused a large and significant reduction in synaptic marker staining, which was fully rescued by cytosine. Scale bar 50 μ m.

Conclusion

Cytisine is a low affinity full agonist of the $\alpha 7$ nicotinic acetylcholine receptor ($\alpha 7$ nAChR) and a high affinity partial agonist of $\alpha 4/\beta 2$ nAChR (K_i 1,400–1,700 nM and 0.3–1 nM, respectively)⁴. It has high systemic bioavailability and when taken for smoking cessation has only limited and mild adverse reactions⁵.

- Existing amyloid- β targeting treatments for AD are costly and carry considerable risk
- Although they remove plaques their ability to slow cognitive decline is limited
- Cost and global scalability remain major challenges
- Repurposing provides a fast translational path to find cheap, effective alternatives

- Tabex™ has decades of use with good safety record
- Highly CNS-penetrant
- Ultra-low cost and globally deployable
- Halts amyloid deposition and protects synapses



20p/tablet

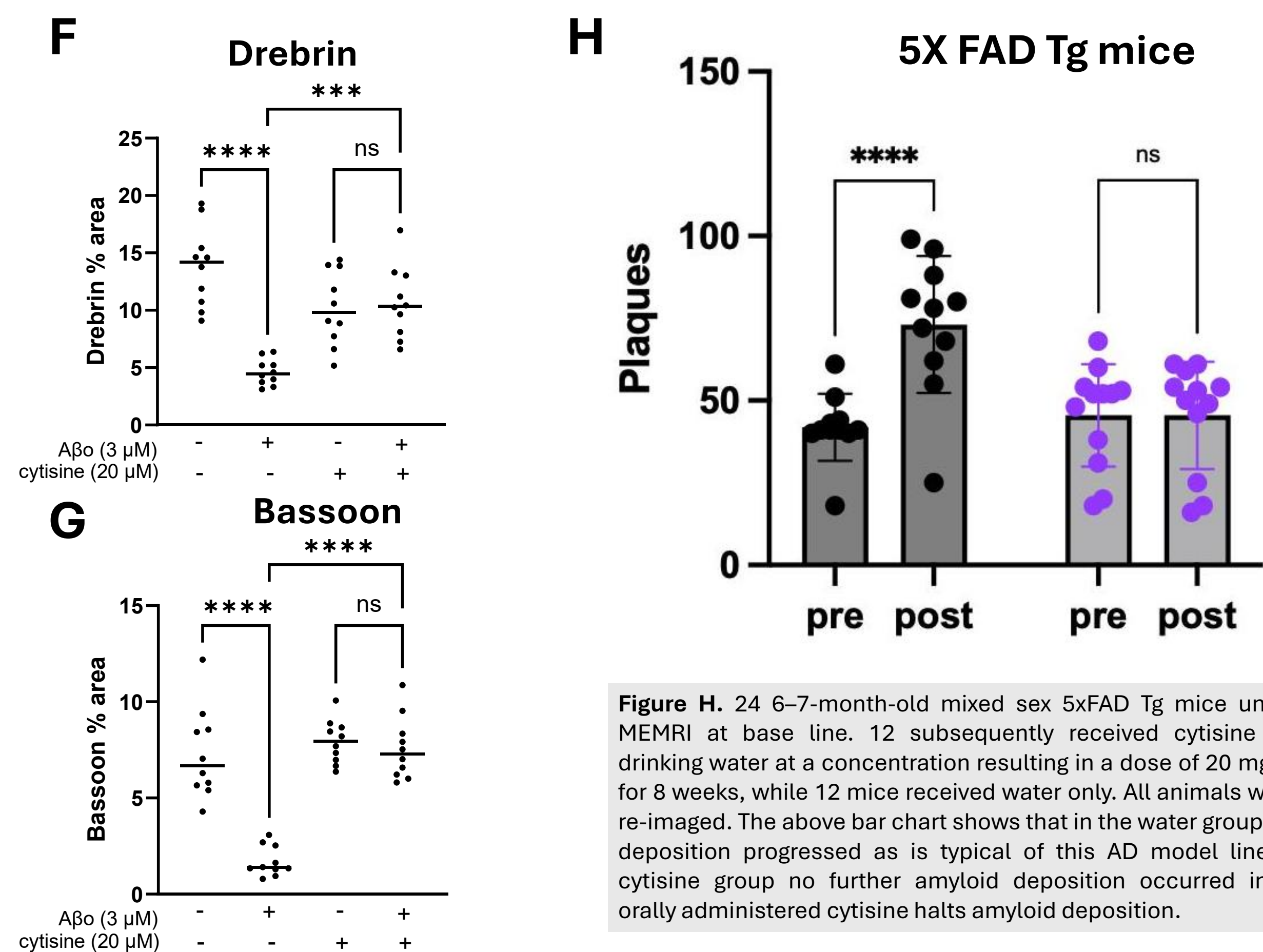


Figure H. 24 6–7-month-old mixed sex 5xFAD Tg mice underwent MEMRI at base line. 12 subsequently received cytosine in their drinking water at a concentration resulting in a dose of 20 mg/kg/day, for 8 weeks, while 12 mice received water only. All animals were then re-imaged. The above bar chart shows that in the water group amyloid deposition progressed as is typical of this AD model line. In the cytosine group no further amyloid deposition occurred indicating orally administered cytosine halts amyloid deposition.

References

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