



McGill

Faculty of
Medicine

Department of
PHARMACOLOGY & THERAPEUTICS

Targeting M1 muscarinic and sigma-1 receptors in Alzheimer's disease

Reversal of pathological hallmarks and associated cognitive dysfunction
in McGill-R-Thy1-APP rats

Hélène Hall, PharmD, PhD

Postdoctoral fellow | Cuello lab

McGill university

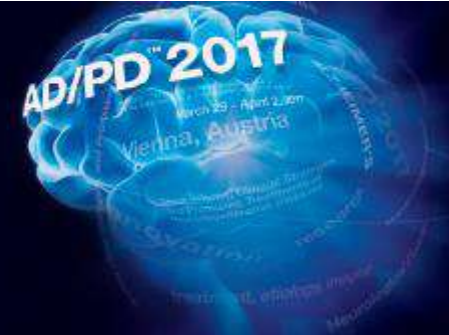
Montreal, Canada



The 13th International Conference on Alzheimer's & Parkinson's Diseases

Mechanisms, Clinical Strategies, and Promising Treatments of Neurodegenerative Diseases

March 29 - April 2, 2017 | Vienna, Austria



Faculty Disclosure

<input checked="" type="checkbox"/>	No, nothing to disclose
<input type="checkbox"/>	Yes, please specify:

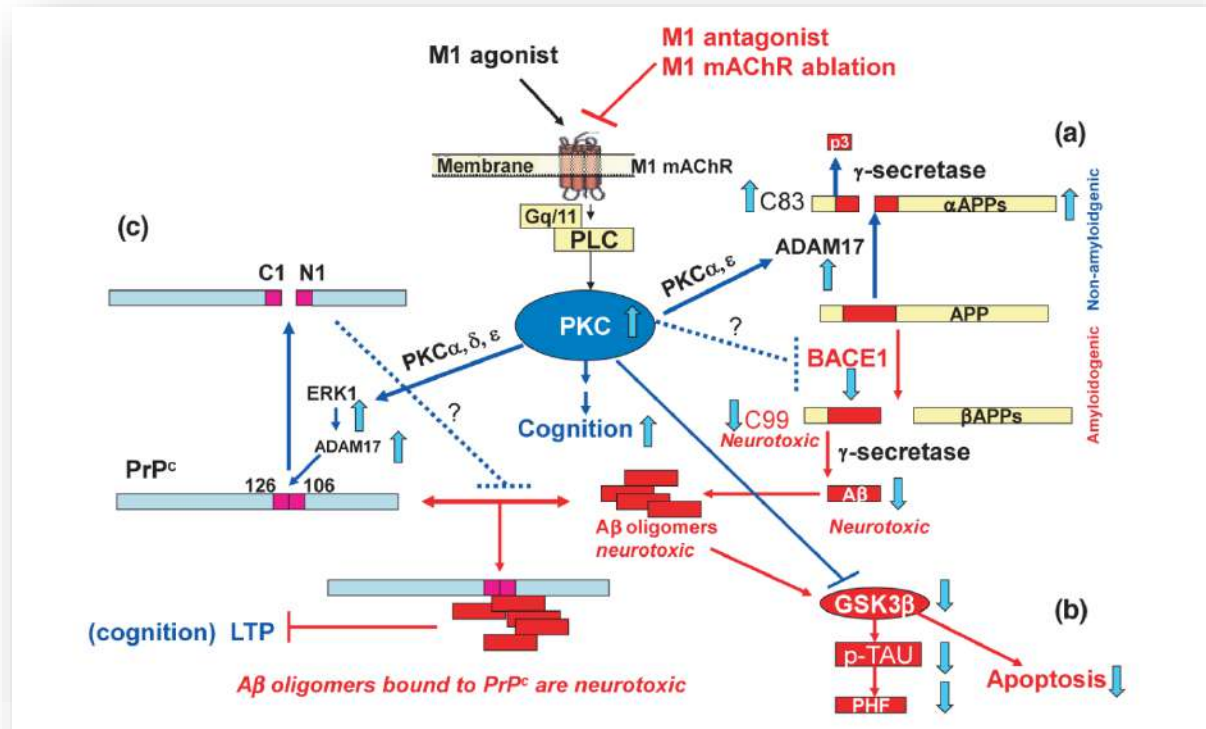
<i>Company Name</i>	<i>Honoraria/ Expenses</i>	<i>Consulting/ Advisory Board</i>	<i>Funded Research</i>	<i>Royalties/ Patent</i>	<i>Stock Options</i>	<i>Ownership/ Equity Position</i>	<i>Employee</i>	<i>Other (please specify)</i>

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M1 muscarinic receptors as a therapeutic target for AD

- Stimulation of M1 muscarinic receptors
 - ✓ reduces amyloid pathology
 - ✓ decreases tau hyperphosphorylation



Fisher, 2012, Journal of Neurochemistry 120(suppl 1)-22

AF710B (aka ANAVEX 3-71): a novel M1/sigma-1 agonist

➤ AF710B drug (*aka ANAVEX 3-71*)



Allosteric modulator of M1 muscarinic receptor:
modulation of the APP processing

(Nitsch *et al*, 1992, *Science*; Caccamo *et al*, 2006, *Neuron*)

Agonist of sigma 1 receptor:
enhanced cognition, neuroprotection

(Senda *et al*, 1996, *Eur J Pharmacol*, Meunier *et al*, 2006, *J Pharmacol Exp Ther.*)

➤ In 3xtg mice:

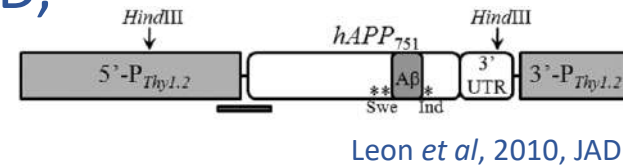
✓ AF710B attenuates cognitive deficits

✓ Reduces AD-like hallmarks

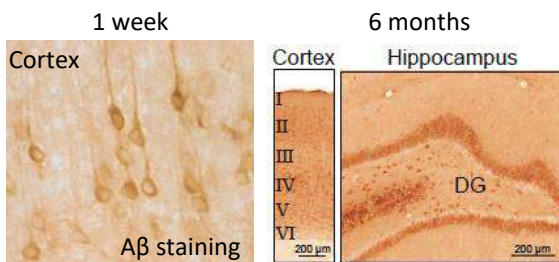
(amyloid pathology, tau hyperphosphorylation, inflammation)

McGill-R-Thy1-APP rats: a model of AD-like amyloid pathology

- Express human *APP*, carrying mutations associated with familial AD, under Thy1 promoter
- One single transgene insertion per allele, minimal genetic charge

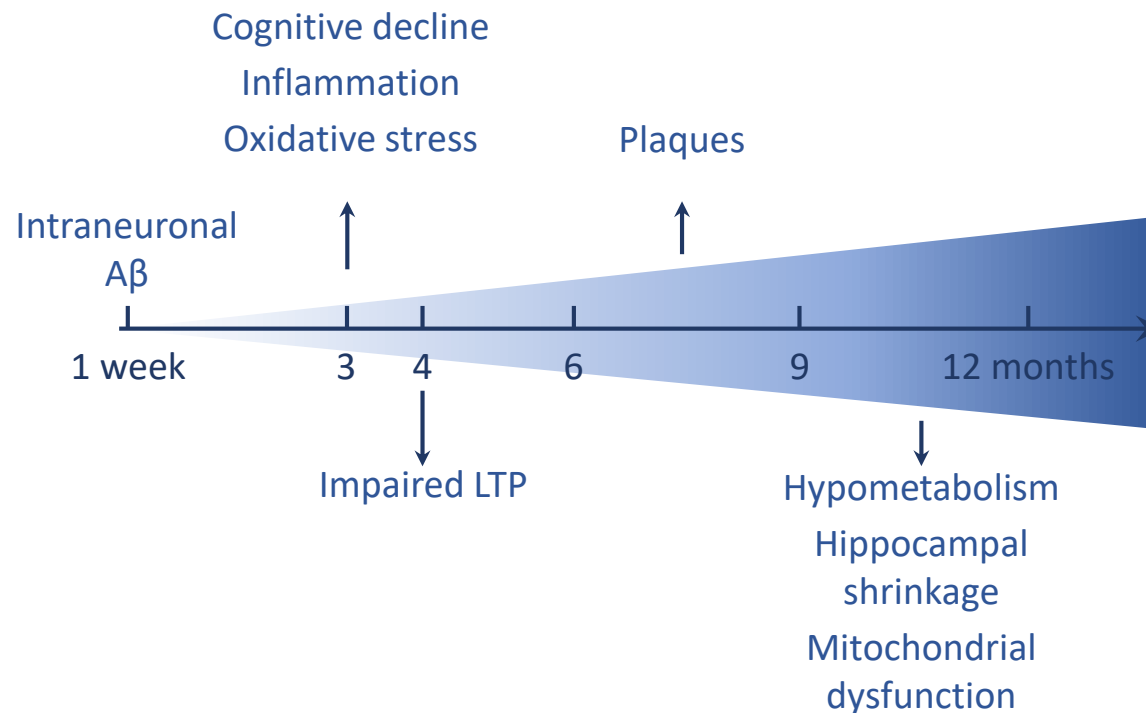


Pre-plaque (early):
Accumulation of intraneuronal Aβ

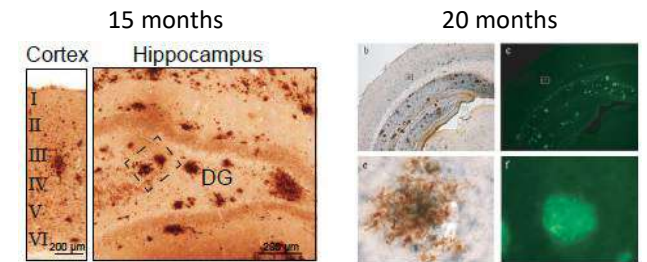


Leon *et al*, 2010, JAD 20(1)-113

Wilson *et al*, 2016 Cerebral Cortex



Post-plaque (late, >9mo):
Deposition of Aβ into extracellular plaques



Wilson *et al*, 2016 Cerebral Cortex

Blue: Aβ (McSA1)
Brown: microglia (MHCII)
Green: plaques (ThioS)

Leon *et al*, 2010, JAD 20(1)-113

Experimental design

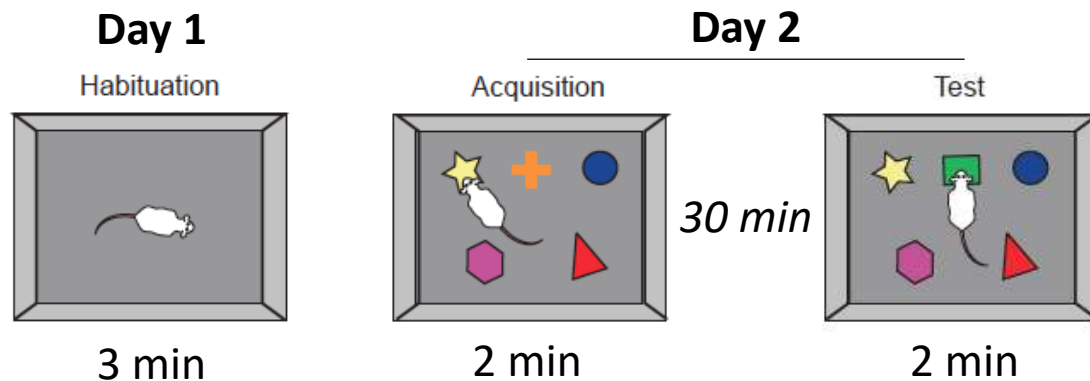
- Long-term treatment of McGill transgenic rats with AF710B, at an advanced stage of pathology



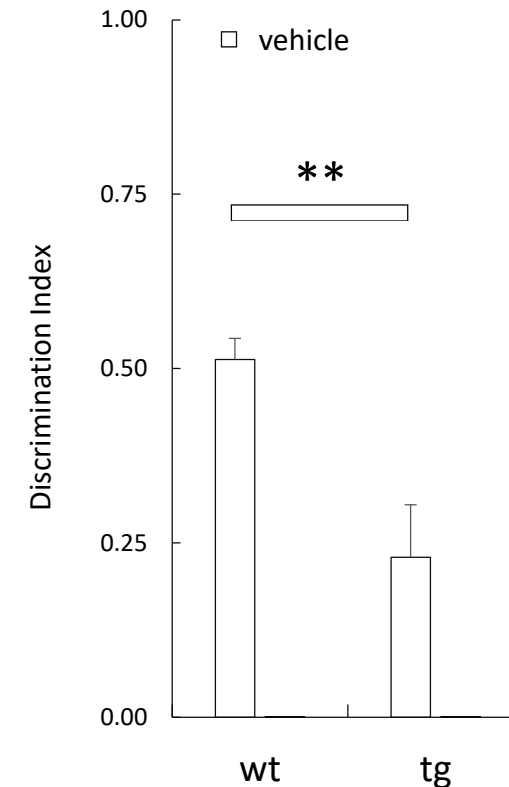
AF710B fully reverts the cognitive deficit in aged McGill tg rats

➤ Novel Object Recognition

- ✓ Based on spontaneous differential exploration of familiar and new objects
- ✓ Rodents will naturally spend more time exploring novel objects over familiar objects



Wilson *et al*, 2017
submitted

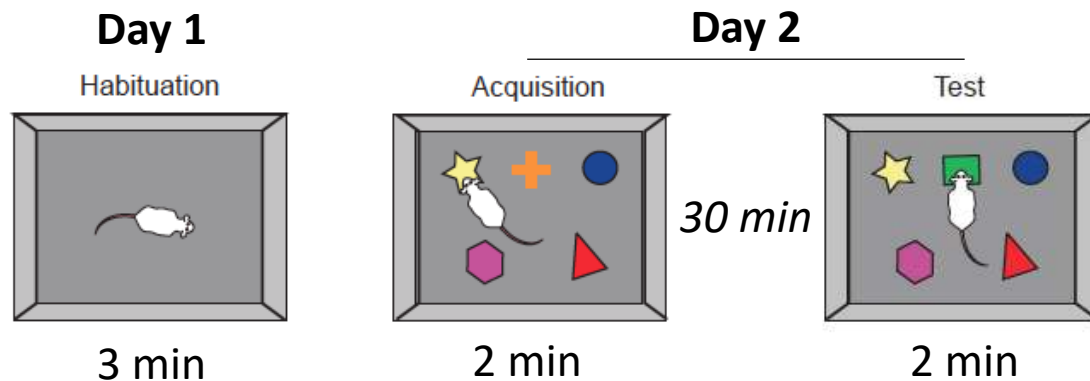


** $p < 0.01$, *** $p < 0.001$
two-way ANOVA

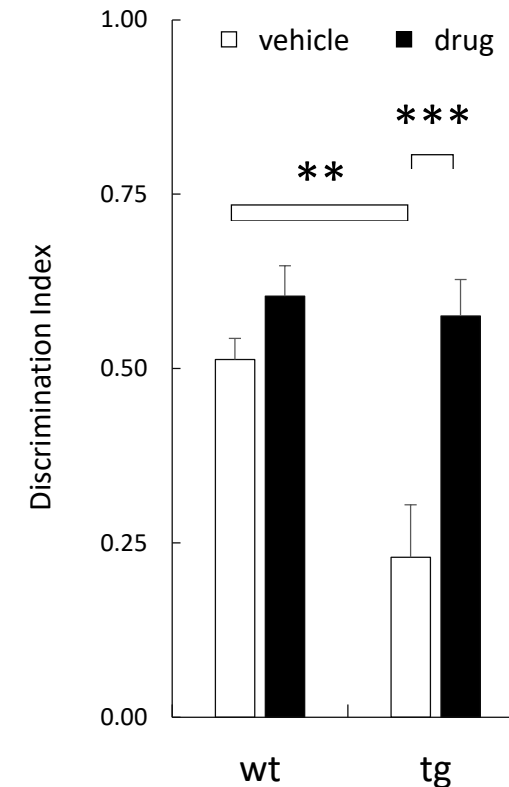
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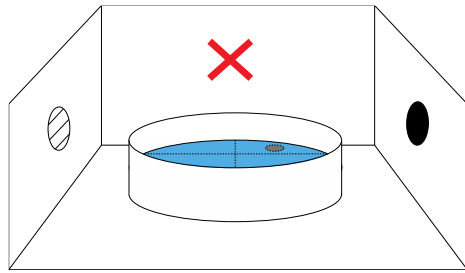
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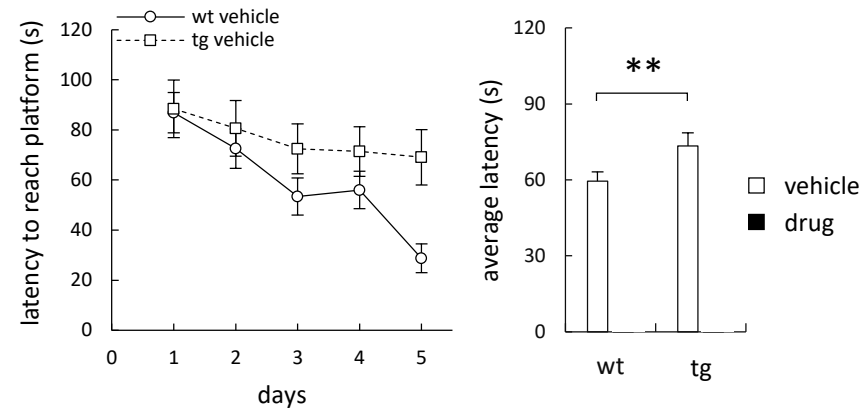
AF710B fully reverts the cognitive deficit in aged McGill tg rats

➤ Morris Water Maze



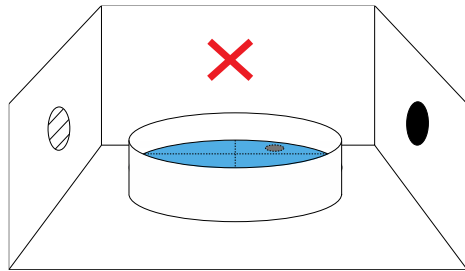
- ✓ 5 days of training to locate hidden platform
- ✓ 4 trials/day
- ✓ Probe trial on day 6

Acquisition phase



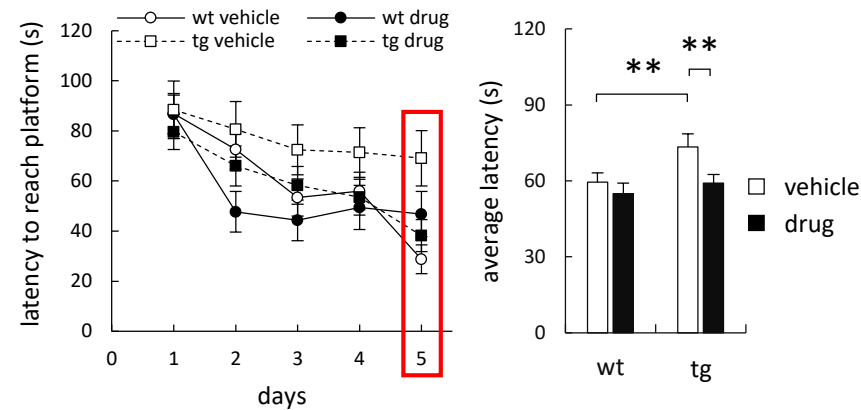
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➤ Morris Water Maze



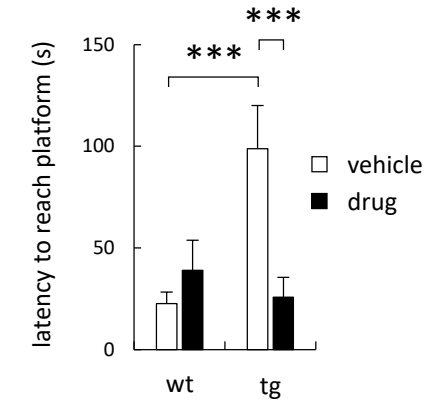
- ✓ 5 days of training to locate hidden platform
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Acquisition phase

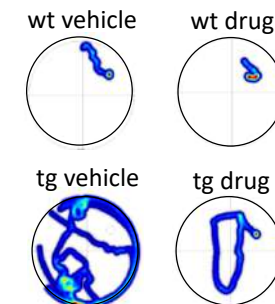


* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; two-way ANOVA

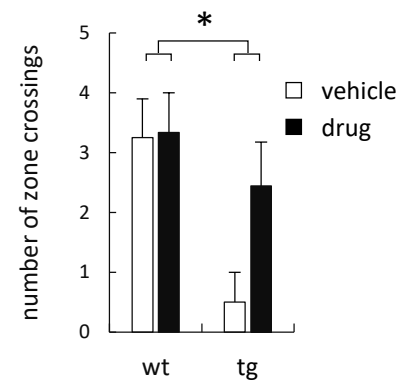
Performance on trial 4 - day 5



Representative swim paths

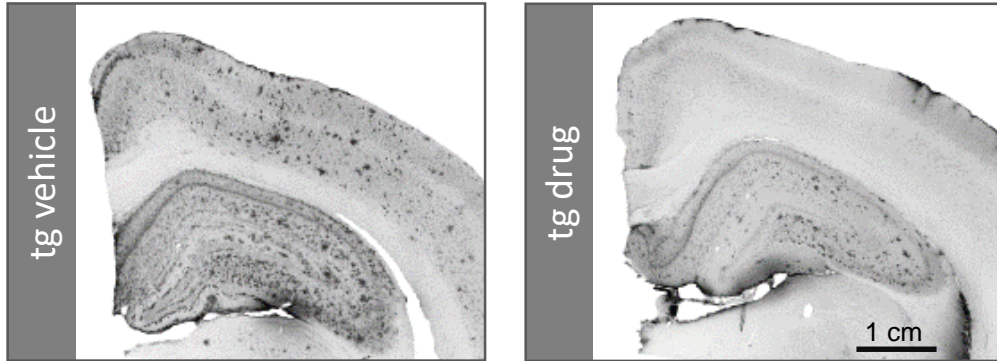


Probe test

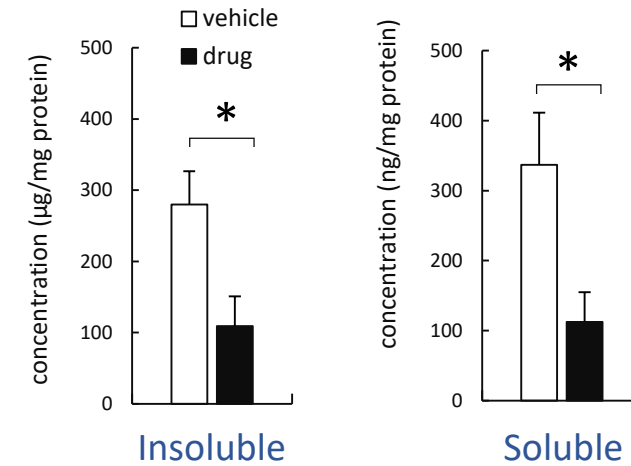


AF710B reduces AD-like amyloid pathology in McGill tg rats

➤ Decrease in A β immunoreactivity (McSA1 staining)

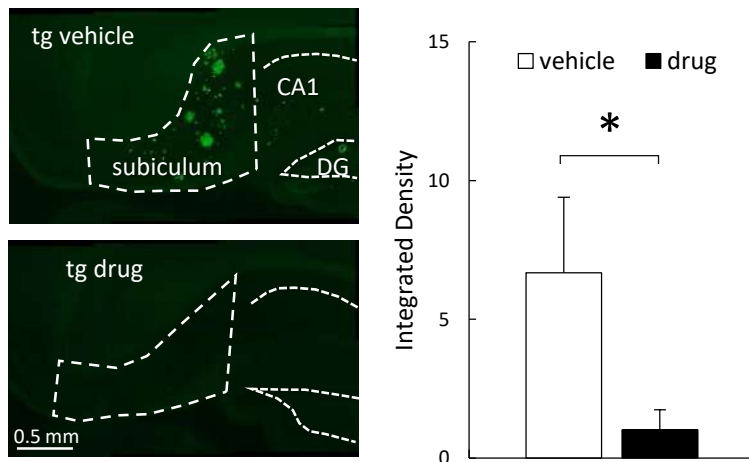


➤ Decrease in cortical A β 42 (ELISA)

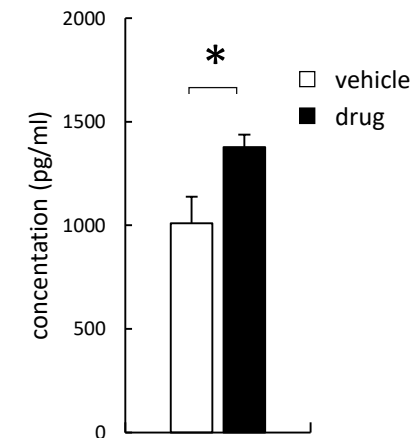


* $p < 0.05$; two-tailed t-test

➤ Decrease in mature plaques (ThioS+)



➤ Increase in CSF A β 42



AF710B reduces inflammation in McGill tg rats

➤ Early and late inflammation in McGill rats

- ✓ Increase in cytokines and chemokines

	wt 4 months	tg 4 months	tg 13 months
Cytokines			
TNF- α	+	+++	++++
IFN γ	-	+	+++
IL-1 α	-	+	+
IL-1 β	-	+	+++
IL-8	-	+	+++
IL-6	-	++	++
IL-12	-	+	++
IL-13	-	++	++
IL-2	+	++	++
IL-4	+	+	++
IL-10	+	+	+
TGF- β	+	+	+
IL-17 α	+	+	+
Chemokines			
GCSF	-	+	++
GMCSF	+	++	++
IP-10	-	++	+++
Rantes	-	++	++
Resistin	-	+	++

Hanzel *et al*, 2014, *Neurobiol Aging*
35(10)-2249

AF710B reduces inflammation in McGill tg rats

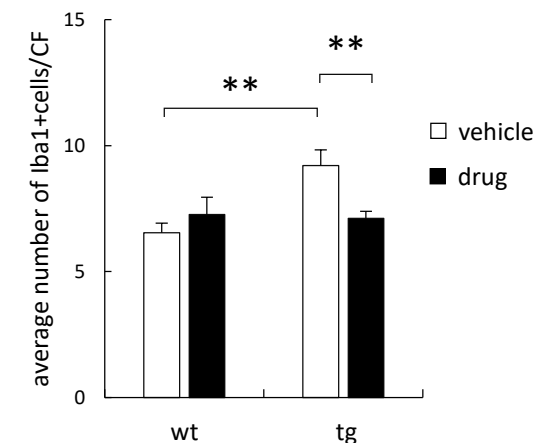
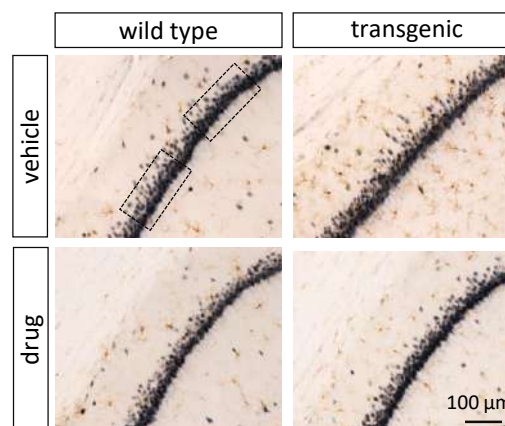
- Early and late inflammation in McGill rats
- AF710B has anti-inflammatory properties

✓ Increase in cytokines and chemokines

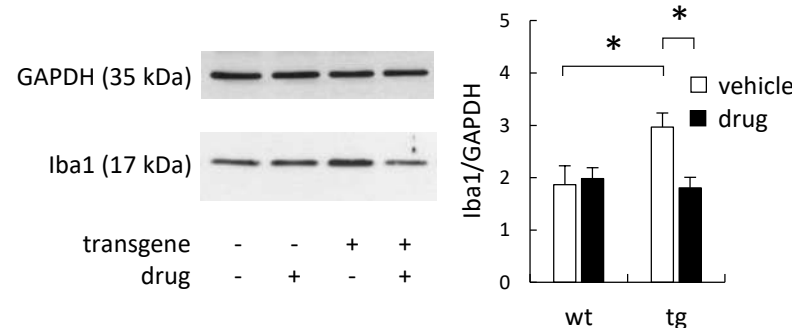
	wt 4 months	tg 4 months	tg 13 months
Cytokines			
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IL-6	-	++	++
IL-12	-	+	++
IL-13	-	++	++
IL-2	+	++	++
IL-4	+	+	++
IL-10	+	+	+
TGF- β	+	+	+
IL-17 α	+	+	+
Chemokines			
GCSF	-	+	++
GMCSF	+	++	++
IP-10	-	++	+++
Rantes	-	++	++
Resistin	-	+	++

Hanzel *et al*, 2014, *Neurobiol Aging* 35(10)-2249

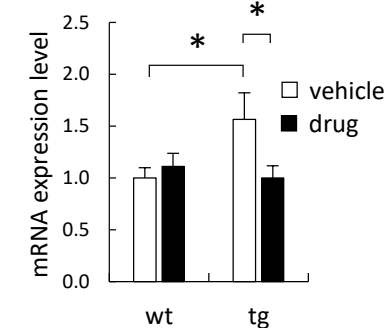
✓ Microglia recruitment to A β -burdened neurons in CA1



✓ Hippocampal levels of Iba1



✓ Cortical IL 10 mRNA

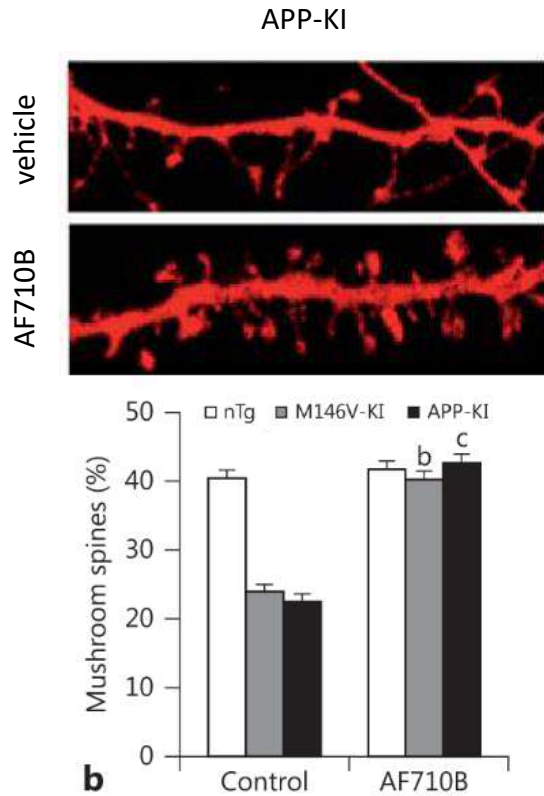


* $p < 0.05$, ** $p < 0.01$; two-way ANOVA

Hall *et al*, 2017, in preparation

AF710B has synaptogenic properties

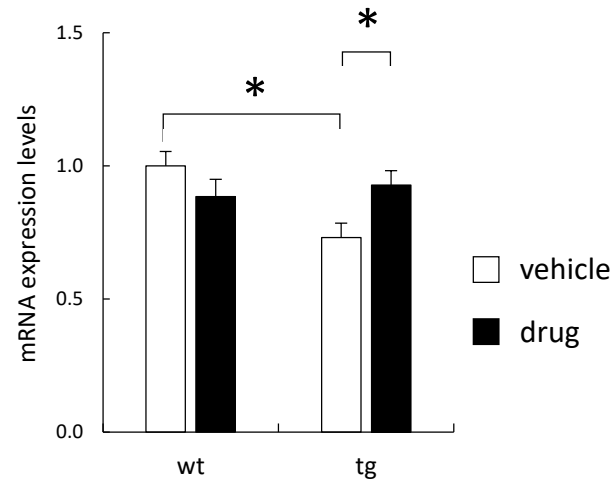
- *At the postsynaptic level*
AF710B restores synaptic spines



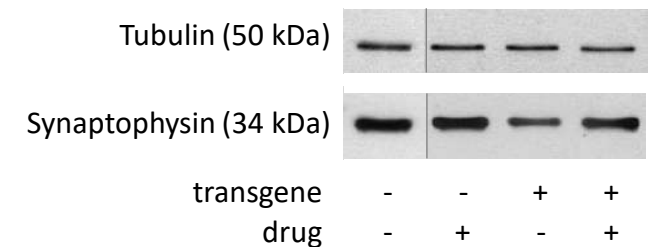
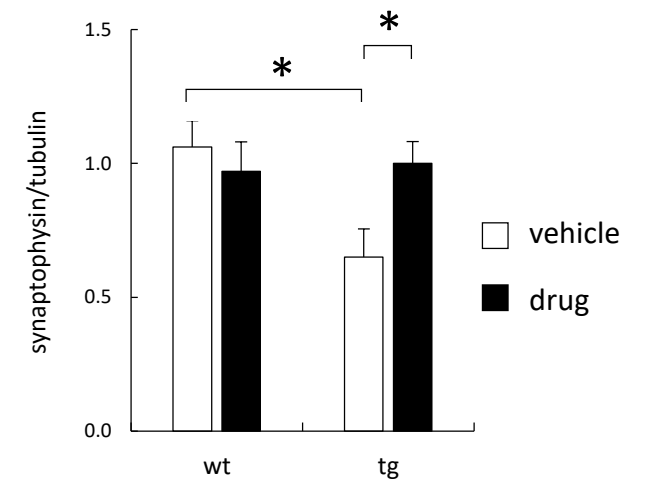
Fisher *et al*, *Neurodegener Dis*
16(1-2)-95

- *At the presynaptic level*
AF710B restores synaptophysin levels

Synaptophysin mRNA levels

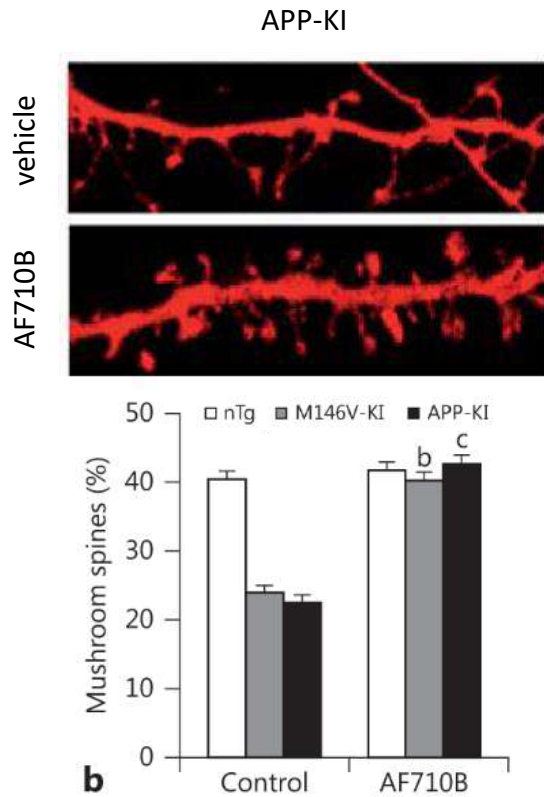


Synaptophysin protein levels



AF710B has synaptogenic properties

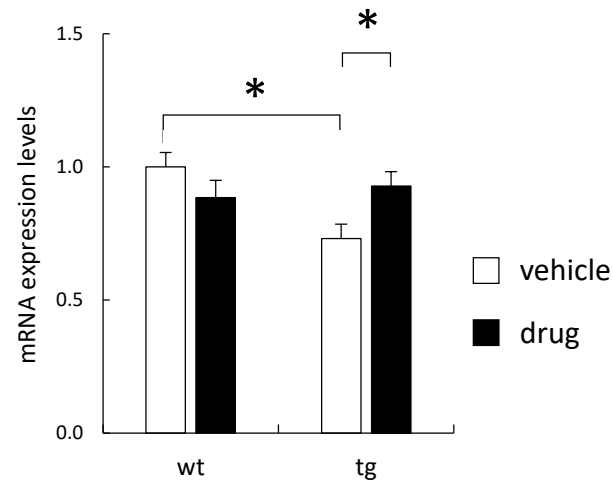
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AF710B restores synaptic spines



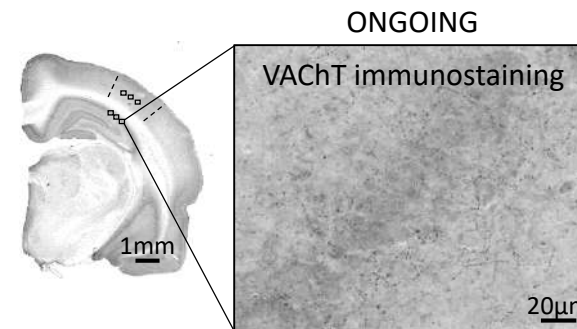
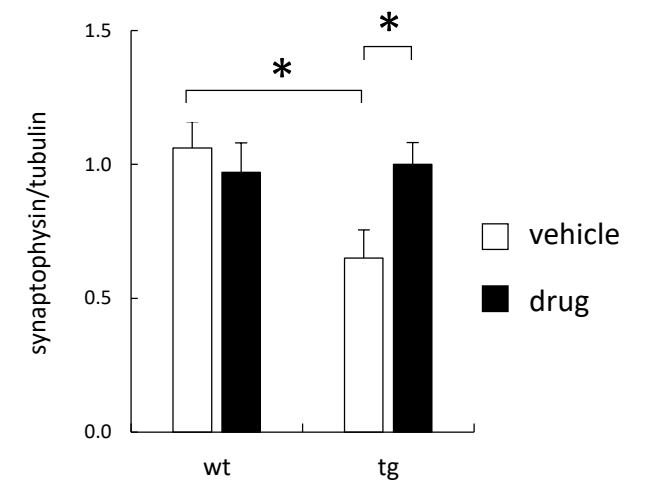
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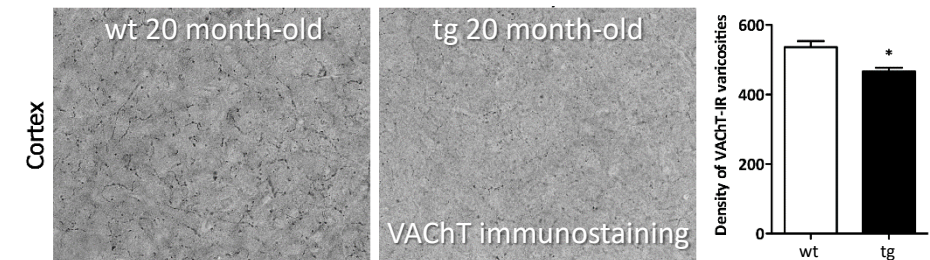
Synaptophysin mRNA levels



Synaptophysin protein levels



Decrease in cholinergic boutons in McGill tg rats



Hall *et al*, 2017, in preparation

Iulita, Bistue *et al*, 2017, submitted

Conclusions

- AF710B (a dual M1/Sigma-1 agonist) has long-lasting procognitive effects at the late stages of the amyloid neuropathology in McGill-R-Thy1-APP rats.
- *Disease-modifying effects:*
 - Decrease in AD-like amyloid pathology
 - Anti-inflammatory effect
 - Synaptogenic activity
- Effects maintained following a 5- week interruption in the treatment, suggesting true disease-modifying properties.

Acknowledgements



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