Mouse astrocytes exhibit agonist-induced functional $S1P_1$ receptor antagonism

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Disclosures

Nada Ben Yakoub, Tatjana Uffelmann, Sarah Tisserand and **Marc Bigaud** are employees of Novartis.

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Background and objective

- Sphingosine-1-phosphate (S1P) receptor subtype 1 ($S1P_1$) plays a key role in regulation of lymphocyte trafficking[1]
- In multiple sclerosis, $S1P_1$ agonists such as fingolimod and siponimod induce $S1P_1$ down modulation (i.e. receptor internalization and degradation) inhibiting the egress of pathogenic lymphocytes to the CNS - a phenomenon also known as functional $S1P_1$ antagonism[2,3,4]
- However, no evidence of this phenomenon exists in the cells of the CNS

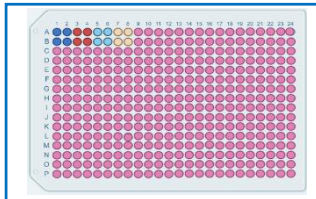
Objective	**To investigate the presence of $S1P_1$-functional antagonism by assessing agonist-induced $S1P_1$ down modulation in the astrocytes using a Ca^{2+} signaling assay**

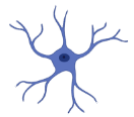
Ca^{2+}, calcium; CNS, central nervous system; $S1P_1$, sphingosine-1-phosphate receptor subtype 1

1. Tiper IV, et al. *Pathog Dis.* 2016;74:ftw063; 2. Chun J, et al. *Clin Neuropharmacol.* 2010;33:91–101; 3. McGinley M, et al. *Ther Adv Neurol Disord.* 2018;11:1–2; 4. Lukas S, et al. *J Biomol Screen.* 2014;19:407–416.

Methodology: Agonist-induced Ca^{2+} signaling

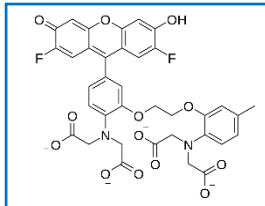
Fluorescent Ca^{2+} probe and Fluorescent Imaging Plate Reader (FLIPR)





Cell plates with murine astrocytes

Pre-incubation of murine astrocytes (C8-D1A) with vehicle or an $S1P_1$ agonist[#] (S1P, AUY954, fingolimod or siponimod) at different concentrations (0.0001 µM up to 30 µM) (overnight incubation)



Fluo4-AM, a dye that becomes fluorescent upon Ca^{2+} binding

Washing and Ca^{2+} probe loading (incubation for an hour) followed by ATP priming (10 µM) to activate the Ca^{2+} pumps (incubation for 30 minutes)

Compound stimulation and FLIPR readout (3 minutes - Quadruplicate)

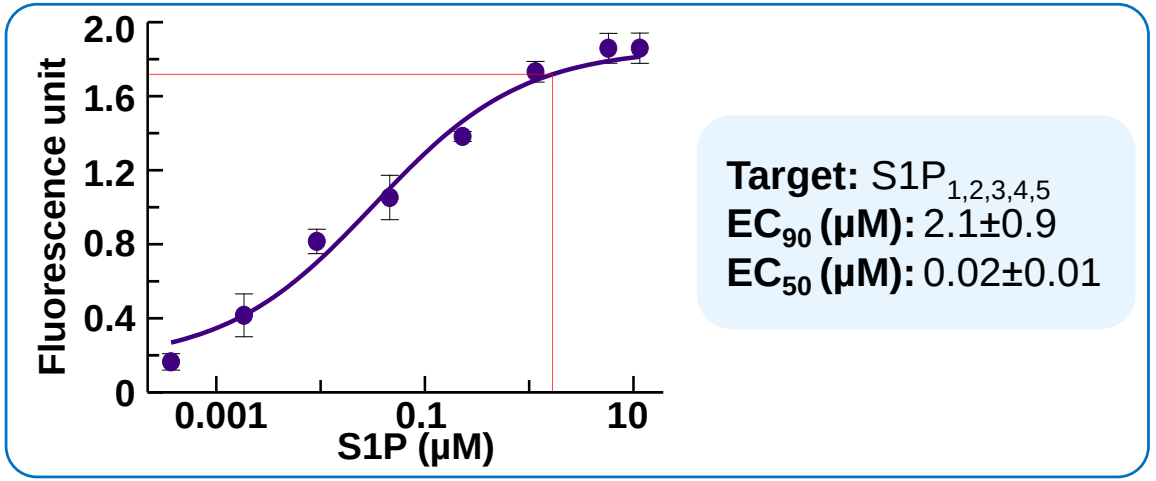
Outcomes

Dose response curves using agonist-induced Ca^{2+} signaling, measured as an increase in the intracellular fluorescence via FLIPR

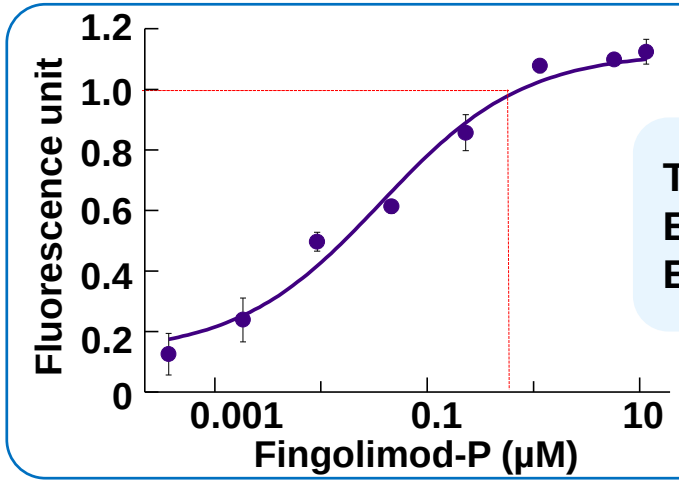
[#]S1P (natural ligand for S1PRs), AUY954 (selective $S1P_1$ agonist), fingolimod ($S1P_{1,3,4,5}$ agonist), or siponimod ($S1P_{1,5}$ agonist)
ATP, adenosine triphosphate; Ca^{2+}, calcium; FLIPRs, fluorescent imaging plate reader; S1P, sphingosine-1-phosphate; $S1P_1$, sphingosine-1-phosphate receptor subtype 1; S1PR, sphingosine-1-phosphate receptor

Results

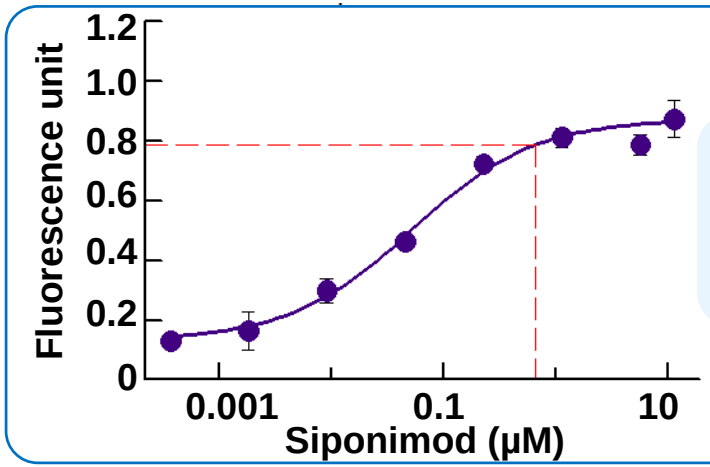
Astrocytes pre-incubated with vehicle (n=3 each)



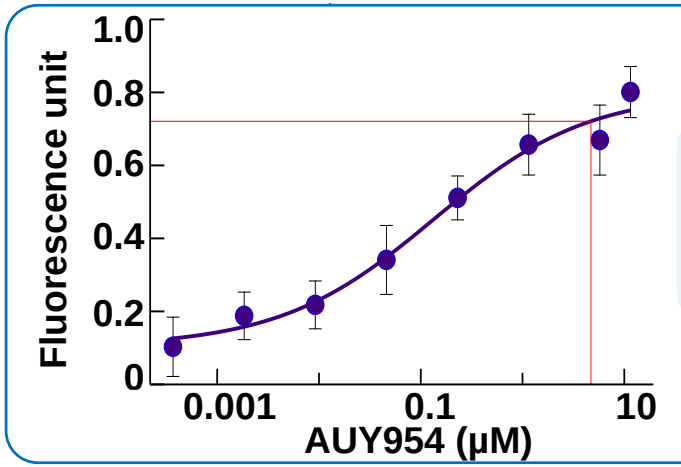
Target: $S1P_{1,2,3,4,5}$
EC_{90} (μM): 2.1±0.9
EC_{50} (μM): 0.02±0.01



Target: $S1P_{1,3,4,5}$
EC_{90} (μM): 0.9±0.3
EC_{50} (μM): 0.008±0.004



Target: $S1P_{1,5}$
EC_{90} (μM): 0.6±0.2
EC_{50} (μM): 0.03±0.03



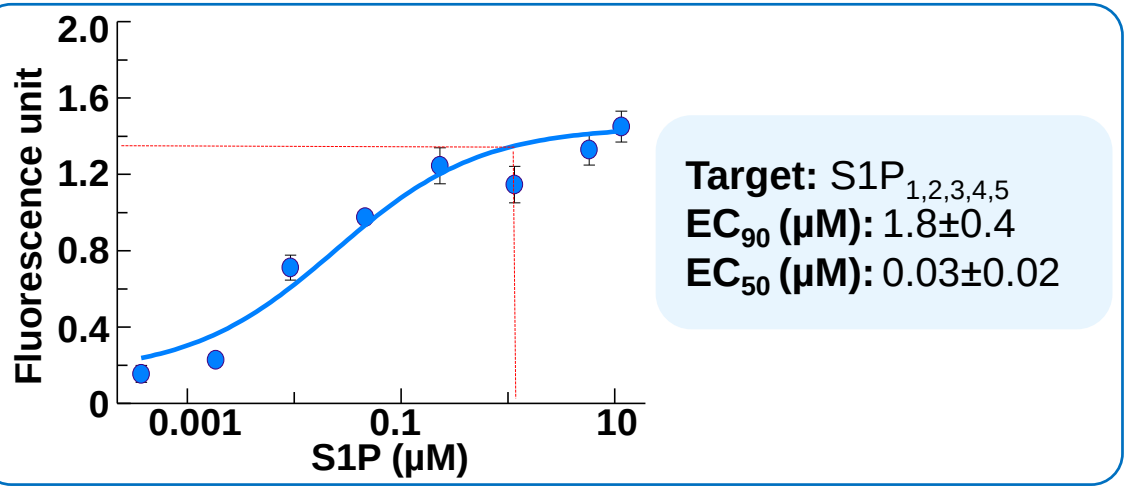
Target: $S1P_{1}$
EC_{90} (μM): 7.0±1.2
EC_{50} (μM): 0.07±0.03

Dose-dependent increase in the intracellular Ca^{2+} signals in response to all tested S1PR agonists, with EC_{50} values being within the range of 8–70 nM

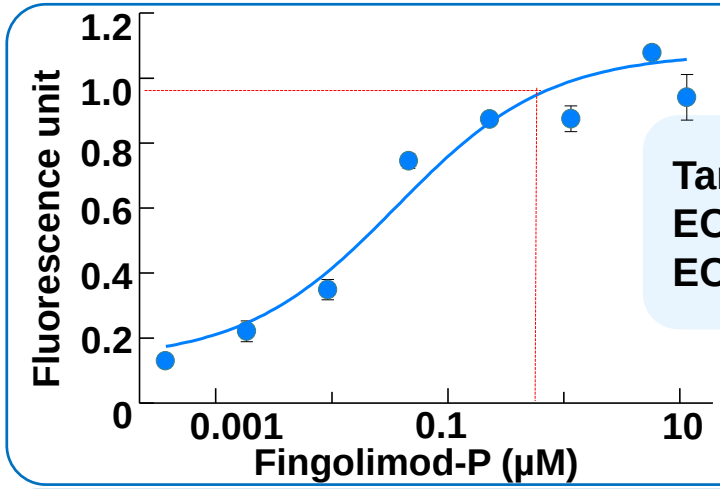
Ca^{2+}, calcium; EC_{50}, concentration of a drug that gives half-maximal response; EC_{90}, concentration of a drug that gives 90% response; fingolimod-P, fingolimod-phosphate; S1P, sphingosine-1-phosphate receptor; S1PR, sphingosine-1-phosphate receptor

Results

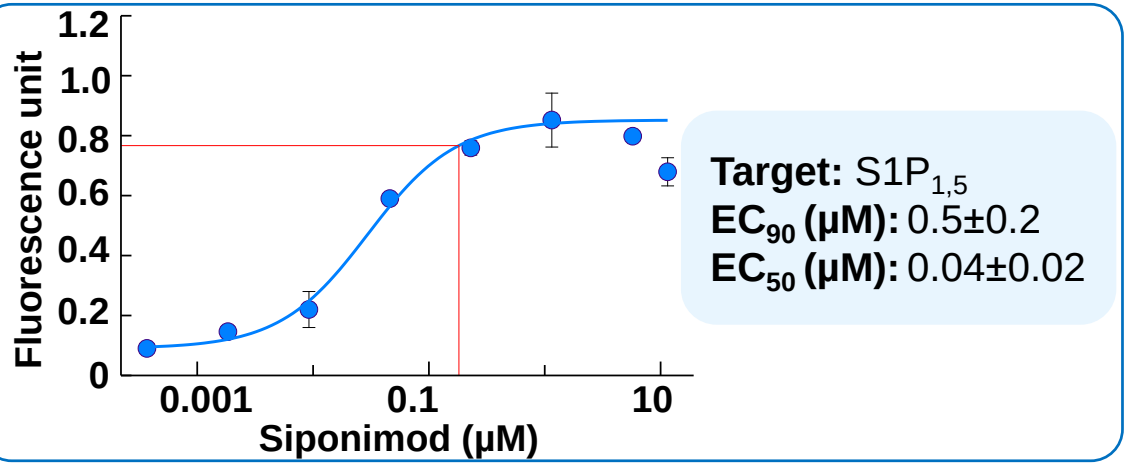
Astrocytes pre-incubated with S1P (1µM) (n=3 each)



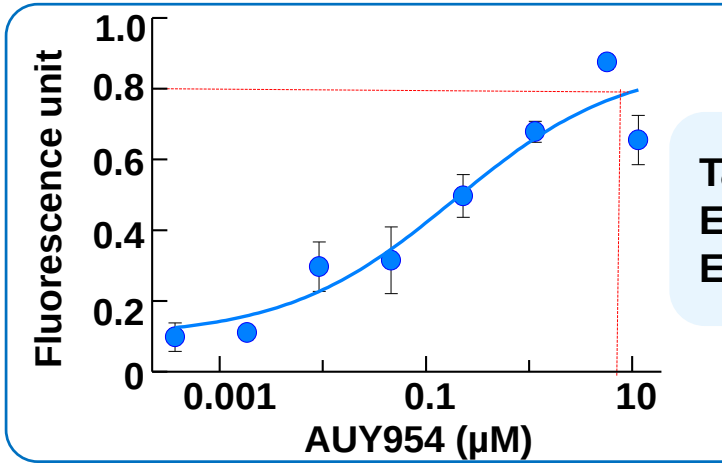
Target: $S1P_{1,2,3,4,5}$
EC_{90} (µM): 1.8±0.4
EC_{50} (µM): 0.03±0.02



Target: $S1P_{1,3,4,5}$
EC_{90} (µM): 0.7±0.3
EC_{50} (µM): 0.05±0.02



Target: $S1P_{1,5}$
EC_{90} (µM): 0.5±0.2
EC_{50} (µM): 0.04±0.02



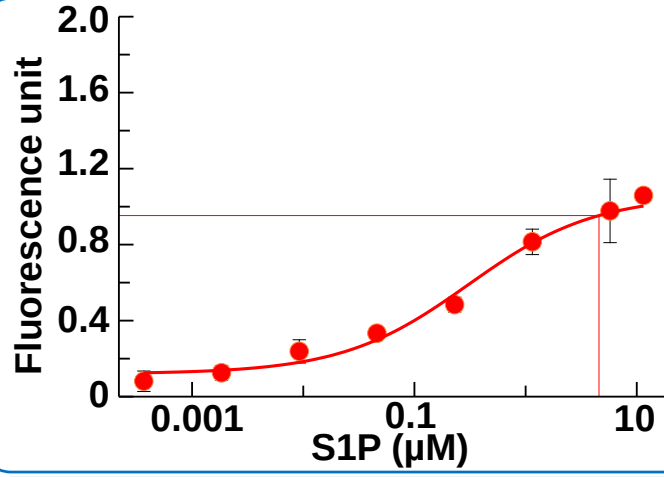
Target: $S1P_1$
EC_{90} (µM): 8.0±1.4
EC_{50} (µM): 0.09±0.04

Pre-incubation with natural ligand S1P ($S1P_{1,2,3,4,5}$ agonist) did not alter the effects of the S1PR agonists confirming that S1P does not induce down modulation of its own receptors (S1PRs)

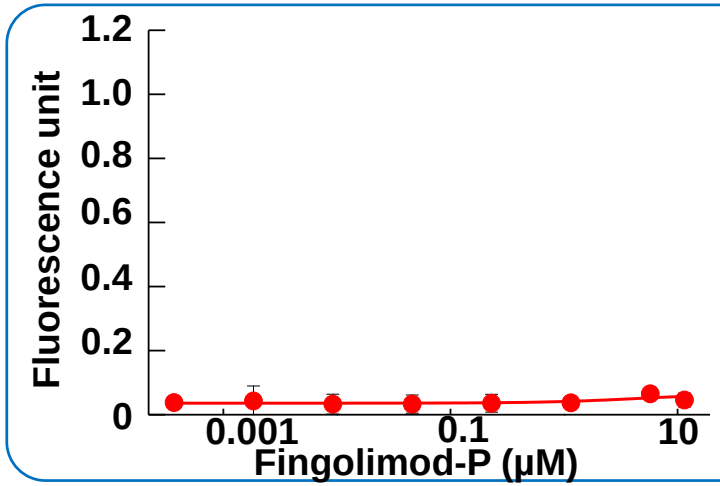
Ca^{2+}, calcium; EC_{50}, concentration of a drug that gives half-maximal response; EC_{90}, concentration of a drug that gives 90% response; fingolimod-P, fingolimod-phosphate; S1P, sphingosine-1-phosphate; $S1P_1$, sphingosine-1-phosphate receptor subtype; S1PR, sphingosine-1-phosphate receptor

Results

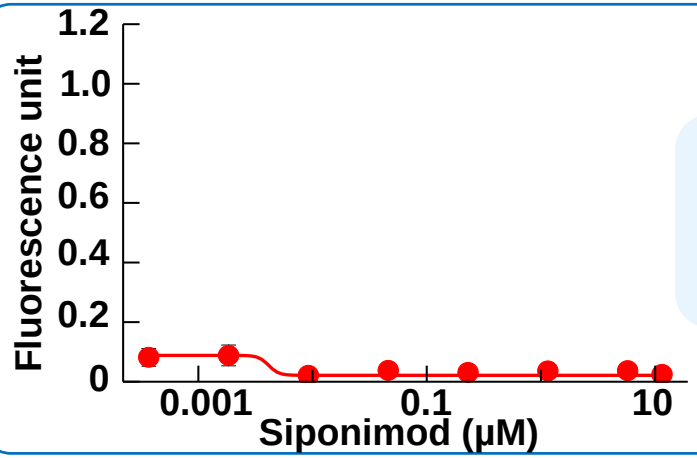
Astrocytes pre-incubated with AUY954 (1µM) (n=3 each)



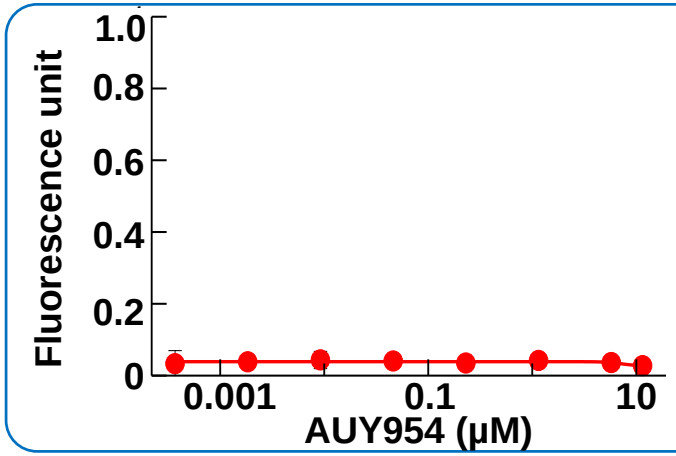
Target: $S1P_{1,2,3,4,5}$
EC_{90} (µM): 7.4±1.8
EC_{50} (µM): 0.12±0.09



Target: $S1P_{1,3,4,5}$
EC_{90} (µM): n.a.
EC_{50} (µM): n.a.



Target: $S1P_{1,5}$
EC_{90} (µM): n.a.
EC_{50} (µM): n.a.



Target: $S1P_1$
EC_{90} (µM): n.a.
EC_{50} (µM): n.a.

- **Pre-incubation with AUY954 abolished effects of fingolimod, siponimod, AUY954, and induced down modulation of $S1P_1$ receptors**
- **In astrocytes, effects of fingolimod and siponimod were principally $S1P_1$-dependent**
- **Similar observations were observed after pre-incubation with fingolimod or siponimod**

Ca^{2+}, calcium; EC_{50}, concentration of a drug that gives half-maximal response; EC_{90}, concentration of a drug that gives 90% response; fingolimod-P, fingolimod-phosphate; n.a., not applicable; S1P, sphingosine-1-phosphate; $S1P_1$, sphingosine-1-phosphate receptor subtype 1

Conclusions

- First evidence of agonist-induced $S1P_1$ down modulation in murine astrocytes
- Similar investigations on other neural and glial cell types are warranted to establish agonist-induced $S1P_1$ down modulation as a general phenomenon in the CNS
- Translational and clinical studies are warranted to further validate this hypothesis

$S1P_1$, sphingosine-1-phosphate receptor subtype 1

Thank you