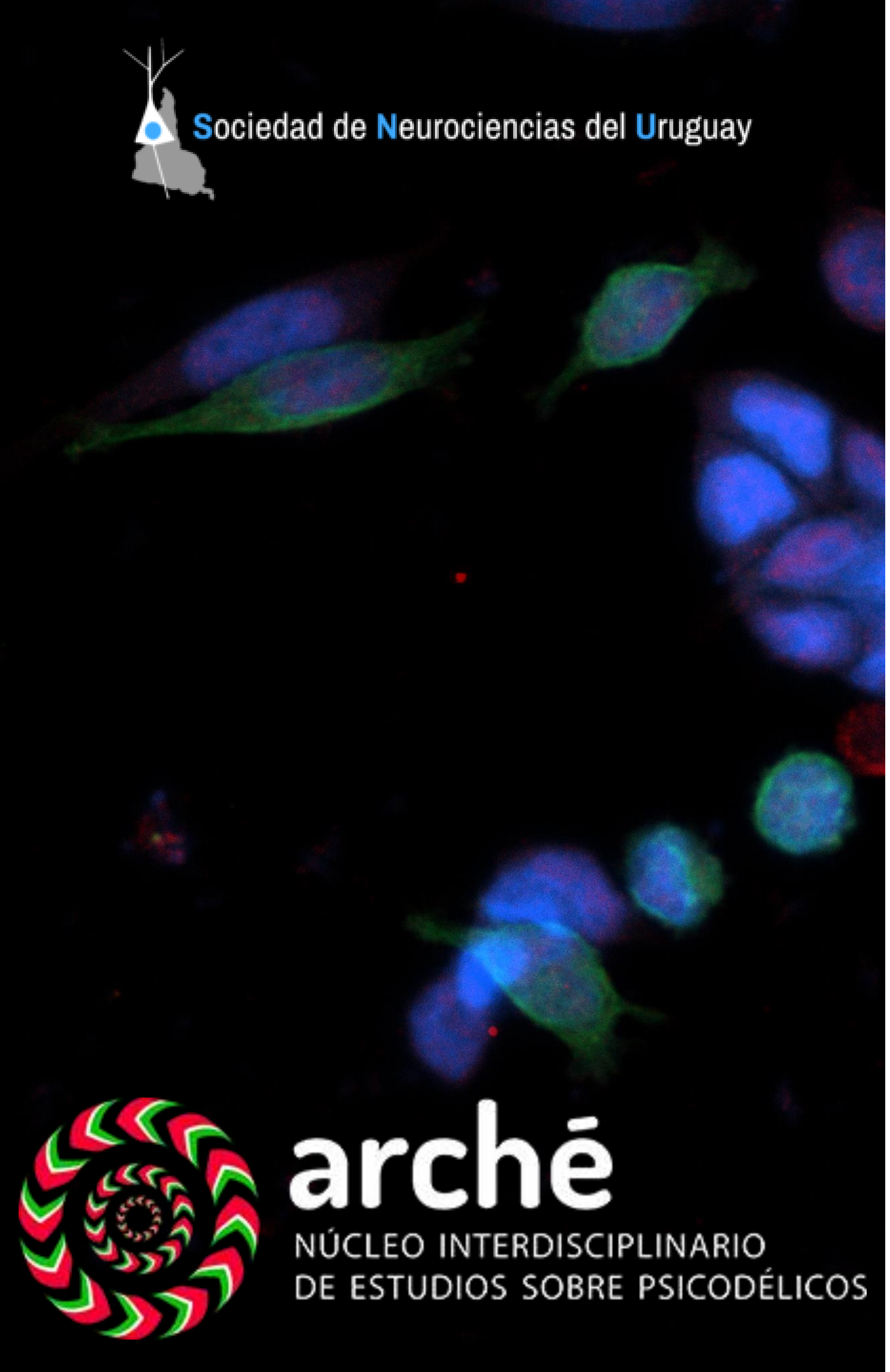


XX Jornadas de la Sociedad de Neurociencias del Uruguay
7 -9 de noviembre de 2024

EFFECT OF *N,N*-DIMETHYLTRYPTAMINE ON NEURITE OUTGROWTH IN PC12 CELL LINE: CHARACTERIZATION AND MECHANISMS

DRA. MARIANA PAZOS

LAB. DE MECANISMOS DE NEURODEGENERACIÓN Y NEUROPROTECCIÓN,
CÁTEDRA UNESCO DE PRODUCTOS NATURALES NEUROACTIVOS,
INSTITUTO DE INVESTIGACIONES BIOLÓGICAS CLEMENTE ESTABLE

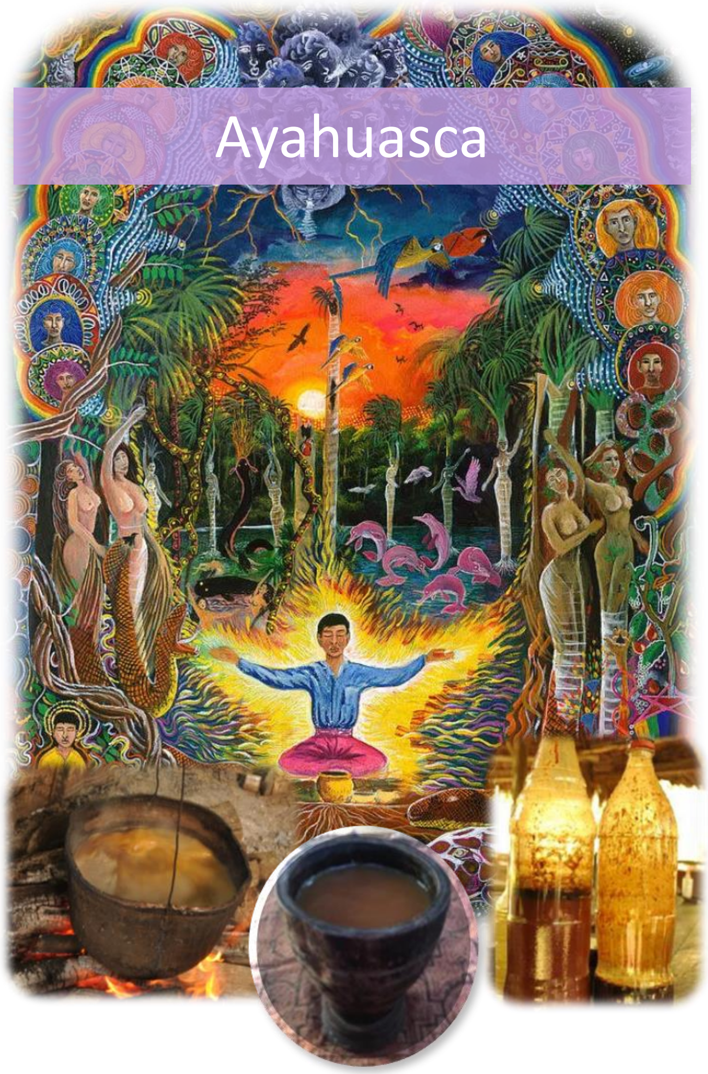
UNESCO

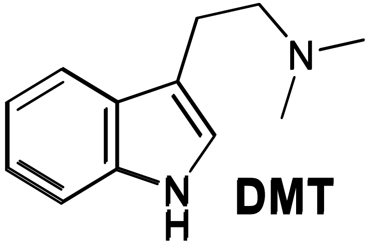
Ministerio de Educación y Cultura

iibce
INSTITUTO DE INVESTIGACIONES BIOLÓGICAS CLEMENTE ESTABLE

INTRODUCTION

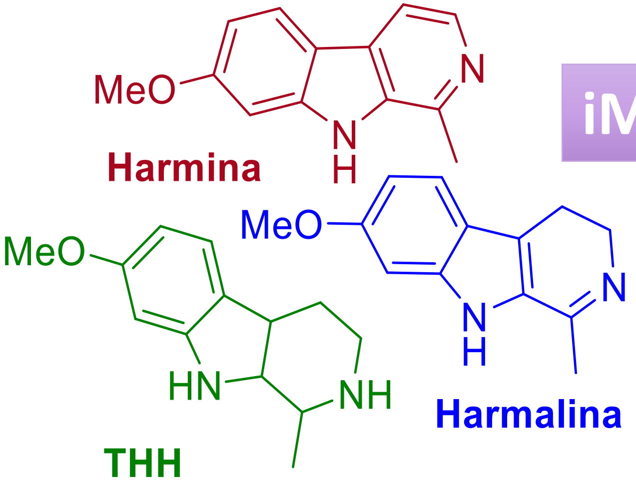
DMT and its therapeutic potential





DMT

Psicodélico clásico



Harmina

Harmalina

THH

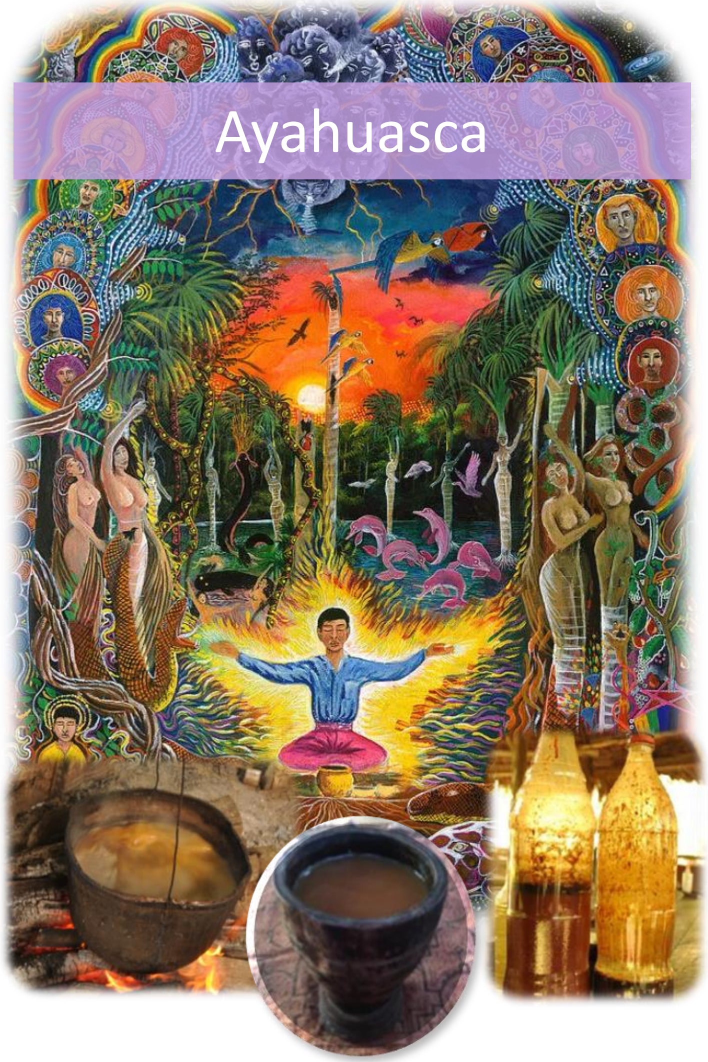
iMAO-A

β-carbolinas

L. Rodríguez, A. López, G. Moyna, G. A. Seoane, D. Davyt, Á. Vázquez, G. Hernández, I. Carrera, *ACS Omega* **2022**, *7*, 12307–12317.

INTRODUCTION

DMT and its therapeutic potential



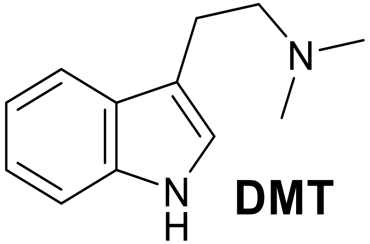
Therapeutic potential

Preclinical:

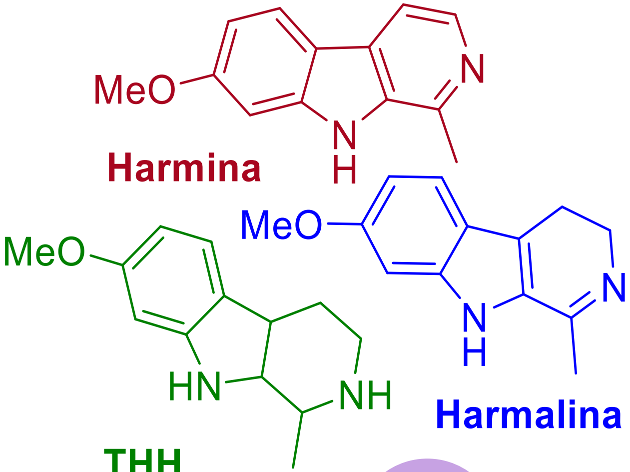
- Depression
- Anxiety
- Substance use disorder
- Alzheimer's disease

Clinical:

- Phase I trials: overall safety, PK, subjective effects, etc.



DMT



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D. Cheng, et el., *Alzheimers. Res. Ther.* **2024**, *3*, 1–16. F. A. Colosimo, et al., *Psychoactives* **2024**, *3*, 93–122.

INTRODUCTION

DMT and its therapeutic potential

Psychedelics Promote Structural and Functional Neural Plasticity

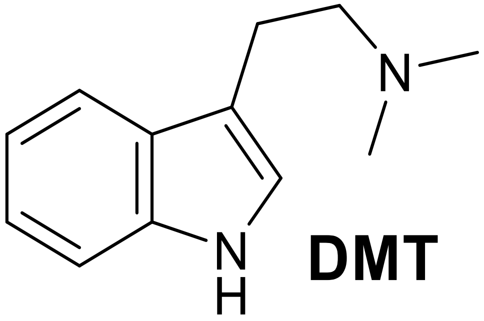
Calvin Ly,[1] Alexandra C. Greb,[1] Lindsay P. Cameron,[2] Jonathan M. Wong,[2] Eden V. Barragan,[2] Paige C. Wilson,[3] Kyle F. Burbach,[4] Sina Soltanzadeh Zarandi,[1] Alexander Sood,[5] Michael R. Paddy,[3] Whitney C. Duim,[1] Megan Y. Dennis,[4,6,7] A. Kimberley McAllister,[5,8,9] Kassandra M. Ori-McKenney,[3] John A. Gray,[5,8] and David E. Olson[1,5,6,10,*]

C. Ly *et al.*, *Cell Rep.*, vol. 23, no. 11, pp. 3170–3182, **2018**.

Psychoplastogens: A Promising Class of Plasticity-Promoting Neurotherapeutics

David E Olson[1,2,3]

D. E. Olson, *J. Exp. Neurosci.* **2018**, *12*, 1–4.



DMT

Regulates adult **neurogenesis** *in vitro* and *in vivo*

J. A. Morales-Garcia, et al., *Transl. Psychiatry* **2020**, *10*, 331.

Anti-AD effects *in vivo* and *in vitro*

D. Cheng, et el., *Alzheimers. Res. Ther.* **2024**, *3*, 1–16.

Modulates innate and adaptive **inflammatory responses** *in vitro*

A. Szabo, et al., *PLoS One* **2014**, *9*, 1–12.

Anti-ischemic properties in rat brain

N. Ye, et al., *J. Med. Chem.* **2020**, *63*, 15187–15217;
Í. Szabó, et al., *Neuropharmacology* **2021**, *192*, 108612.

- **Neuritogenesis** and **spinogenesis** in rat cortical cultures
- **Spinogenesis** in rat's PFC

C. Ly *et al.*, *Cell Rep.*, vol. 23, no. 11, pp. 3170–3182, **2018**.

Increases cell survival **against hypoxia** *in vitro*

A. Szabo, et al., *Front. Neurosci.* **2016**, *10*, 1–11.

INTRODUCTION

DMT and its therapeutic potential: *main pharmacological targets*

Psychedelics Promote Structural and Functional Neural Plasticity

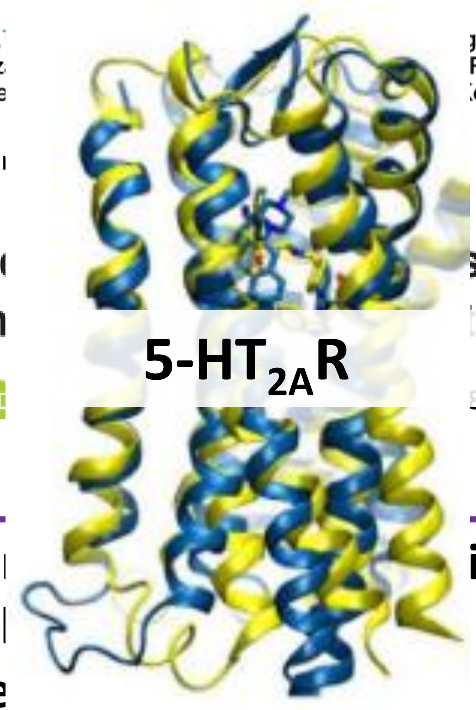
Calvin Ly,[1] Alexandra C. Greb,[1] ...,[2] Eden V. Barragan,[2] Paige C. Wilson,[3] Kyle F. Burbach,[4] Sina Soltanz... R. Paddy,[3] Whitney C. Duim,[1] Megan Y. Dennis,[4,6,7] A. Kimbe... ...enney,[3] John A. Gray,[5,8] and David E. Olson[1,5,6,10,*]

C. Ly *et al.*, *Cell Rep.*, vol. 23, ...

Psychoplastog... ...s of Plasticity-Prom... ...ics

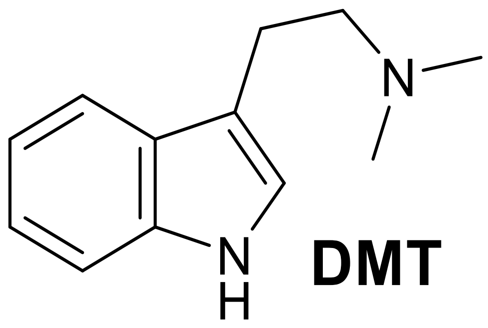
David E Olson[1,2,3]

..., *12*, 1–4.



- **Neuritoge... ...is in** rat cortical...
- **Spinogene...**

C. Ly *et al.*, *Cell Rep.*, vol. 23, no. 11, pp. 3170–3182, **2018**.



DMT

Regulates adult **neurogenesis** *in vitro* and *in vivo*

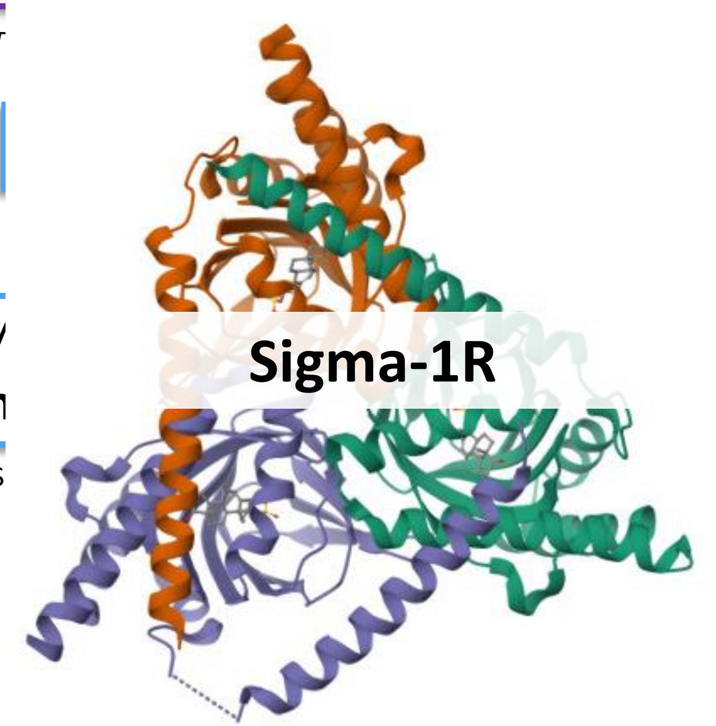
J. A. Morales-Garcia, et al., *T*...

...*tro*

..., *3*, 1–16.

M... in...

A. S...



...t brain

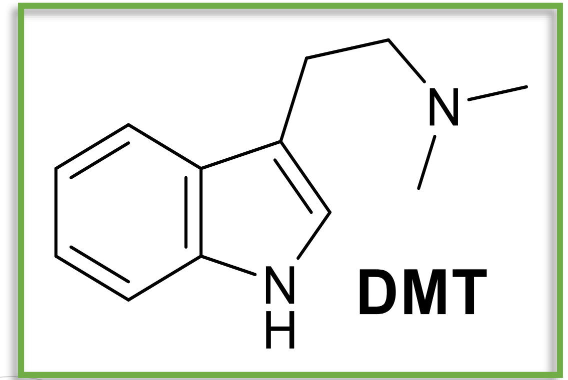
...5187–15217; ...*192*, 108612.

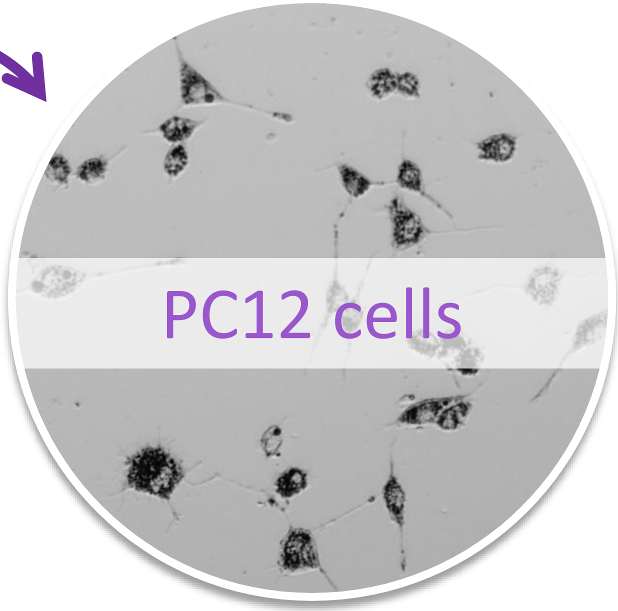
Increases cell survival **against hypoxia** *in vitro*

A. Szabo, et al., *Front. Neurosci.* **2016**, *10*, 1–11.

DMT IN PC12 CELL LINE

Neuritogenesis?





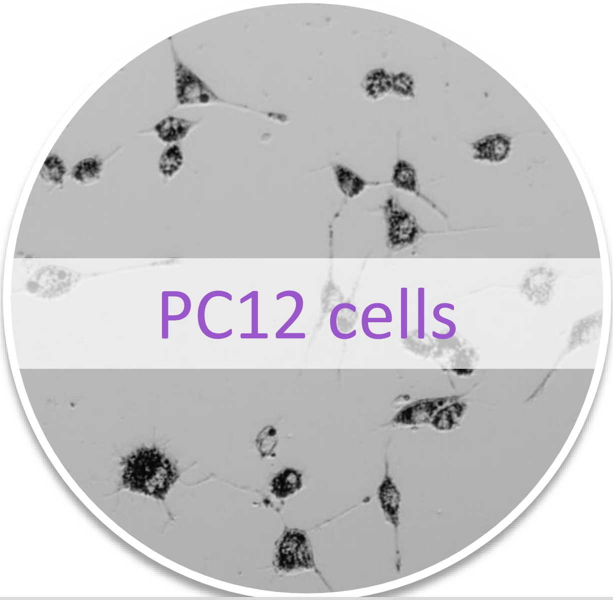
DMT IN PC12 CELL LINE

Well-established cathecolaminergic **cell line** obtained from a rat **pheochromocytoma**

Able to **synthesize, take up, store and release catecholamines** (mainly dopamine and epinephrine).

When stimulated with Nerve Growth Factor (**NGF**), they differentiate to **neuron-like cells** (dPC12):

- cease division
- extend neurites
- form synapse-like structures
- show electrical excitability properties



SUITABLE MODEL TO STUDY

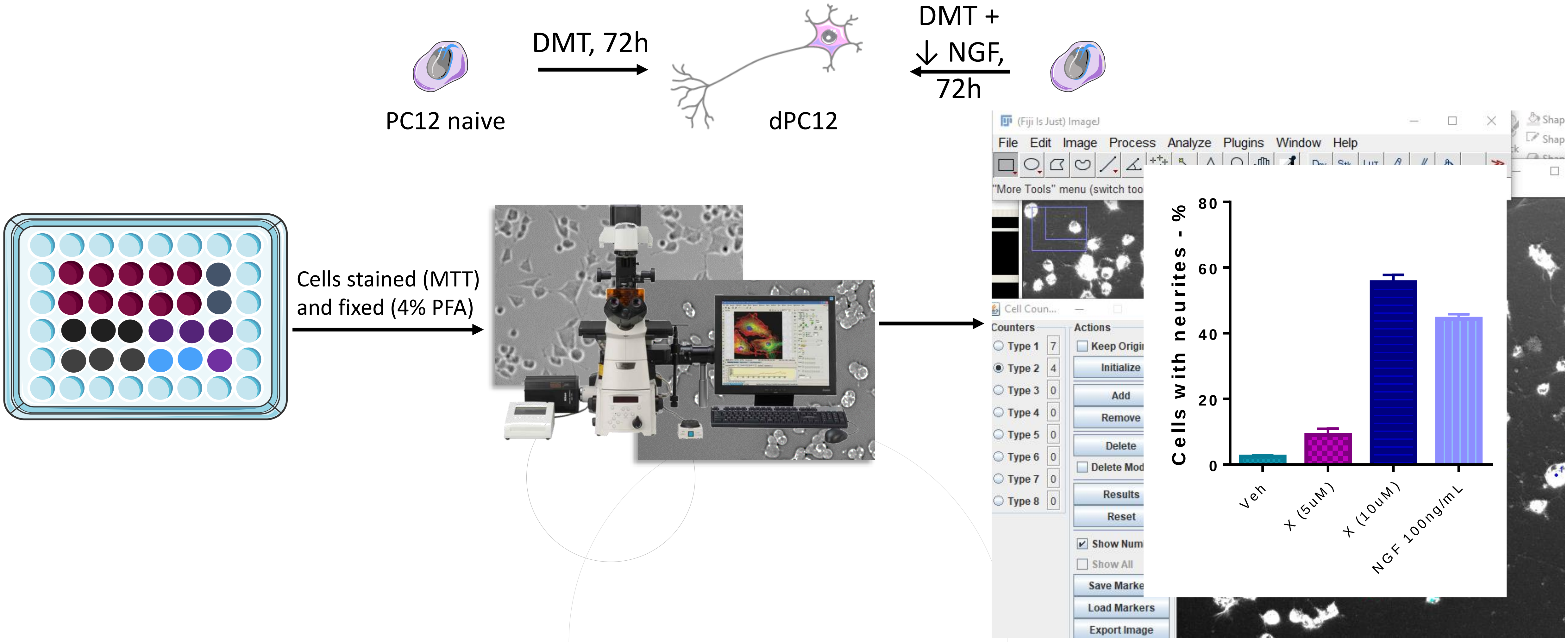
✓ Neuronal differentiation
✓ Neurite outgrowth
✓ Molecular mechanisms

K. K. Teng, et al., *Cell Biol. Four-Volume Set*, **2006**, pp. 171–176.

L. A. Greene, A. S. Tischlert, *Cell Biol.* **1976**, *73*, 2424–2428.

DMT IN PC12 CELL LINE

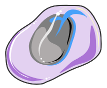
Effect of DMT on neurite outgrowth



DMT IN PC12 CELL LINE

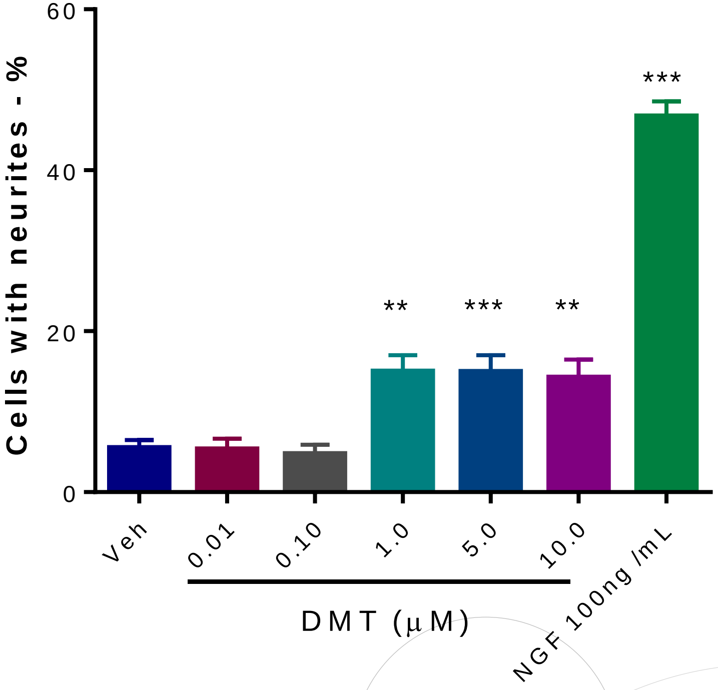
Effect of DMT on neurite outgrowth

- Characterization

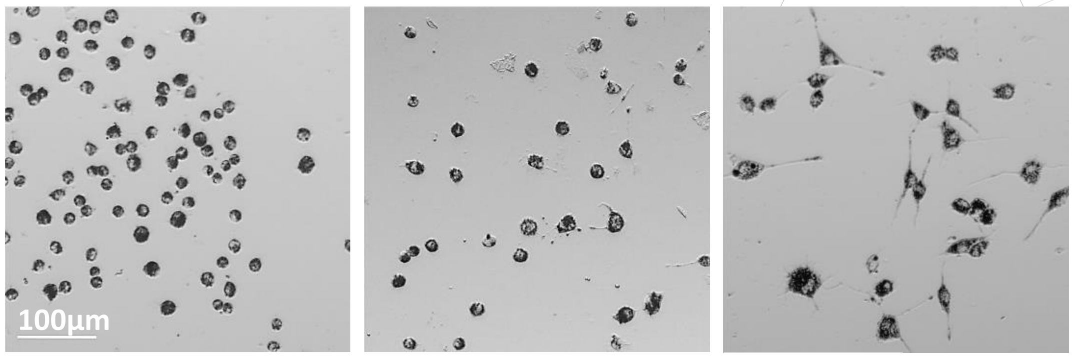


PC12 naive

DMT, 72h



✓ **DMT promotes neuritogenesis in PC12 cells**



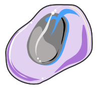
Vehicle

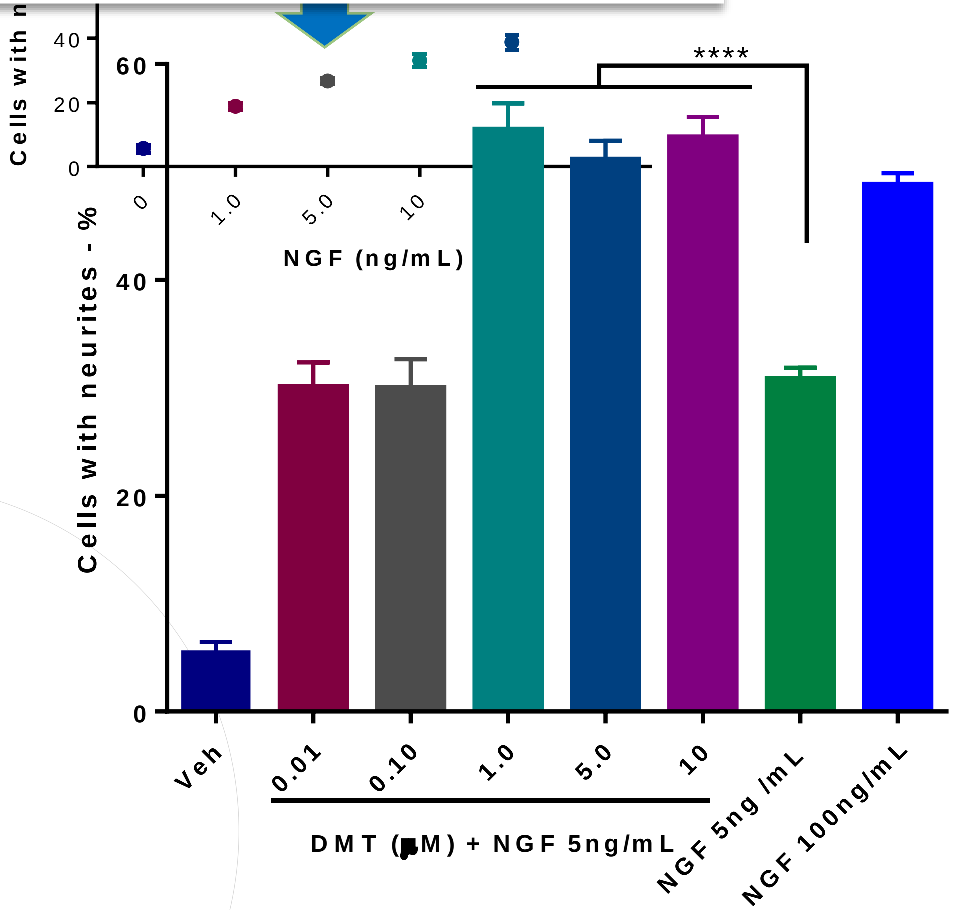
DMT 5µM

NGF 100ng/mL

✓ **DMT shows an additive-like effect with NGF**

DMT + ↓ NGF, 72h





DMT IN PC12 CELL LINE

Effect of DMT on neurite outgrowth

- Towards an understanding of its molecular mechanisms

Pharmacological inhibitors

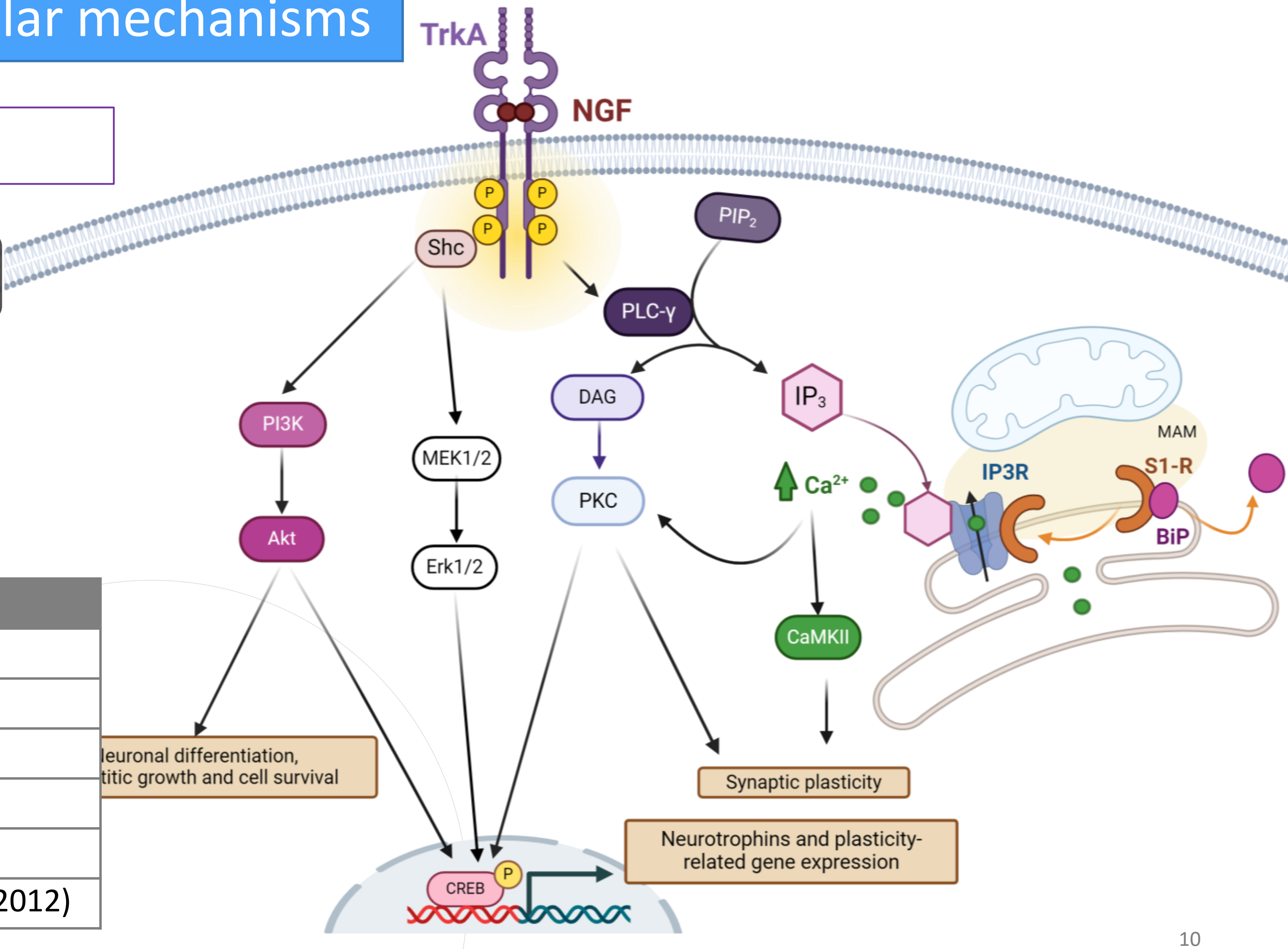
PC12 differentiation by NGF:

- TrkA receptor and pathways:
  - PI3K/Akt
  - MEK/Erk
  - PLCγ

DMT pharmacology:

- $5\text{-}HT_{2A}R$
- Sigma-1R

Pathway/receptor	Inhibitor (µM)	Ref.
PLCγ	U73122 (1µM)	(Gassaway et al., 2016)
PI3K/Akt	LY294002 (10µM)	(Gassaway et al., 2016)
MEK/Erk	PD98059 (10µM)	(Lee and Chao, 2001)
Trk(A,B,C)	GNF-5837 (1µM)	(Albaugh et al., 2012)
$5\text{-}HT_{2A}R$	Ketanserin (10µM)	(Vargas et al., 2023)
S1-R	NE-100 (10µM)	(Ishima and Hashimoto, 2012)



DMT IN PC12 CELL LINE

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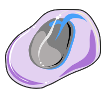
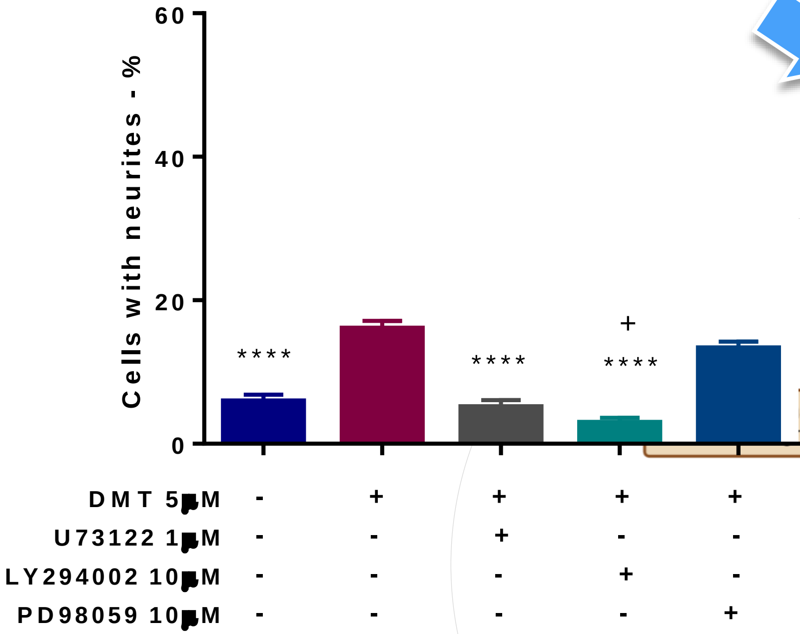
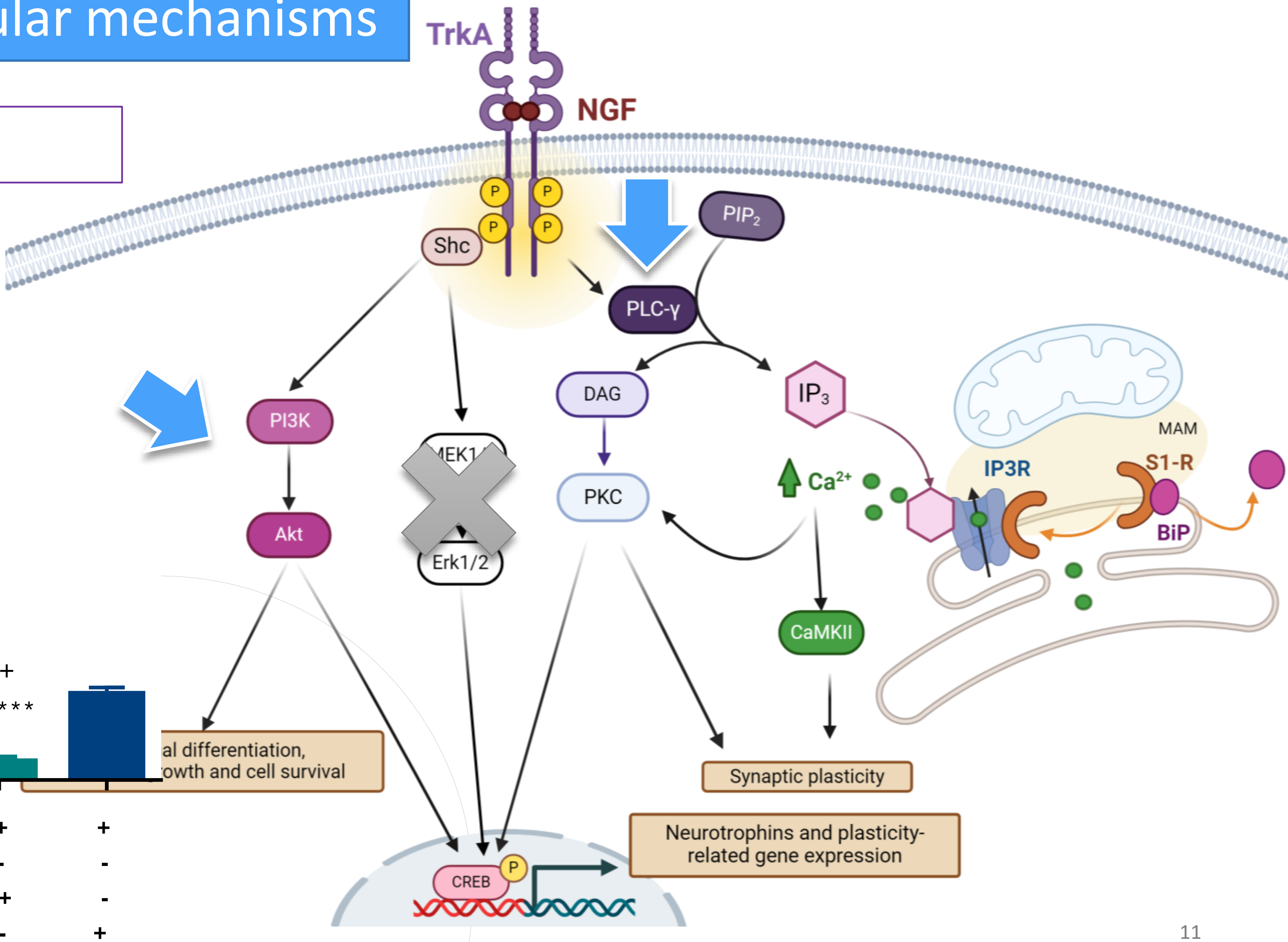


PC12 naive

DMT, 72h

+ inhibitor





DMT IN PC12 CELL LINE

Effect of DMT on neurite outgrowth

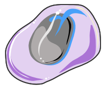
- Towards an understanding of its molecular mechanisms

✓ **Neuritogenesis by DMT depends on PI3K/Akt and PLCγ pathways**

Pharmacological inhibitors

PC12 differentiation by NGF:

- TrkA receptor and pathways:
  - PI3K/Akt
  - MEK/Erk
  - PLCγ



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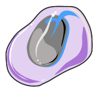
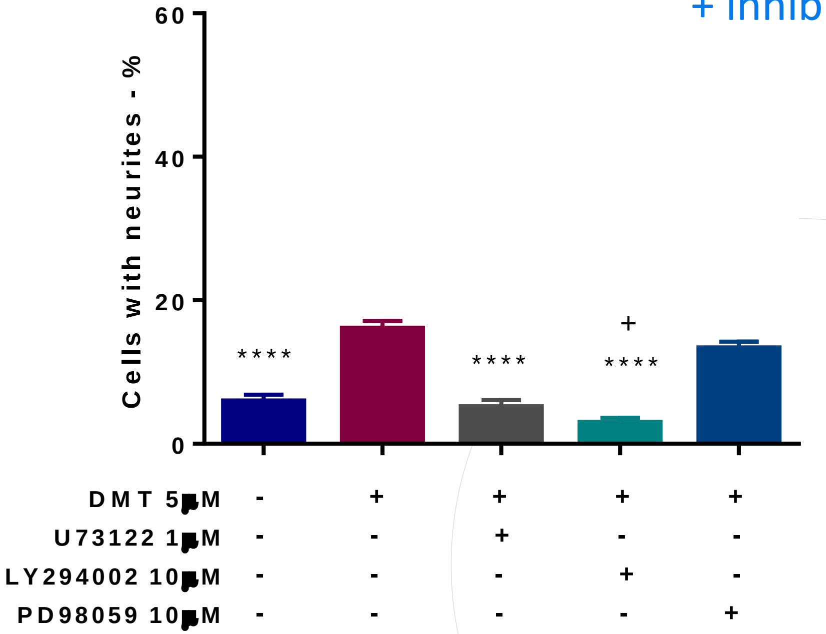
DMT, 72h

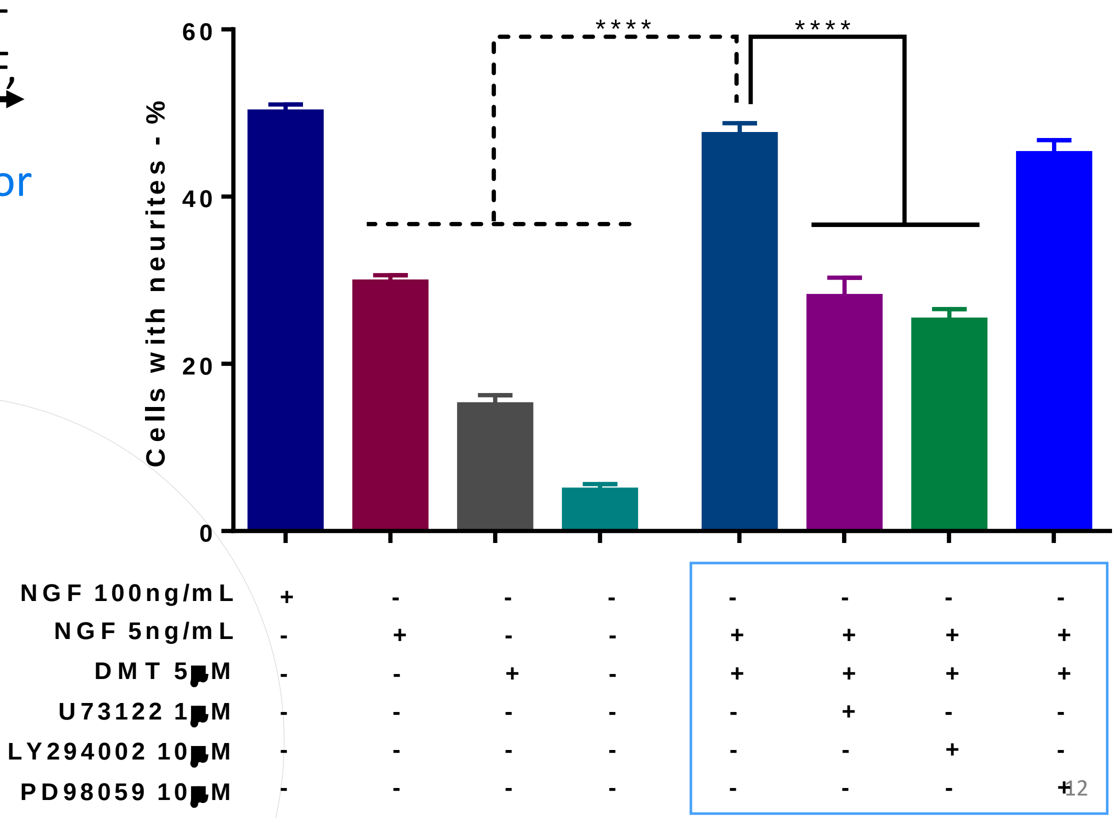
+ inhibitor



DMT + ↓ NGF, 72h

+ inhibitor





DMT IN PC12 CELL LINE

Effect of DMT on neurite outgrowth

- Towards an understanding of its molecular mechanisms

Pharmacological inhibitors

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- Sigma-1R

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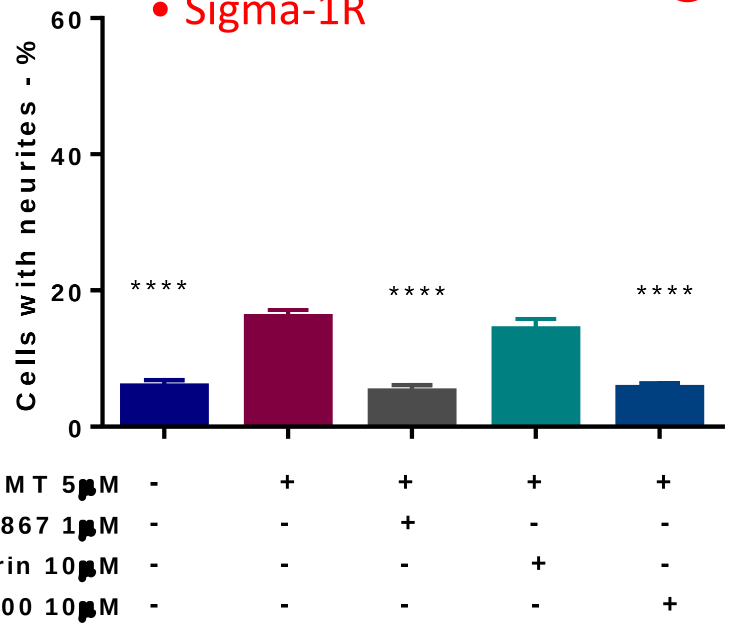
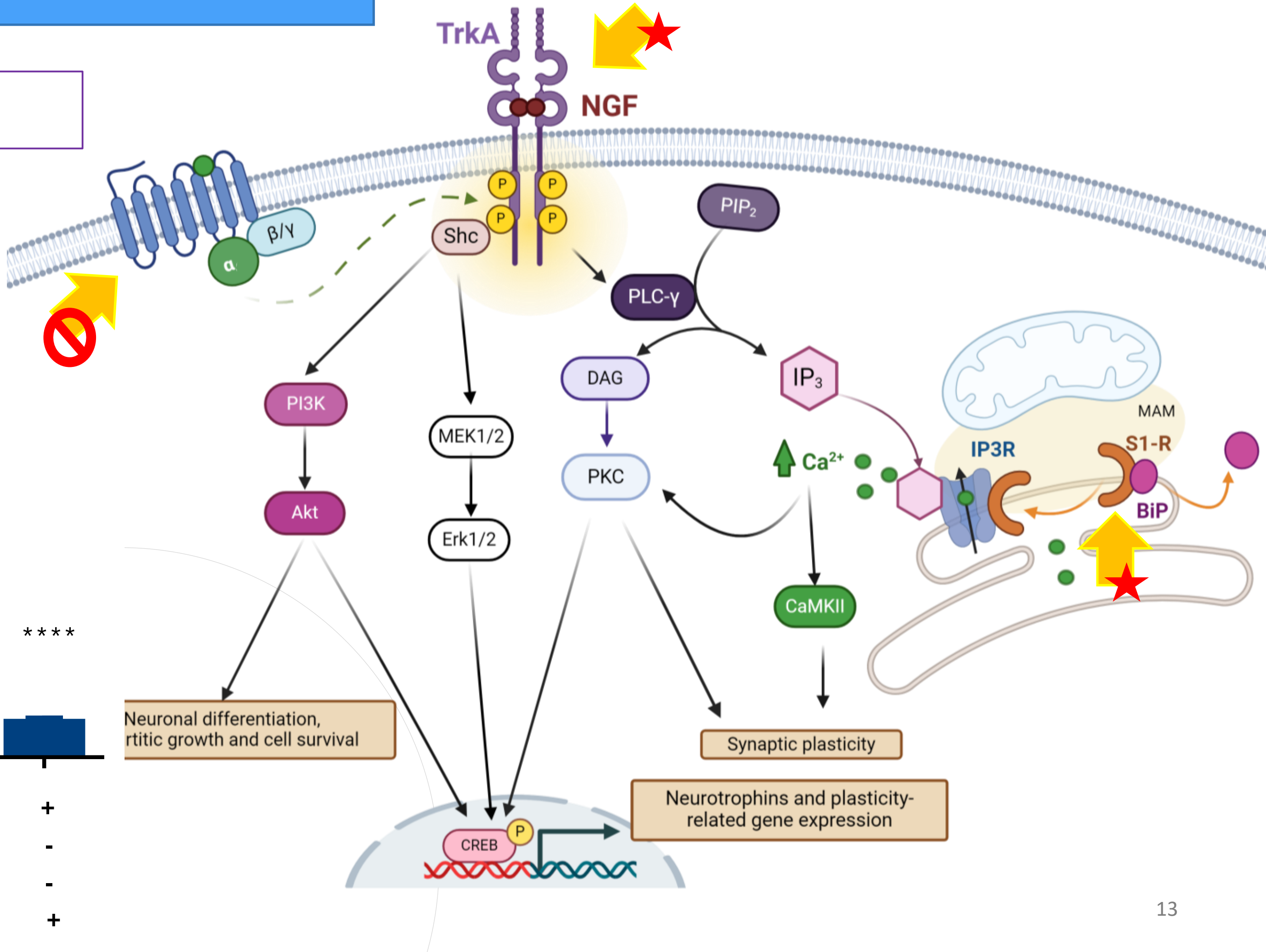
DMT, 72h

+ inhibitor





DMT IN PC12 CELL LINE

Effect of DMT on neurite outgrowth

- Towards an understanding of its molecular mechanisms

Pharmacological inhibitors

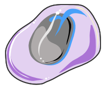
- ✓ **Trks are fundamental for PC12 differenciation**
- ✓ **S1-R is required for DMT-mediated neuritogenesis**
- ✓ **S1-R might play a secondary role when NGF is present**

PC12 differentiation by NGF:

- TrkA receptor and pathways:
  - PI3K/Akt
  - MEK/Erk
  - PLCγ

DMT pharmacology:

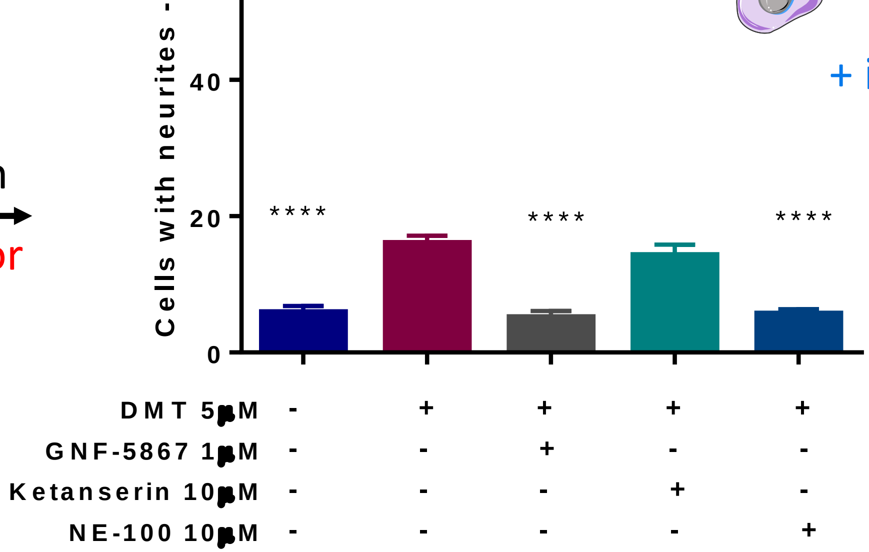
- $5\text{-}HT_{2A}R$
- Sigma-1R

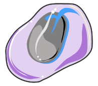


PC12 naive

DMT, 72h

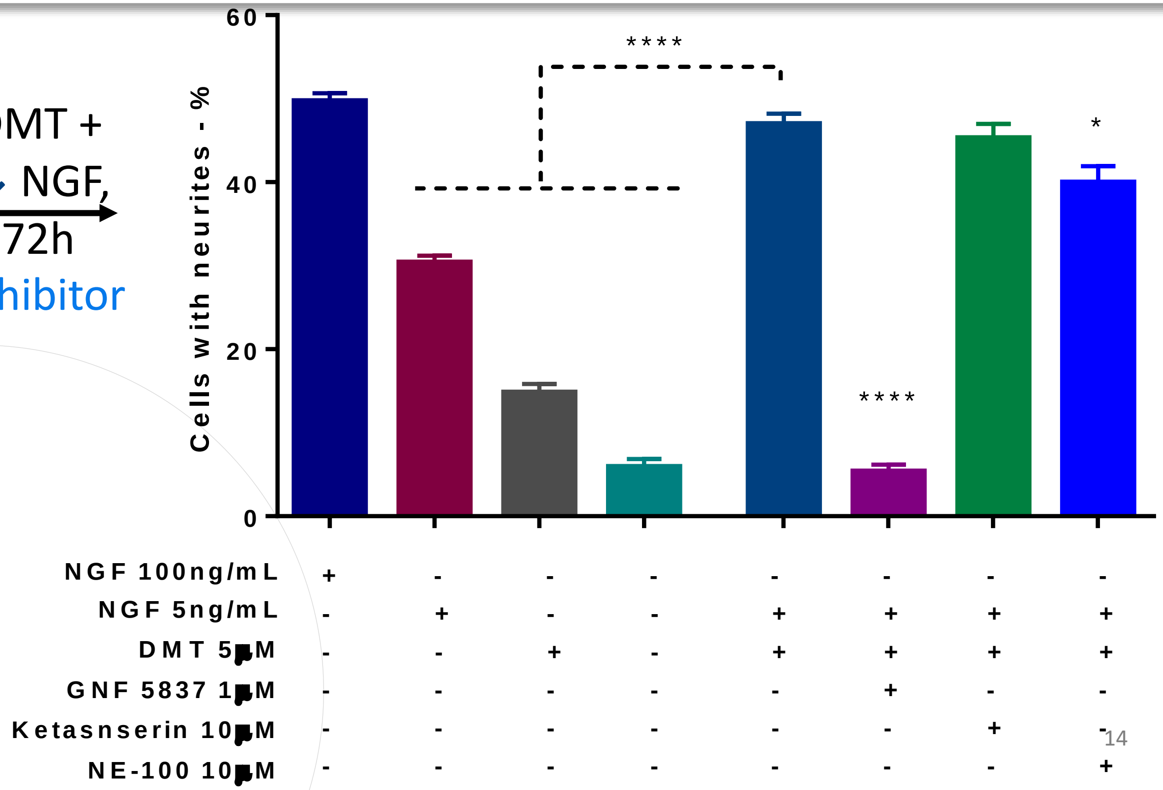
+ inhibitor





DMT + ↓ NGF, 72h

+ inhibitor



DMT IN PC12 CELL LINE

Effect of DMT on neurite outgrowth

- Towards an understanding of its molecular mechanisms

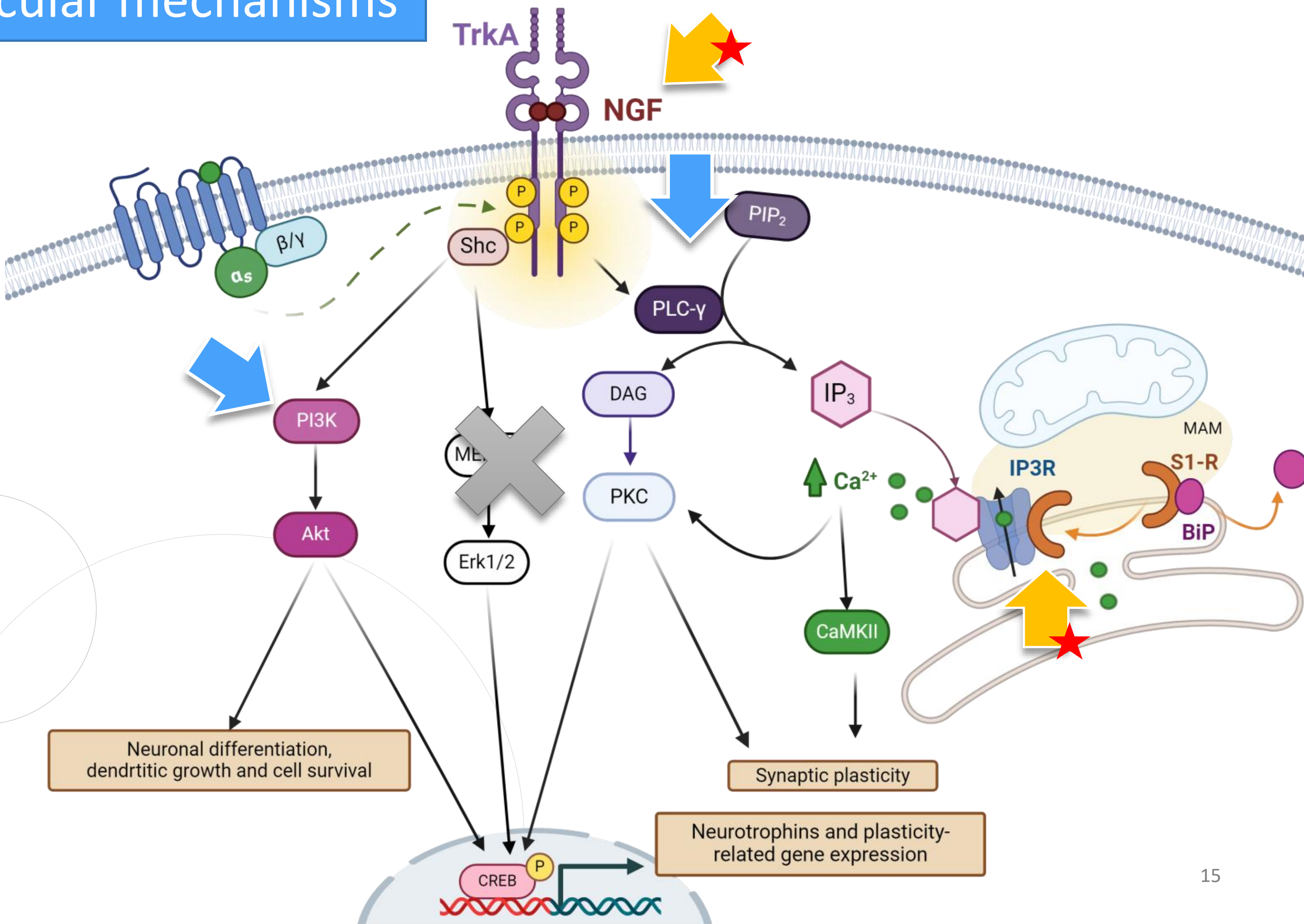
DMT:
- Trk ****
- S1R ****

DMT + NGF:
- Trk ****
- S1R *

Different molecular mechanisms

➢ DMT diectly activates TrkA?

➢ DMT inderectly activates Trk(A)?



DMT IN PC12 CELL LINE

PERSPECTIVES

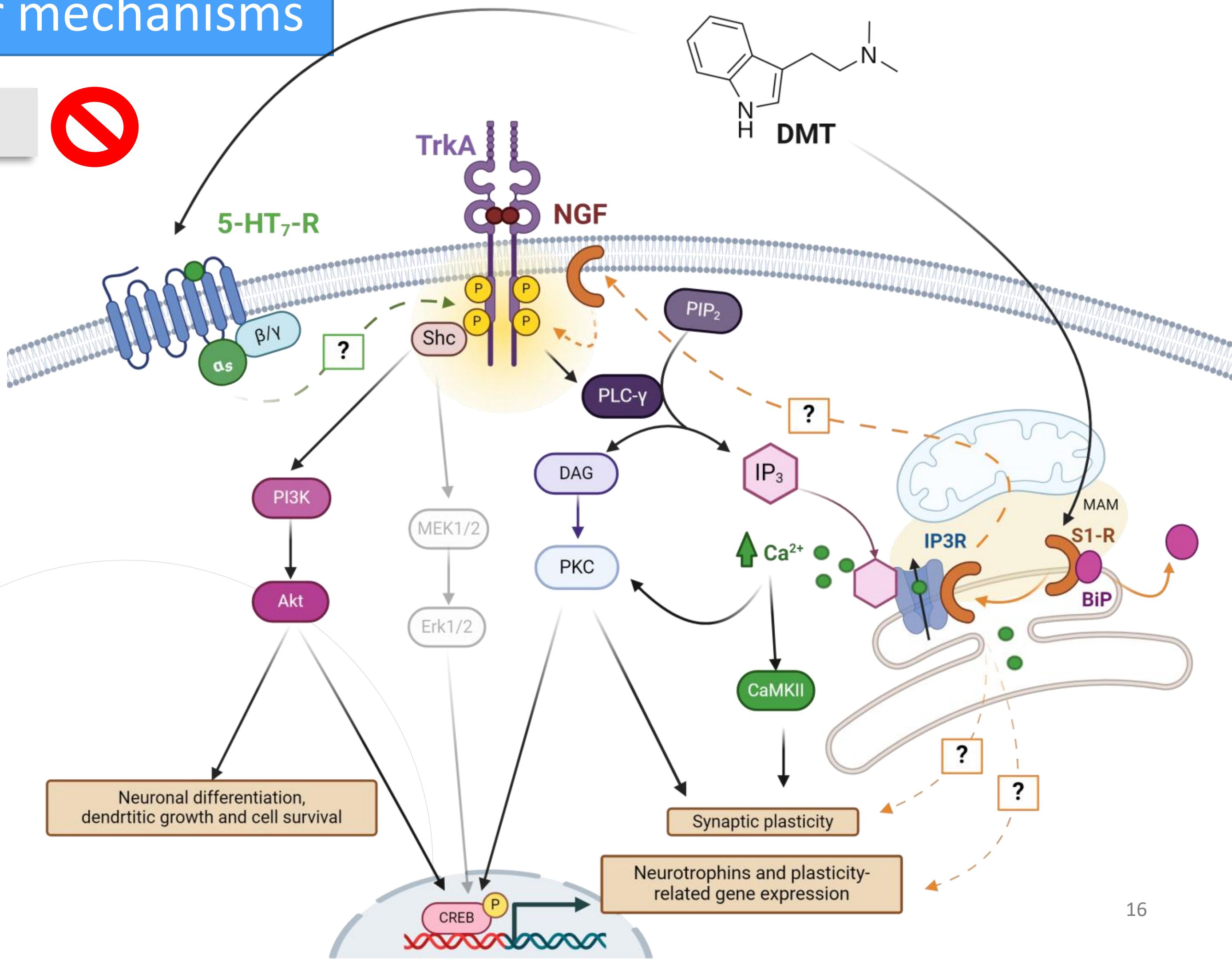
Effect of DMT on neurite outgrowth

- Towards an understanding of its molecular mechanisms

Trk activation

- GPCRs
- S1-R
- Other?

5-HT$_{2A}$ ó 5-HT$_{7}$



DMT IN PC12 CELL LINE

PERSPECTIVES

Effect of DMT on neurite outgrowth

- Towards an understanding of its molecular mechanisms

Trk activation

- GPCRs — 5-HT$_{2A}$ ó 5-HT$_7$
- S1-R
- Other?

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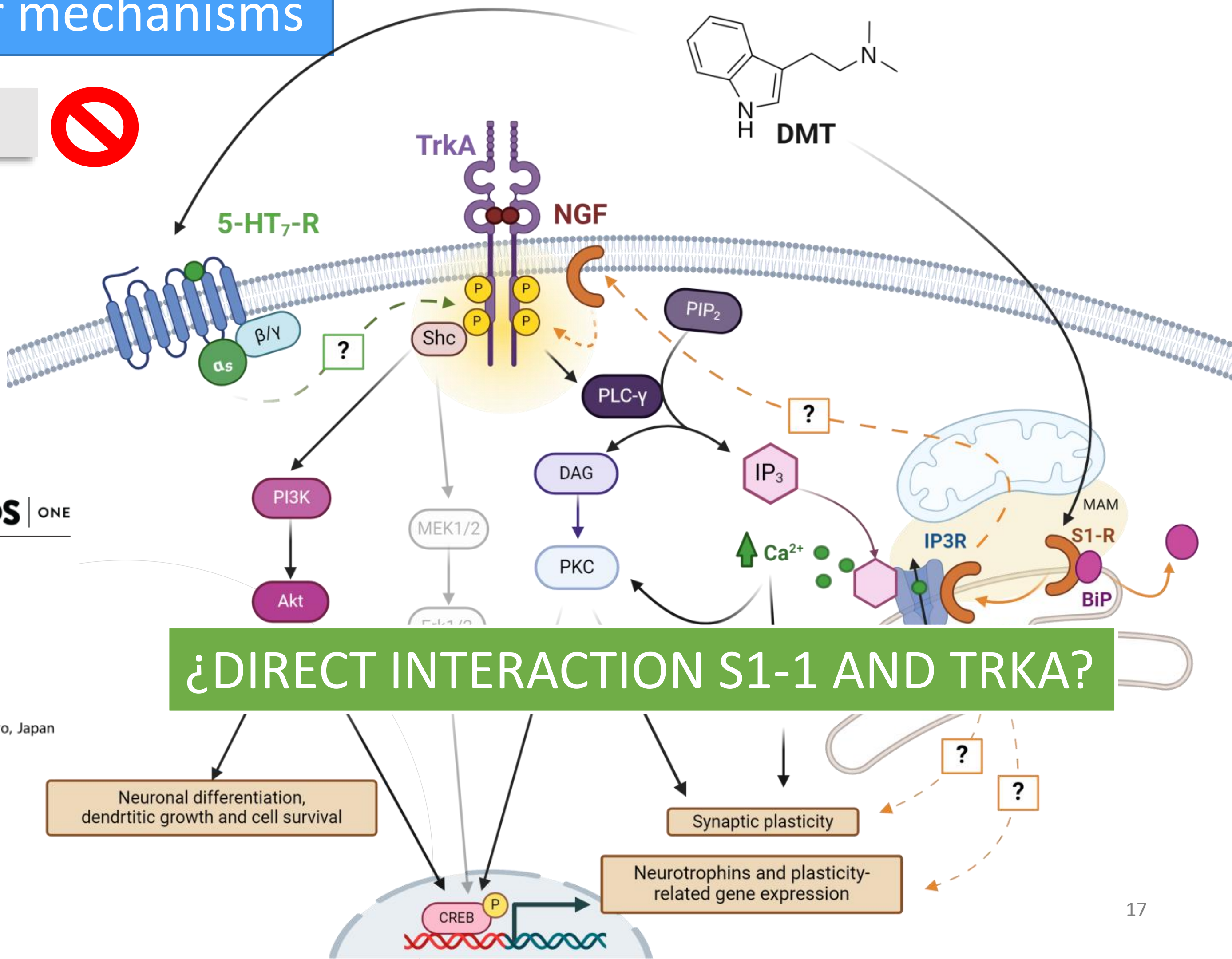
Sigma-1 Receptor Enhances Neurite Elongation of Cerebellar Granule Neurons via TrkB Signaling

Yuriko Kimura[1,2], Yuki Fujita[1,2], Kumi Shibata[1], Megumi Mori[1], Toshihide Yamashita[1,2]*

1 Department of Molecular Neuroscience, Graduate School of Medicine, Osaka University, Suita, Osaka, Japan, 2 JST, CREST, 5, Sanbancho, Chiyoda-ku, Tokyo, Japan

Y. Kimura, Y. Fujita, K. Shibata, M. Mori, T. Yamashita, *PLoS One* **2013**, *8*, 75760.



¿DIRECT INTERACTION S1-1 AND TRKA?

DMT IN PC12 CELL LINE

PERSPECTIVES

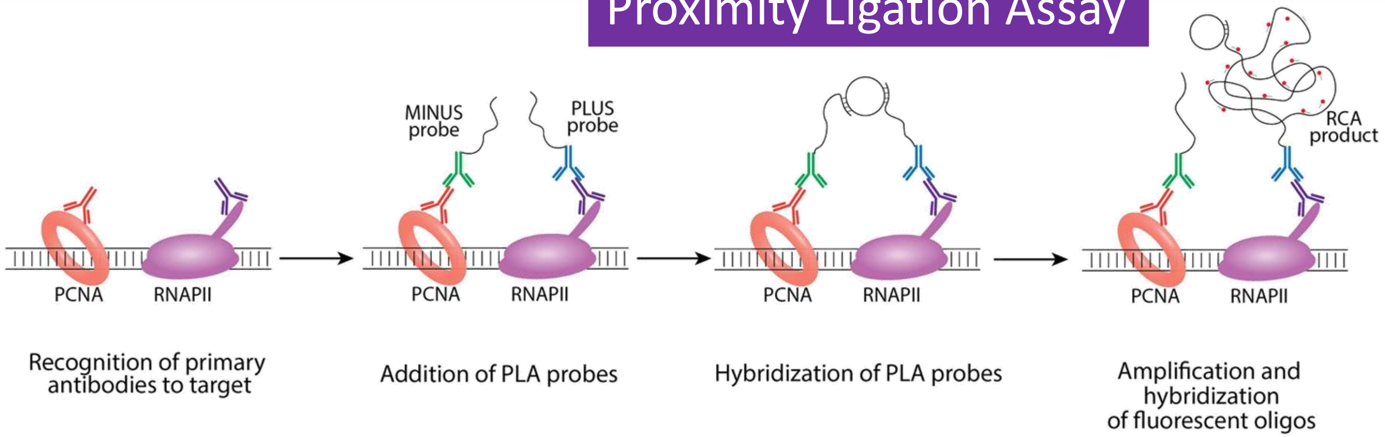
Effect of DMT on neurite outgrowth

- Towards an understanding of its molecular mechanisms

Trk activation

- GPCRs
- S1-R
- Other?

Proximity Ligation Assay



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¿DIRECT INTERACTION S1-1 AND TRKA?

DMT IN PC12 CELL LINE

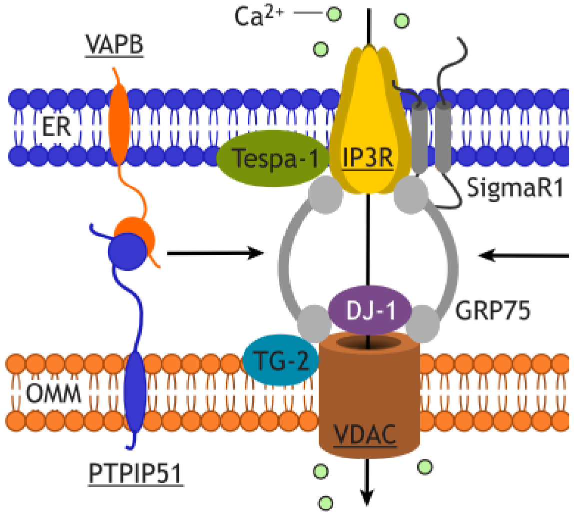
PERSPECTIVES

Effect of DMT on neurite outgrowth

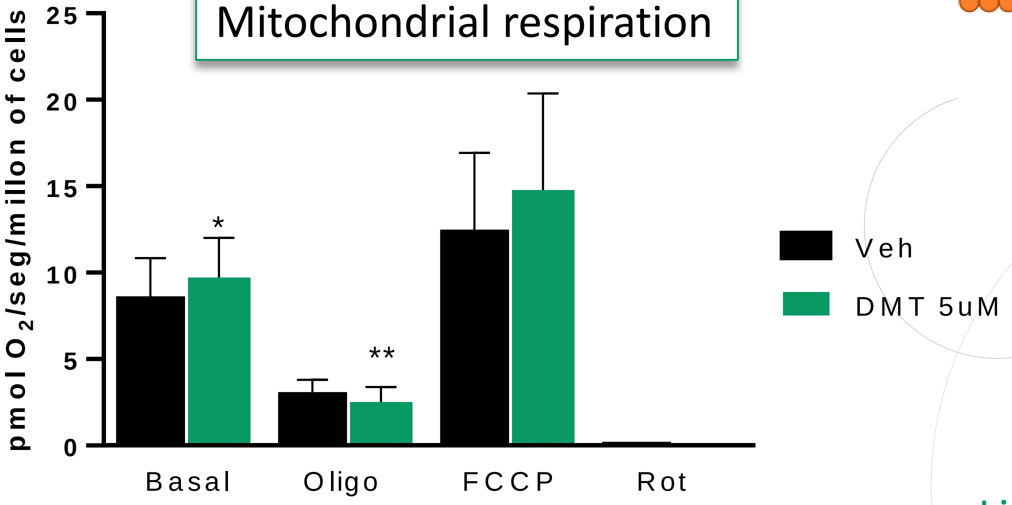
- Towards an understanding of its molecular mechanisms

Trk activation

- GPCRs
- S1-R
- Other?



Mitochondrial respiration



Lic. Camila Narbondo

Differentiation by nerve growth factor (NGF) involves mechanisms of crosstalk between energy homeostasis and mitochondrial remodeling

Francesca Martorana[1,2], Daniela Gaglio[2,3], Maria Rosaria Bianco[4], Federica Aprea[1,2], Assunta Virtuoso[4], Marcella Bonanomi[2], Lilia Alberghina[1,2,5], Michele Papa[2,4] and Anna Maria Colangelo[1,2,5]

F. Martorana, et al., *Cell Death Dis.* **2018**, *9*, DOI 10.1038/s41419-018-0429-9.

DMT IN PC12 CELL LINE

PERSPECTIVES

Effect of DMT on neurite outgrowth

- Towards an understanding of its molecular mechanisms

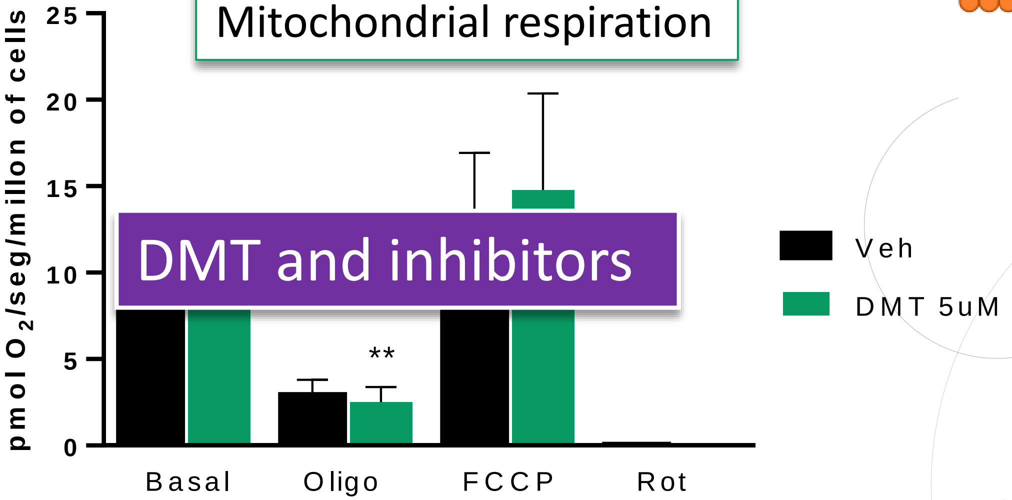
Trk activation

GPCRs

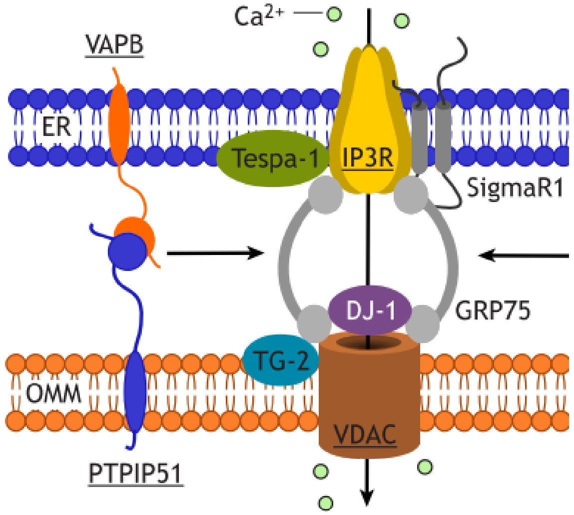
S1-R

Other?

Mitochondrial respiration



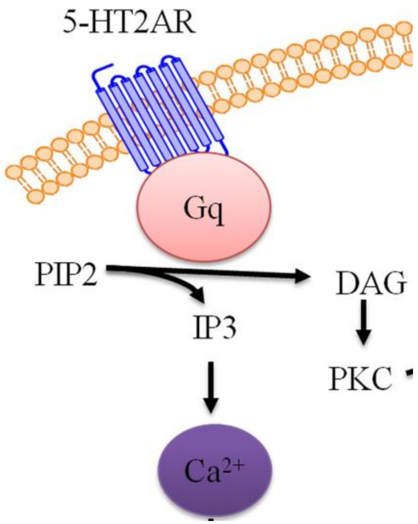
DMT and inhibitors



Lic. Camila Narbondo

$[Ca^{2+}]i$

- Fura-2 in PC12 with DMT and inhibitors



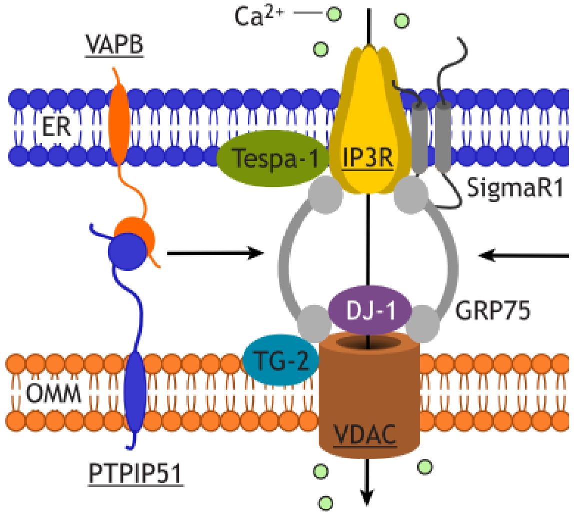
PERSPECTIVES

DMT IN PC12 CELL LINE

Effect of DMT on neurite outgrowth

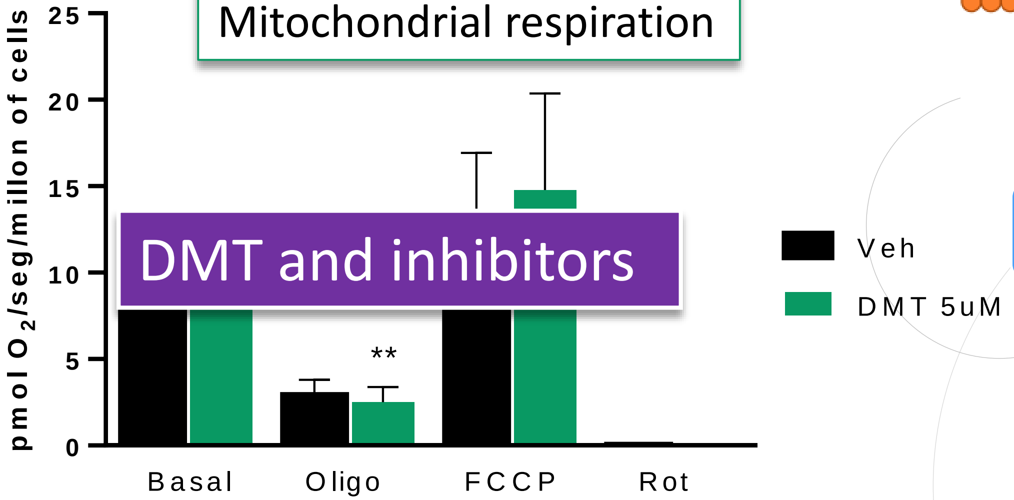
- Towards an understanding of its molecular mechanisms

Trk activation
- GPCRs
- S1-R
- Other?



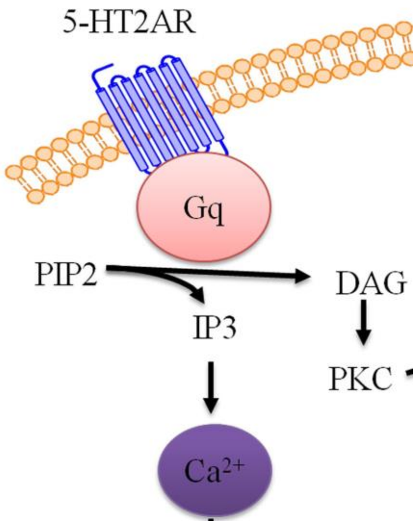
$[Ca^{2+}]i$
- Fura-2 in PC12 with DMT and inhibitors

Mitochondrial respiration



DMT and inhibitors

GAP-43 – immunocytochemitry, WB.
- Increases expresion upon NGF stimulation
- **Ca^{2+} modulated**
- Modulates neurite outgrowth in PC12
- Modulates synaptic plasticity



THANK YOU

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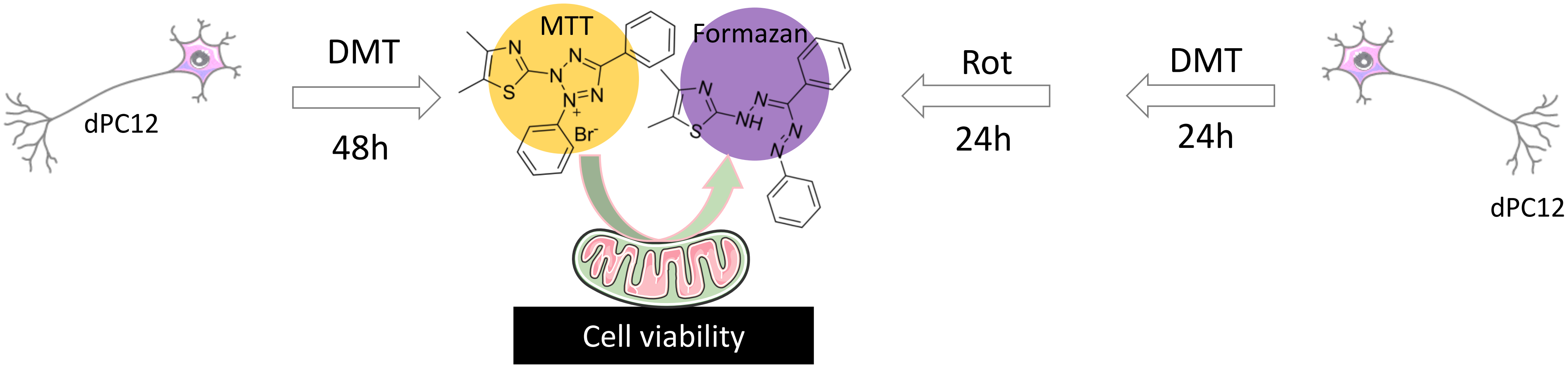
https://arche.ei.udelar.edu.uy/

DMT IN PC12 CELL LINE

Effect of DMT on cell viability and protection

Cell viability

Neuroprotection

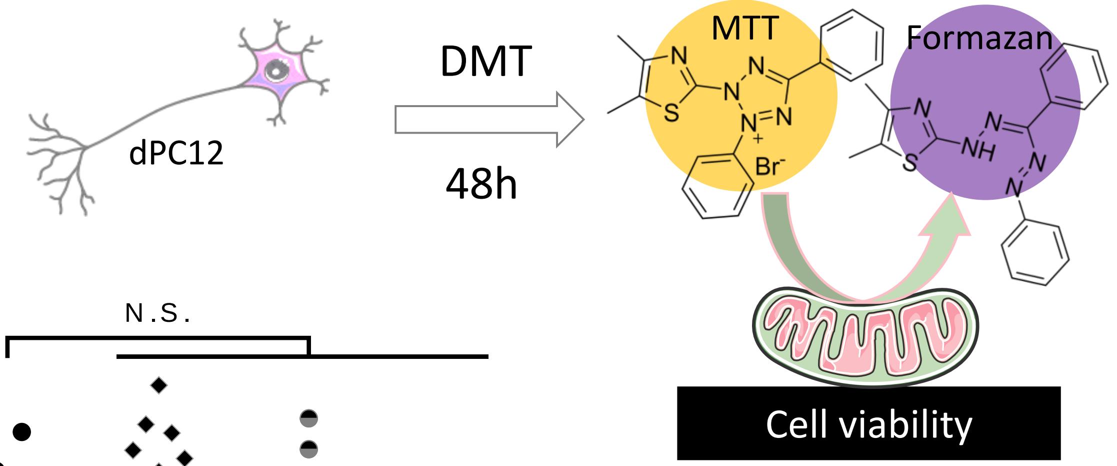


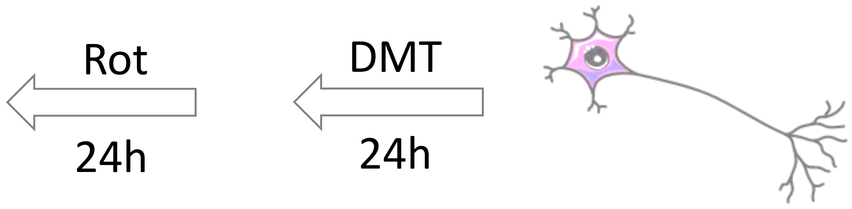
DMT IN PC12 CELL LINE

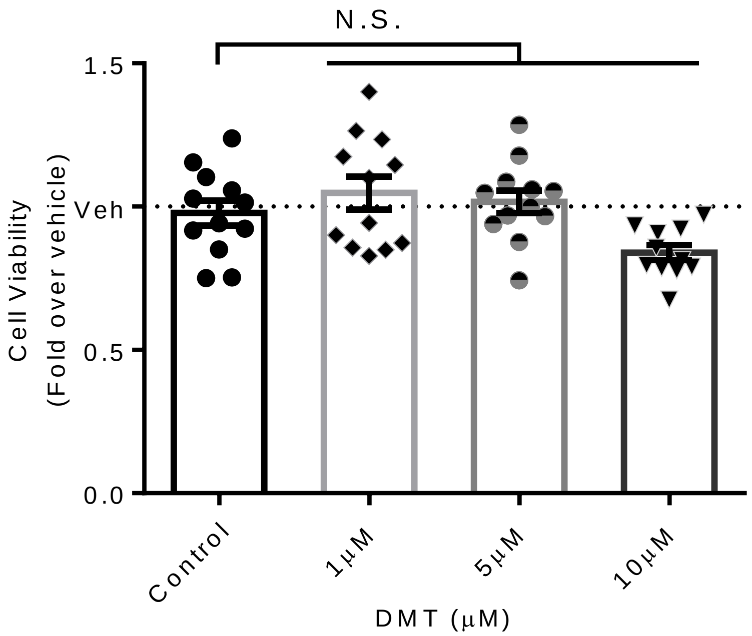
Effect of DMT on cell viability and protection

Cell viability

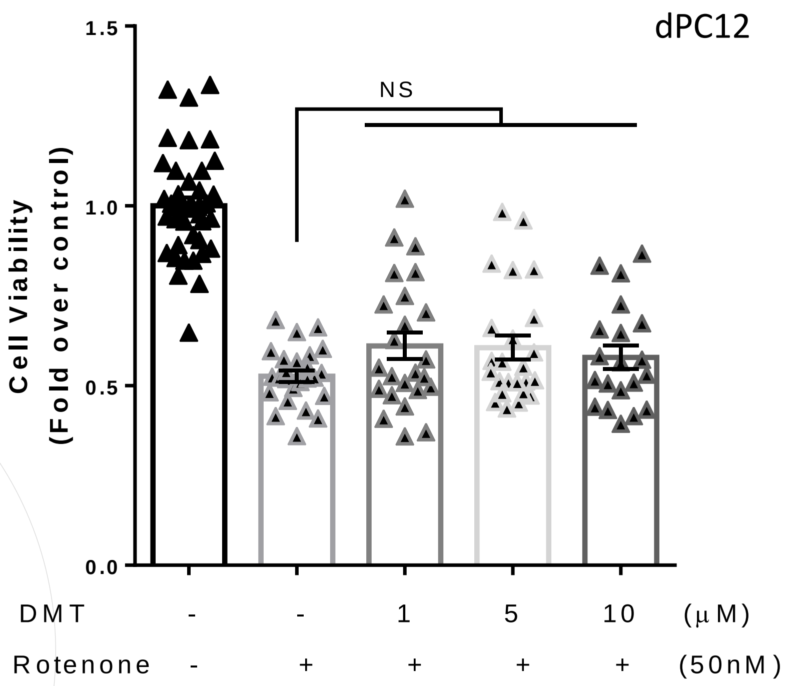
Neuroprotection





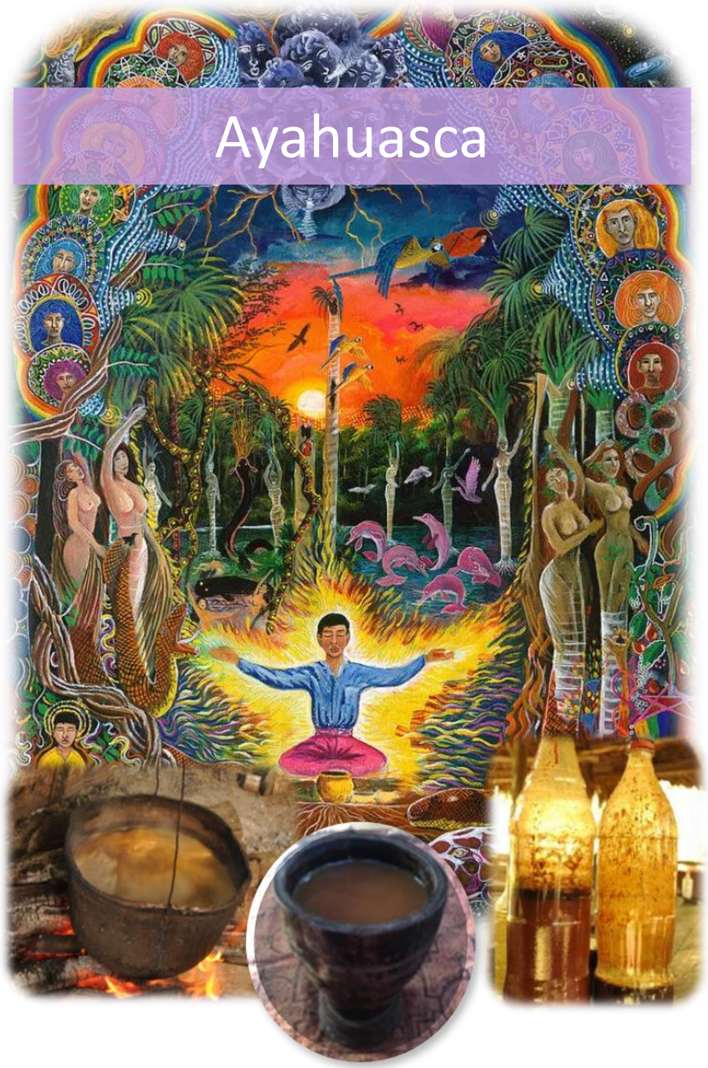


Lic. Francisca Baroffio



INTRODUCTION

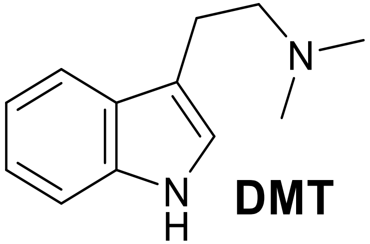
Ayahuasca, DMT and their therapeutic potential

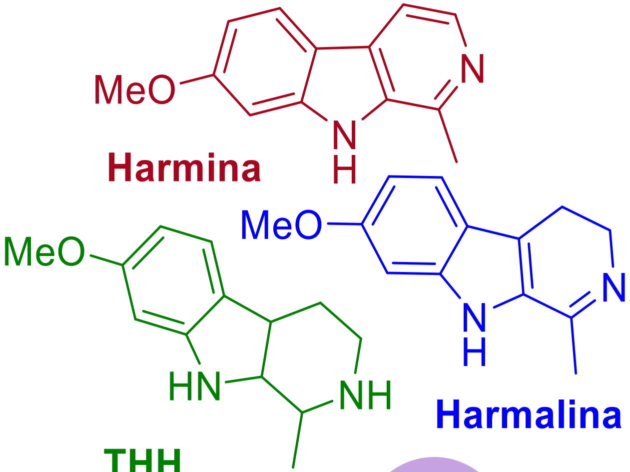


Therapeutic potential

Ayahuasca or β-carbolines:

- Depression
- Anxiety
- Substance use disorder
- PTSD
- Parkinson's disease
- Alzheimer's disease





Color code:

Pre-clinical | Observational | Clinical trials

L. O. Maia, D. Daldegan-Bueno, I. Wießner, D. B. Araujo, L. F. Tófoli, *Eur. Neuropsychopharmacol.* **2023**, *66*, 45–61.

INTRODUCTION

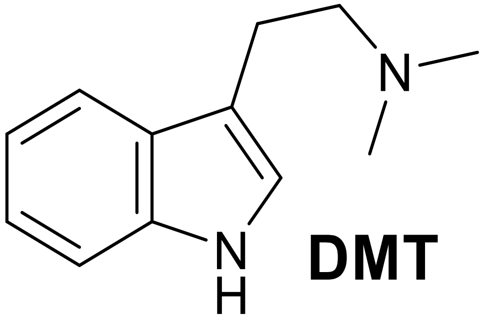
Main mediators of DMT biological activities

DMT modulates innate and adaptive inflammatory responses *in vitro* (moDC):
- ↓ pro-inflammatory cytokines IL-1b, IL-6, TNFa and the chemokine IL-8
- ↑ anti-inflammatory cytokine IL-10
- ↓ differentiation of T-cells to Th1 and Th17 inflammatory effectors

A. Szabo, et al., *PLoS One* **2014**, *9*, 1–12.

Increases cell survival against hypoxia (0.5% O_2) *in vitro* (cultured human cortical neurons derived from iPSCs, monocyte-derived macrophages, moMACs, and dendritic cells, moDCs.

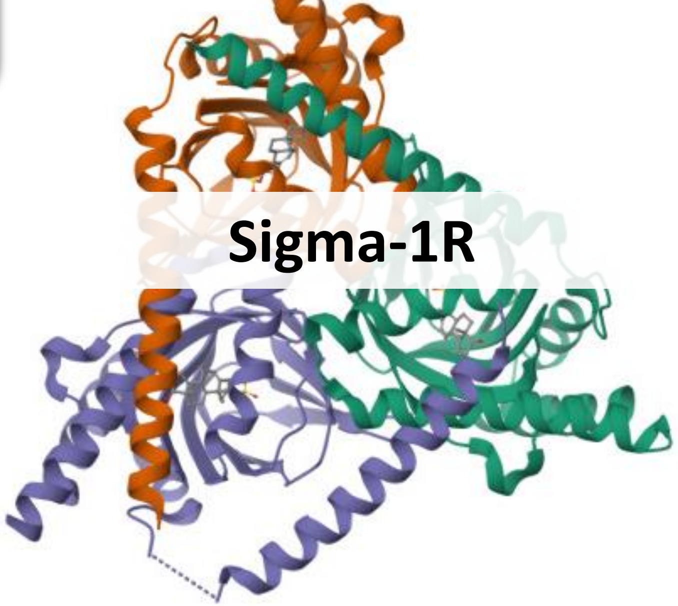
A. Szabo, et al., *Front. Neurosci.* **2016**, *10*, 1–11.



DMT

Anti-ischemic properties in rat brain:
↓ infarct size, ↑ recovery, ↓ neurodegeneration

N. Ye, et al., *J. Med. Chem.* **2020**, *63*, 15187–15217;
Í. Szabó, et al., *Neuropharmacology* **2021**, *192*, 108612.



INTRODUCTION

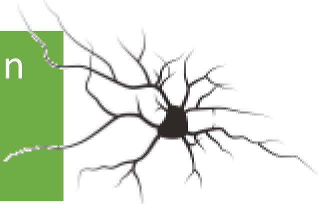
DMT, neuroprotection and neuroplasticity

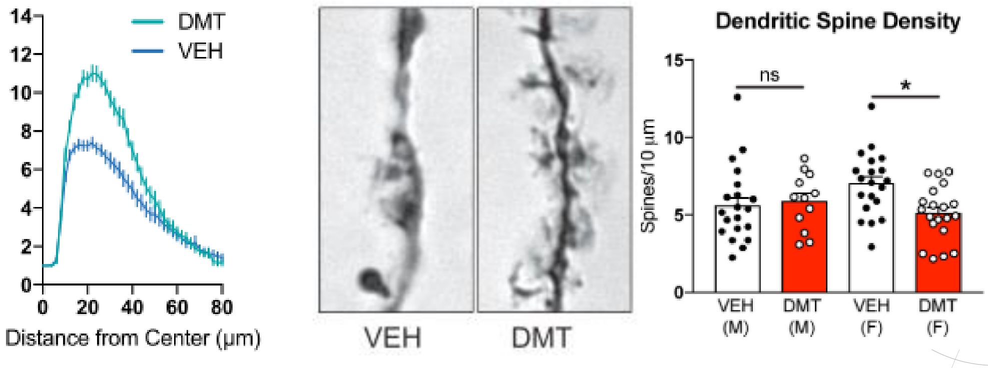
Psychedelics Promote Structural and Functional Neural Plasticity

Calvin Ly,[1] Alexandra C. Greb,[1] Lindsay P. Cameron,[2] Jonathan M. Wong,[2] Eden V. Barragan,[2] Paige C. Wilson,[3] Kyle F. Burbach,[4] Sina Soltanzadeh Zarandi,[1] Alexander Sood,[5] Michael R. Paddy,[3] Whitney C. Duim,[1] Megan Y. Dennis,[4,6,7] A. Kimberley McAllister,[5,8,9] Kassandra M. Ori-McKenney,[3] John A. Gray,[5,8] and David E. Olson[1,5,6,10,*]

C. Ly *et al.*, *Cell Rep.*, vol. 23, no. 11, pp. 3170–3182, **2018**.

- Neuritogenesis and spinogenesis in rat cortical cultures
- Spinogenesis in rat rat's PFC





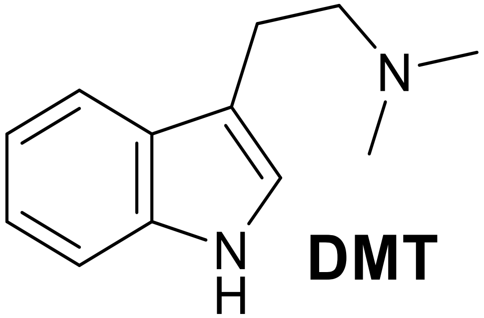
Psychoplastogens: A Promising Class of Plasticity-Promoting Neurotherapeutics

David E Olson[1,2,3]

D. E. Olson, *J. Exp. Neurosci.* vol 12, pp. 1-4, **2018**,

Marina Sanz-SanCristobal[1,2], Jordi Riba[6] and Ana Perez-Castillo[1,2,4]

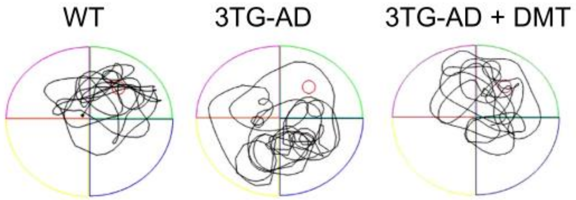
Transl. Psychiatry **2020**, *10*, 331.



DMT

Chronic, Intermittent Microdoses of the Psychedelic *N*,*N*-Dimethyltryptamine (DMT) Produce Positive Effects on Mood and Anxiety in Rodents

Lindsay P. Cameron,[†] Charlie J. Benson,[‡] Brian C. DeFelice,[§] Oliver Fiehn,[§,∥] and David E. Olson[*,‡,⊥,#]



Cheng *et al. Alzheimer's Research & Therapy* (2024) 16:95
https://doi.org/10.1186/s13195-024-01462-3
Alzheimer's Research & Therapy
RESEARCH Open Access

N, N-Dimethyltryptamine, a natural hallucinogen, ameliorates Alzheimer's disease by restoring neuronal Sigma-1 receptor-mediated endoplasmic reticulum-mitochondria crosstalk

Dan Cheng[1,2], Zhuo-Gui Lei[3], Kin Chu[4], Oi Jin Honey Lam[5], Chun Yuan Chiang[6] and Zhang-Jin Zhang[1,2*]