The Ayahuasca's psychedelic component DMT potentiates neuritogenesis in PC12 cells

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INTRODUCTION

Ayahuasca is a **psychedelic beverage** originally from the Amazon rainforest used for a variety of **medicinal**, **spiritual**, and **cultural** purposes. It is prepared by decoction of the vine *Banisteriopsis caapi*, source of β-carboline alkaloids, and *Psychotria viridis* containing the classic psychedelic *N,N*-dimethyltryptamine (**DMT**). (Figure 1)

In the context of the ***"renaissance of psychedelics"*** several recent reports highlight its potential **therapeutic** applications for the treatment of depression and substance use disorders.[1] The **DMT** present in Ayahuasca is a potent agonist of the serotoninergic receptor **5-HT$_{2A}$** and interacts with other serotonin receptors as well as with the **sigma-1** receptor. DMT has been recently categorized as a **"psychoplastogen"**, able to promote structural and functional neuroplastic changes in **cortical** cell cultures.[2,3] This suggests that the therapeutic potential of DMT might include **pathologies** where neuroplasticity of other neuronal populations is compromised, as in several **neurodegenerative diseases.**

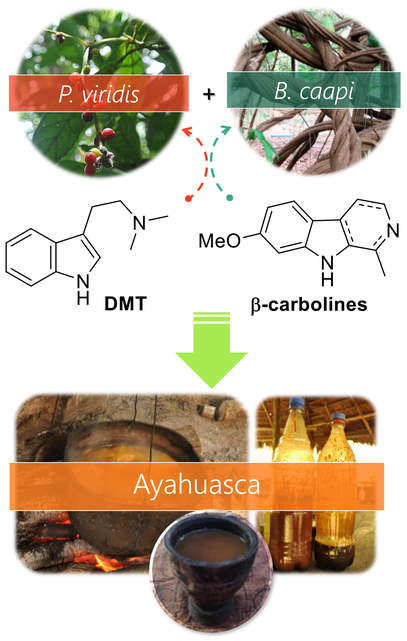

Figure 1. Ayahausca and its main components, DMT and β-carbolines.

AIMS AND STRATEGY

AIMS: The main **objective** of this work was to study **DMT's ability** to promote **neuritogenesis** in the catecholaminergic **cell line PC12**. Additionally, we aimed to determine the **implication** in this effect **of** the intracellular pathways **Erk**, **Akt** and **PLCγ**, as well as of the serotonin receptor **5-HT$_{2A}$**.

STRATEGY: When cultured in the presence of nerve growth factor (NGF), PC12 cells differentiate and acquire neuronal characteristics both morphologically and functionally.[4] We used **PC12 cells as a model** to characterize DMT's ability to promote **neuritogénesis** by itself and under **NGF deficient conditions**, as a potential treatment for **neurodegeneration** of **catecholaminergic** systems. Percentage of cells that developed neurites was determined by manually counting treated PC12 previously dyed with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and fixed. Using **pharmacological inhibitors** we characterize the **molecular mechanisms** involved. (Figure 2)

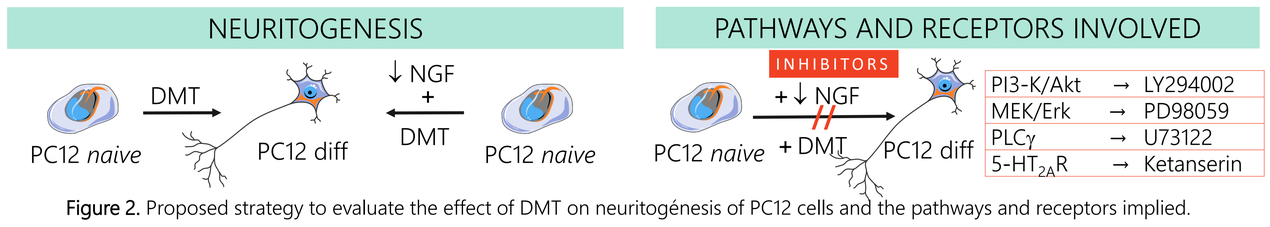

Figure 2. Proposed strategy to evaluate the effect of DMT on neuritogénesis of PC12 cells and the pathways and receptors implied.

RESULTS AND DISCUSSION

1. DMT promotes neuritogenesis in PC12 cells

Firstly, we evaluated DMT's **effect on cell viability** to identify toxic concentrations. Undifferentiated PC12 cells (naïve PC12 – PC12n) were treated with a range of concentrations of DMT for 72h. Cell viability was evaluated by the MTT assay. DMT at 10μM showed a mild yet significant effect on cell viability. (Figure 3A).

Analogue conditions were used to evaluate **DMT's ability to promote neurite outgrowth**. We determined the percentage of PC12n that developed neurites and found that DMT promotes neuritogénesis at **1, 5 and 10 μM** concentrations. (Figure 3B)

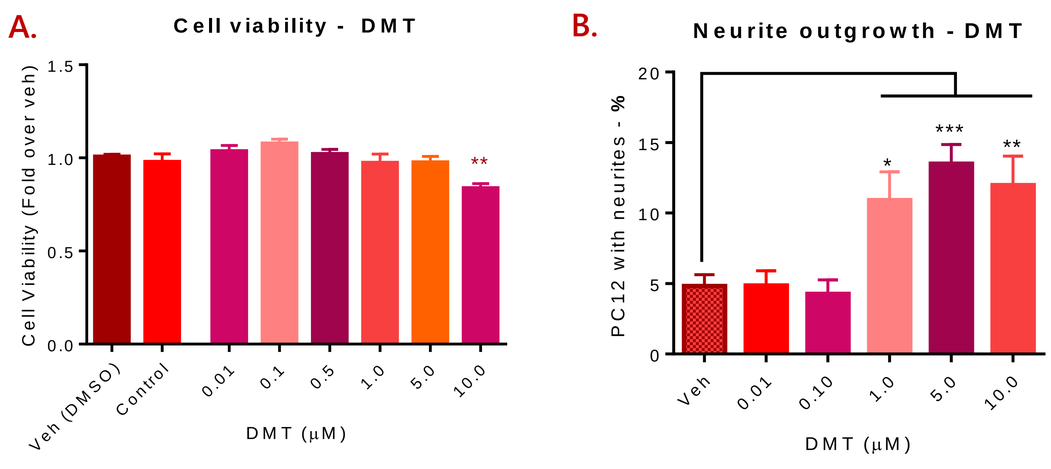

Figure 3. A. Cell viability of PC12n cell treated with DMT at different concentrations. **B.** Percentage of PC12n cells that grow neurites after DMT treatment. Data is presented as mean ± SEM of N=5; One-way ANOVA followed by Dunnett's multiple comparisons test is shown (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

2. DMT enhances NGF-mediated neuritogénesis in PC12 cells

To assess DMT's neuritogenic effect under NGF-deficient conditions, we started by **determining optimal NGF concentration** so that its effect on neuritogenesis was **lower than** positive control (**NGF 100ng/mL**) and **higher** than the **vehicle** treatment (culture medium). PC12n were treated for 72h with a dose curve of NGF and percentage of cells with neurites was determined. **NGF 5 ng/mL** concentration (30%±5.6% of PC12 with neurites) **was selected**. (Figure 4A)

Then, PC12n cells were treated with **a dose curve of DMT** in presence of **NGF 5 ng/mL** for 72h. Results showed an **enhancement** of NGF effect on **neuritogenesis** of PC12 cells at **1, 5 and 10 μM** concentrations of **DMT**. (Figure 4B)

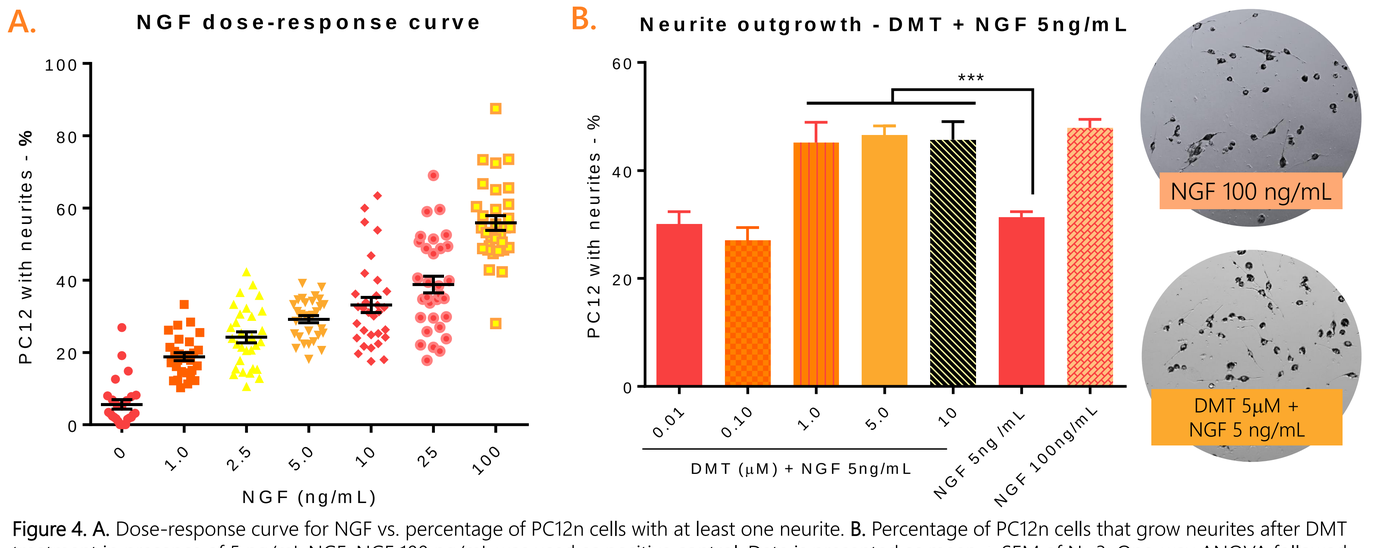

Figure 4. A. Dose-response curve for NGF vs. percentage of PC12n cells with at least one neurite. **B.** Percentage of PC12n cells that grow neurites after DMT treatment in presence of 5 ng/mL NGF; NGF 100 ng/mL was used as positive control. Data is presented as mean ± SEM of N=3; One-way ANOVA followed by Dunnett's multiple comparisons test is shown (*** $p < 0.001$).

3. PLCγ and Akt are involved in PC12 neuritogénesis by DMT + NGF

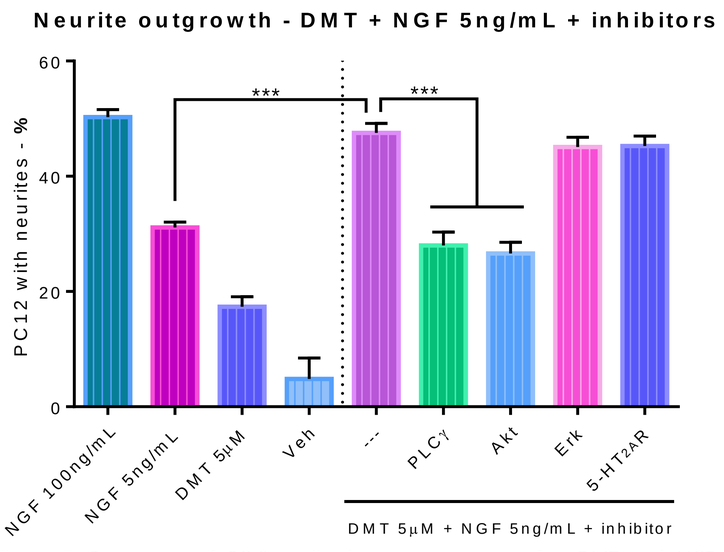

Figure 5. Percentage of PC12n cells that grow neurites after DMT and NGF treatment, in presence and absence of pharmacological inhibitors. Data is presented as mean ± SEM of N=4; One-way ANOVA followed by Dunnett's multiple comparisons test is shown (*** $p < 0.001$).

To evaluate the implication of **PLCγ**, **Erk** and **Akt** pathways and receptor **5-HT$_{2A}$** on the discovered neuritogenic effect, PC12n were treated with **DMT 5μM + NGF 5ng/mL** in presence and absence of the specific **pharmacological inhibitors** listed on Figure 2. NGF 100 ng/mL was used as positive control and vehicle (DMSO) as negative control.

Our results indicate that both **PLCγ** and **Akt** pathways **mediate** the neuritogenic effect of DMT in presence of low NGF concentrations. The corresponding inhibitors seem to **restore** the effect of **NGF 5 ng/mL** alone. **Erk** and **5-HT$_{2A}$ inhibitors** on the other hand, did **not affect** neuritogenesis in our experimental conditions. (Figure 5)

CONCLUSIONS

- ✓ The psychedelic component of Ayahuasca, **DMT**, **doesn't affect** PC12 cells **viability** below 10 μM in 72h treatments.
- ✓ **DMT** promotes **neurite outgrowth** in **PC12n** cells at 1, 5 and 10 μM concentrations in 72h treatments.
- ✓ **DMT** potentiates **neuritogenesis** in PC12 cells under **NGF-deficient** conditions.
- ✓ **Akt** and **PLCγ** pathways activation is **needed** for DMT's neuritogenic effect under NGF-deficient conditions.
- ✓ **Erk** pathway and serotonin receptor **5-HT$_{2A}$** are **not involved** in DMT-mediated neuritogénesis under low NGF concentration.
- ✓ We are now **working** on determining the implication of **receptors sigma-1** and **TrkA in** this effect.

REFERENCES

1. Inserra, A. *et al.*, Psychedelics in Psychiatry: Neuroplastic, Immunomodulatory, and Neurotransmitter Mechanisms. *Pharmacological Reviews*, *73*(1), 2021, 202–277.
2. Rodríguez, L.*et al.*, New Insights into the Chemical Composition of Ayahuasca. *ACS Omega*, *7*, 2022, 12307–12317.
3. Ly, C. *et al.*, Psychedelics Promote Structural and Functional Neural Plasticity. *Cell Reports*, *23*(11), 2018, 3170–3182.
4. K. K. Teng *et al.*, Cultured PC12 cells: A model for neuronal function, differentiation, and survival. *Cell Biology, Four-Volume Set*, vol. 1, 2006, 171–176.

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