The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

Evaluating the potential of methylphenidate and amphetamine acute exposure to promote neurite outgrowth and synaptogenesis in differentiated SH-SY5Y neuronal cells

Chaired by DR. ALFREDO BERZAL-HERRANZ;
Co-Chaired by PROF. DR. MARIA EMÍLIA SOUSA

pharmaceuticals MDPI

Mariana Ferreira[1,2*], Vera Marisa Costa[1,2], Félix Dias Carvalho[1,2] and João Paulo Capela[1,2,3*]

[1] Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[2] UCIBIO - Applied Molecular Biosciences Unit, Laboratory of Toxicology, Department of Biological Sciences, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[3] FP-I3ID, Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Porto, Portugal.

* Corresponding authors: marianalferreira98@gmail.com; joaoc@ufp.edu.pt

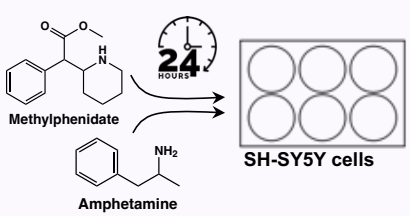
U.PORTO FACULDADE DE FARMÁCIA UNIVERSIDADE DO PORTO
UCIBIO @ REQUIMTE

Evaluating the potential of methylphenidate and amphetamine acute exposure to promote neurite outgrowth and synaptogenesis in differentiated SH-SY5Y neuronal cells

Stroke and traumatic brain injury are neurological diseases without an approved treatment for neural repair.

What if?

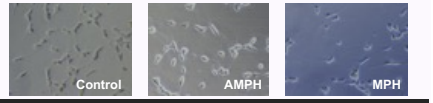
Methylphenidate (MPH) and Amphetamine (AMPH) increase monoamine levels on the synaptic cleft.



01 No cytotoxicity at low concentrations (0.001, 0.01, 0.1, 1 and 10 µM).

02 MPH and AMPH evoked no alterations of synaptophysin, GAP43 and PSD95 levels at low concentrations (0.01 and 0.1 µM).

03 MPH and AMPH promoted no neurite outgrowth at low concentrations (0.01 and 0.1 µM).



Conclusion

Twenty-four hours exposure to MPH and AMPH did not evoke neurite outgrowth in SH-SY5Y cells.

Mariana Ferreira* (marianalferreira98@gmail.com), Vera Marisa Costa, Félix Dias Carvalho, João Paulo Capela* (joaoc@ufp.pt).

Acknowledgments: This work was supported by national funds through from Fundação para a Ciência e a Tecnologia (FCT), I.P., in the scope of the project "EXPL/MED-FAR/0203/2021".

Abstract: **Methylphenidate** (MPH) and **amphetamine** (AMPH) **increase monoamine levels** in **the synaptic cleft**, due to their properties and similarities to monoamine neurotransmitters. **Stroke** and **traumatic brain injury**, common neurological diseases, affect millions of people every year. Their treatment mainly focuses on the focal point and symptoms, **lacking on the curative measures and neural repair**. In *in vitro* and *in vivo* models, **MPH** and **AMPH** showed to **promote neuronal recovery following injury through neurite outgrowth**.

Thus, this study evaluated the **neurite outgrowth** and **synaptogenesis** promoted by **clinical relevant concentrations** of **MPH** and **AMPH** in a neuronal human model, differentiated **SH-SY5Y**. The cells were exposed to **0.001**, **0.01**, **0.1**, **1** and **10µM** of drugs for **24h**. Our results revealed that after 24h, **MPH** and **AMPH** were **not cytotoxic** to differentiated SH-SY5Y, by either the MTT reduction or the NR uptake assays. Also, the concentrations of **0.1** and **0.01µM** did **not affect** the expression of **synaptophysin**, **PSD95** and **GAP43** evaluated by Western blotting. Moreover, neurite outgrowth was evaluated in microphotographs resourcing to the NeuronJ software and **no enhancement of neurite outgrowth** in differentiated SH-SY5Y cells was promoted by **MPH** or **AMPH** at the concentrations of **0.1** and **0.01µM**.

As far as we know, this is the first study evaluating the effect of **clinical relevant concentrations** of **MPH** and **AMPH** in a paradigm of **acute exposure** to neuronal **SH-SY5Y cells**, being **the starting point to our strategy to understand the possible effects of MPH and AMPH on the improvement of neural network**.

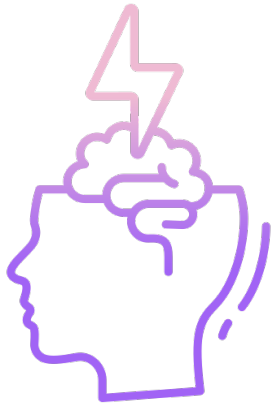
Keywords: Amphetamine; Methylphenidate; Stroke; Traumatic Brain Injury; Neural Repair.

Stroke

12 200 000/year [1]

Traumatic Brain Injury

69 000 000/year [2]



[1]World Stroke Organization (WSO). Global Stroke Fact Sheet 2022. Assessed at 6/10/1011. Available at https://www.world-stroke.org/assets/downloads/WSO_Global_Stroke_Fact_Sheet.pdf

[2]Dewan MC, Rattani A, Gupta S, Baticulon RE, Hung YC, Punchak M, Agrawal A, Adeleye AO, Shrime MG, Rubiano AM, Rosenfeld JV, Park KB. Estimating the global incidence of traumatic brain injury. J Neurosurg. 2018 Apr 1:1-18. doi: 10.3171/2017.10.JNS17352.

Stroke

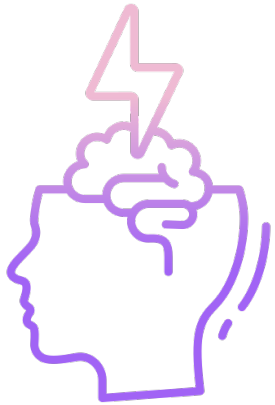
12 200 000/year [1]

- **Cut-off** of the **blood supply** to a **part of the brain**.

Traumatic Brain Injury

69 000 000/year [2]

- **Alteration in brain function**, or other **evidence of brain pathology**, caused by an **external force**.



[1]World Stroke Organization (WSO). Global Stroke Fact Sheet 2022. Assessed at 6/10/1011. Available at https://www.world-stroke.org/assets/downloads/WSO_Global_Stroke_Fact_Sheet.pdf

[2]Dewan MC, Rattani A, Gupta S, Baticulon RE, Hung YC, Punchak M, Agrawal A, Adeleye AO, Shrime MG, Rubiano AM, Rosenfeld JV, Park KB. Estimating the global incidence of traumatic brain injury. J Neurosurg. 2018 Apr 1:1-18. doi: 10.3171/2017.10.JNS17352.

Stroke

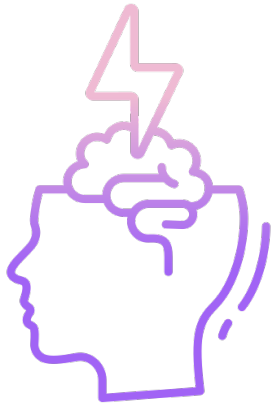
12 200 000/year [1]

- **Cut-off** of the **blood supply** to a **part of the brain**.

Traumatic Brain Injury

69 000 000/year [2]

- **Alteration in brain function**, or other **evidence of brain pathology**, caused by an **external force**.



- Both are in an **urgent need of** a **consistent** and **effective treatment** for the **neural repair after injury**.

[1]World Stroke Organization (WSO). Global Stroke Fact Sheet 2022. Assessed at 6/10/1011. Available at https://www.world-stroke.org/assets/downloads/WSO_Global_Stroke_Fact_Sheet.pdf

[2]Dewan MC, Rattani A, Gupta S, Baticulon RE, Hung YC, Punchak M, Agrawal A, Adeleye AO, Shrime MG, Rubiano AM, Rosenfeld JV, Park KB. Estimating the global incidence of traumatic brain injury. J Neurosurg. 2018 Apr 1:1-18. doi: 10.3171/2017.10.JNS17352.

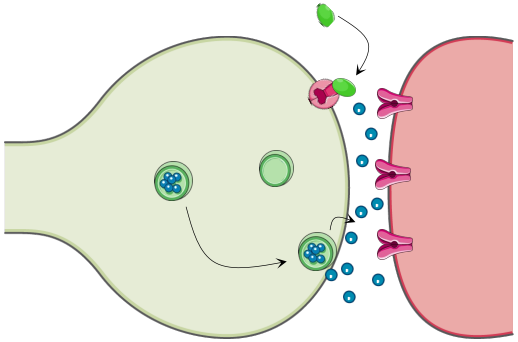
Methylphenidate

- **Methylphenidate is a “Blocker”:**

Inhibition of the monoamines transporter.

↓

⇧ Synaptic monoamines levels.



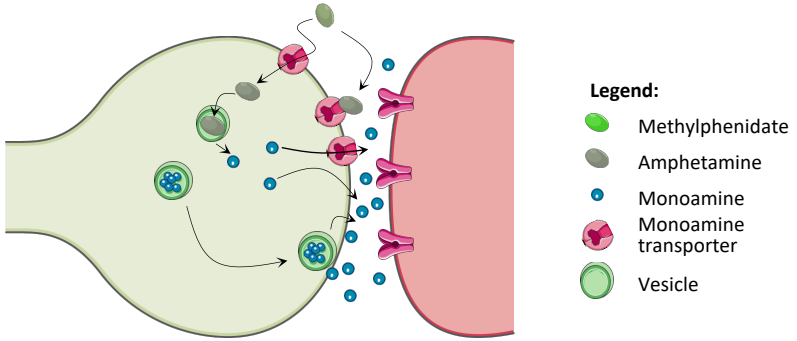
Amphetamine

- **Amphetamine is a “Releaser”:**

Inhibition of the monoamines transporter and vesicular monoamine transporter 2, and promotion of reverse transporter activity.

↓

⇧ Presynaptic monoamines release.



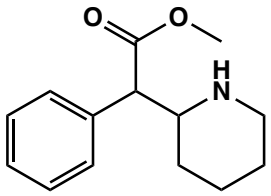
Methylphenidate

- Promotes mostly **short-term** improvements in **attention**, **vigilance**, **working memory**, **speed of processing**, **verbal** and **visual learning** and **memory, reasoning**, and **problem-solving**.

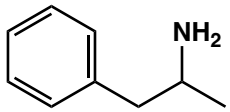
Amphetamine

- Promotes improvement in **cognitive control**, **enhances sociability**, **and induces euphoria**, **speed up reaction times, increase wakefulness**, **muscle strength**, and **reduces fatigue.**

- Both are used in the treatment of **attention deficit hyperactivity disorder**, but also in narcolepsy and other disorders.

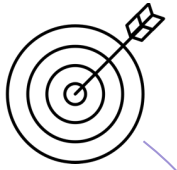


Methylphenidate



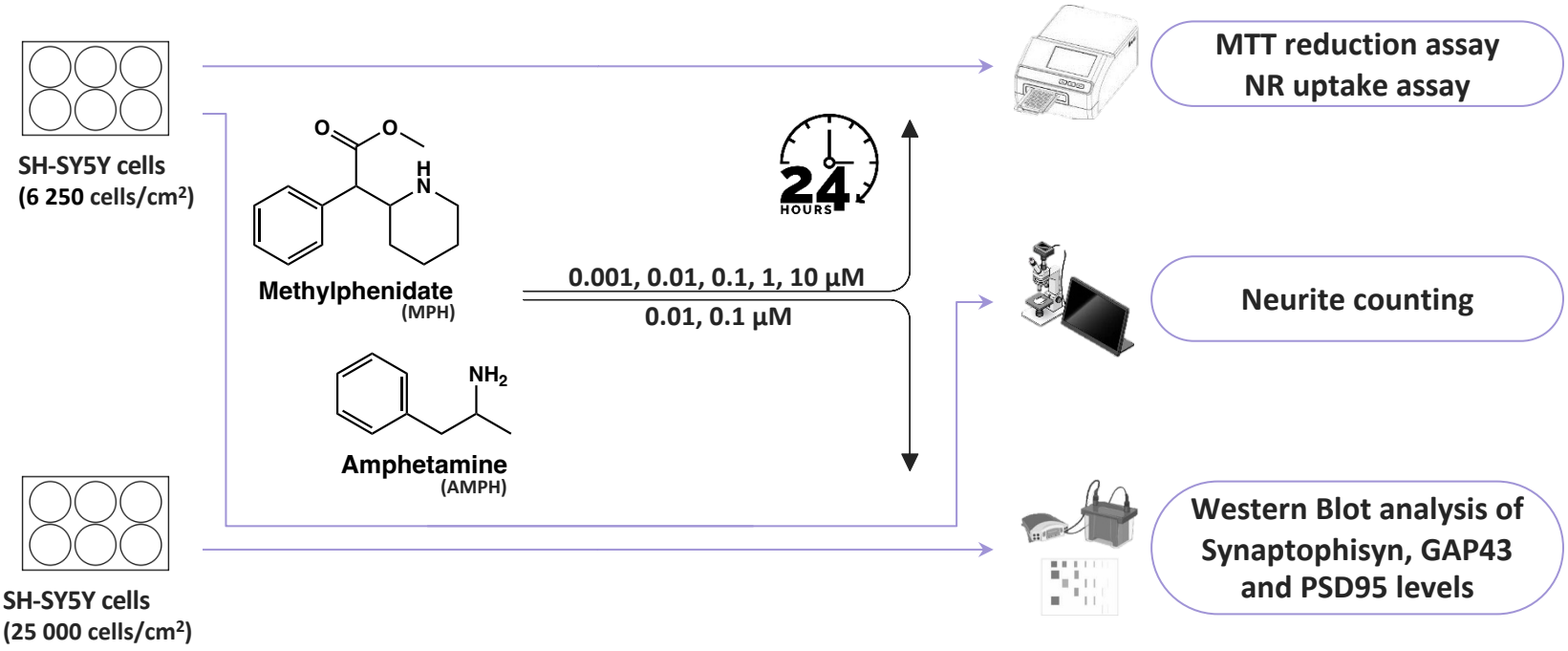
Amphetamine

Aims



This study aimed to investigate the usefulness of **MPH** and **AMPH** in **clinically relevant concentrations** in a paradigm involving the exposure to these drugs in a neuronal human **SH-SY5Y** cell model for **24 hours**, in order to evaluate the possible alterations regarding **neurite outgrowth** and **synaptogenesis**.

Methodology



Both methylphenidate and amphetamine were not cytotoxic to differentiated SH-SY5Y cells when compared to the control group

Table 1 – Mitochondrial and lysosomal dysfunction evaluated by the MTT reduction assay and the NR uptake assay in differentiated SH-SY5Y cells (6 250 cells/cm^2) incubated with MPH (10, 1, 0.1, 0.01, or 0.001 µM) or AMPH (10, 1, 0.1, 0.01 or 0.001 µM) for 24 hours. Results are from 6 independent experiments (total of 24 wells/condition). Statistical analyses were performed using the ANOVA test, followed by Tukey's post hoc test, and the Krustal-Wallis test, followed by Dunn's post hoc test. (MPH – Methylphenidate; AMPH – Amphetamine).

		0.001 µM	0.01 µM	0.1 µM	1 µM	10 µM
MTT reduction assay (% of control)	Methylphenidate					
	Amphetamine					
NR uptake assay (% of control)	Methylphenidate					
	Amphetamine					

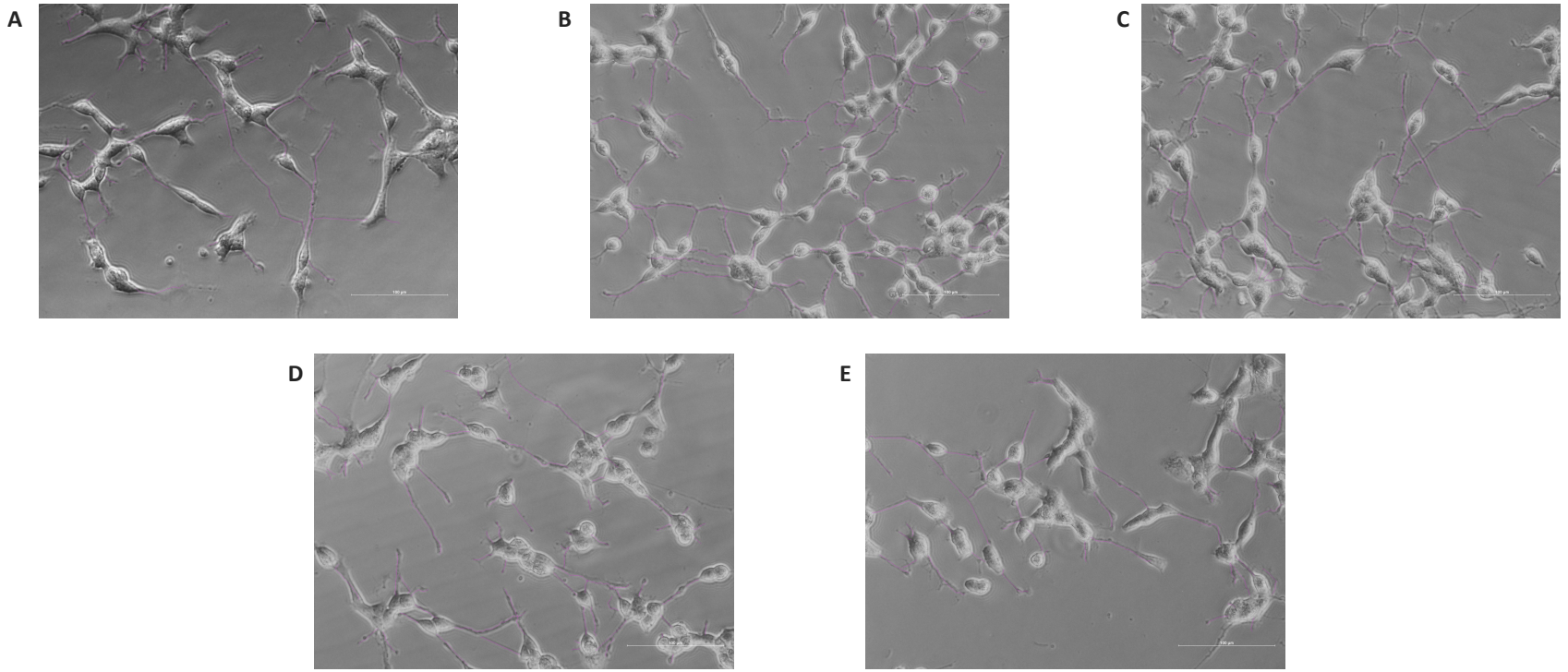
Neither methylphenidate nor amphetamine induced alterations in protein markers of synaptic plasticity and neurite outgrowth

Table 2 – Synaptophysin (34 kDa), PSD95 (95 kDa), and GAP43 (43 kDa) expression in SH-SY5Y cells (25 000 cells/cm2) evaluated by Western blotting after 24 hours of exposure to MPH (0.1 or 0.01 µM) or AMPH (0.1 or 0.01 µM). Values were obtained from 6 independent experiments from each treatment group. Results were normalized by the expression of GAPDH (37 kDa) or α-Tubulin (50 kDa) depending on the respective molecular weight, and also normalized by the control samples of each membrane. Statistical analyses were performed using the ANOVA test, followed by Tukey's post hoc test. (MPH – Methylphenidate; AMPH – Amphetamine).

		GAP43	PSD95	Synaptophysin
Methylphenidate	0.001 µM	=	=	=
	0.01 µM	=	=	=
Amphetamine	0.001 µM	=	=	=
	0.01 µM	=	=	=

Methylphenidate and amphetamine did not promote neurite outgrowth in acute exposure of 24 hours to SH-SY5Y cells

Figure 1 – Neurite outgrowth representative microphotographs in differentiated SH-SY5Y cells (6 250 cells/cm2) after 24 hours of exposure to Control (A), MPH 0.1 µM (B), MPH 0.01 µM (C), AMPH 0.1 (D) and AMPH 0.01 µM (E). Results were obtained from 4 independent experiments from each treatment group. Statistical analyses were performed using the ANOVA test, followed by Tukey's post hoc test, except in the number of cells parameter in which the Krustal-Wallis test was performed, followed by the Dunn's post hoc test. (MPH – Methylphenidate; AMPH – Amphetamine).



Conclusions

- No cytotoxicity at low concentrations (0.001, 0.01, 0.1, 1 and 10 µM).
- MPH and AMPH evoked no changes on the contente of synaptophysin, GAP43 and PSD95 at low concentrations (0.01 and 0.1 µM).
- MPH and AMPH did not promote neurite outgrowth at low concentrations (0.01 and 0.1 µM).

The starting point to our strategy to understand the possible effects of MPH and AMPH on neural repair.

Thanks for your attention!

Acknowledgments:

This work was supported by national funds through from Fundação para a Ciência e a Tecnologia (FCT), I.P., in the scope of the project “EXPL/MED-FAR/0203/2021”.

U.PORTO
FACULDADE DE FARMÁCIA
UNIVERSIDADE DO PORTO

UCIBIO
@ REQUIMTE