Exposure to nicotine and reprogramming of neuronal development

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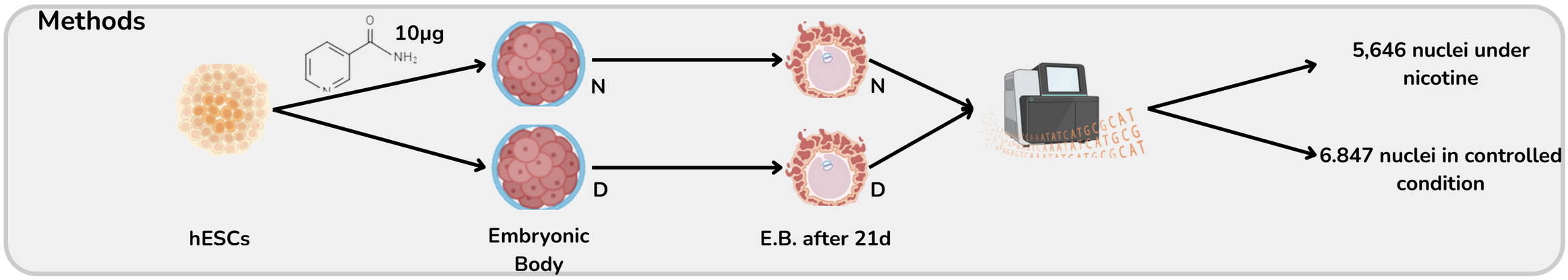
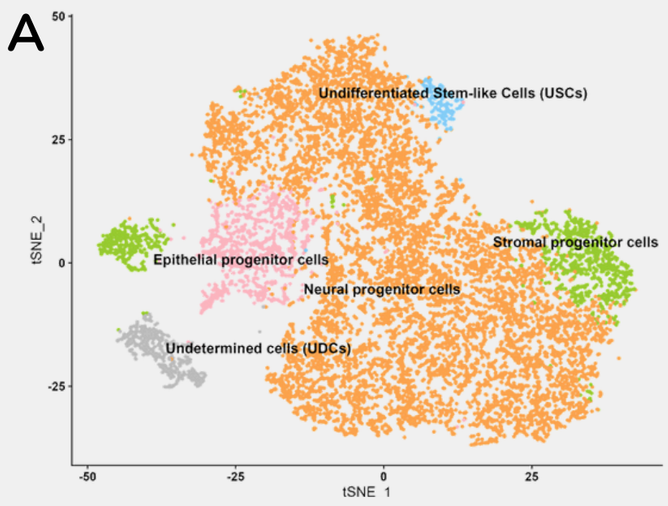
Exposure to nicotine during development profoundly alters neural differentiation pathways. From a single-cell RNA-seq analysis, it was identified a reorganization of the progenitor states, an alteration of the neuronal subtypes as well as an increased activation of metabolic and proliferative programs. The inference of developmental trajectories suggests a biased acceleration of neurogenesis under nicotine exposure.

Methods

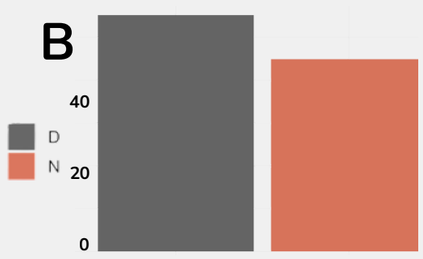


1 Presentation of the complete dataset



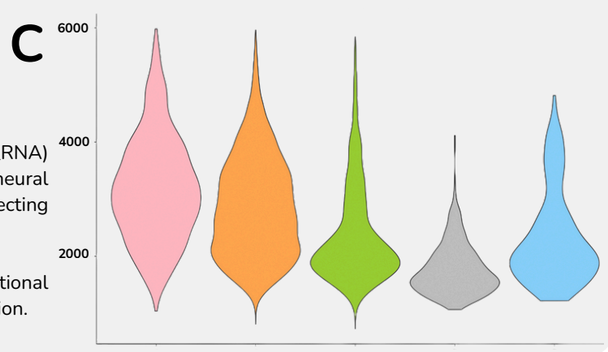
t-SNE representation of the entire single-cell dataset, showing a structured cellular organization corresponding to the main neural developmental states. This projection highlights a strong cellular heterogeneity, compatible with a continuum of differentiation.

→ The dataset captures the entire neurodevelopmental landscape



Overall distribution of cells between the Nicotine condition (N) and the Control (D), showing a comparable distribution between the two conditions (~45% vs 55%). This homogeneity limits the biases related to the global cell composition.

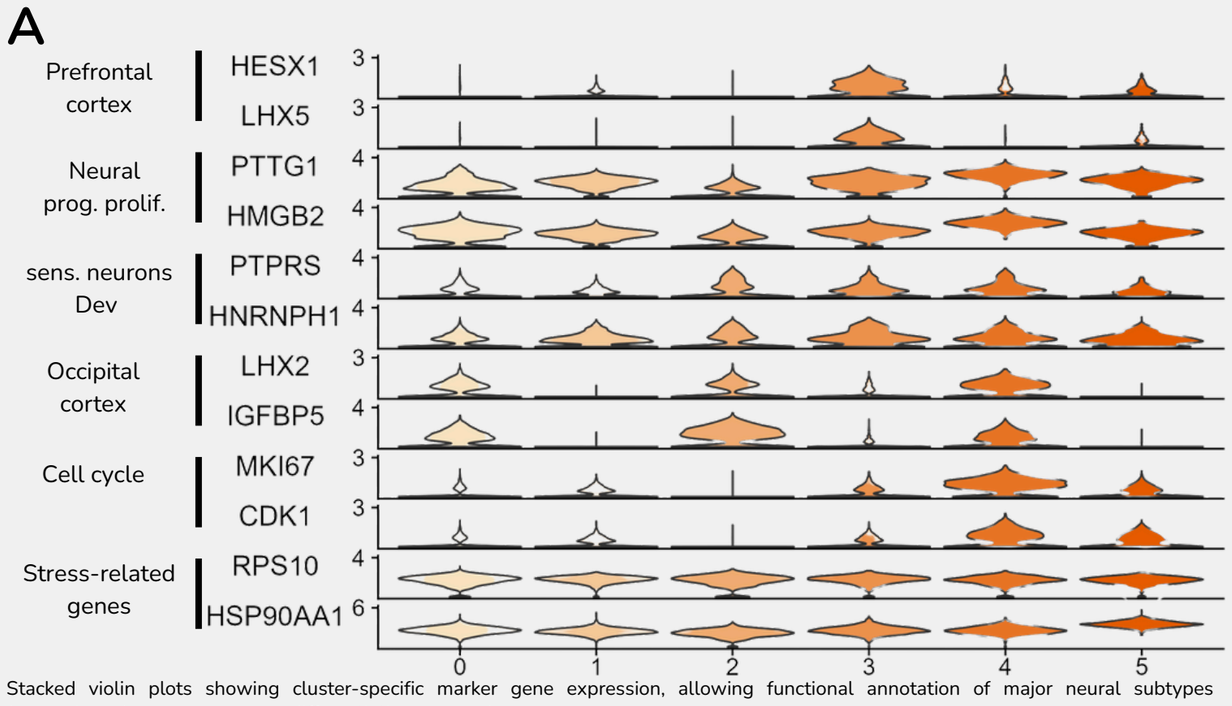
→ The observed differences reflect biological changes and not a sampling imbalance.



Distribution of the number of genes detected per cell (nFeature_RNA) according to the main identified cell types. Epithelial and neural progenitors exhibit the highest nFeature_RNA values, reflecting significant transcriptional activity.

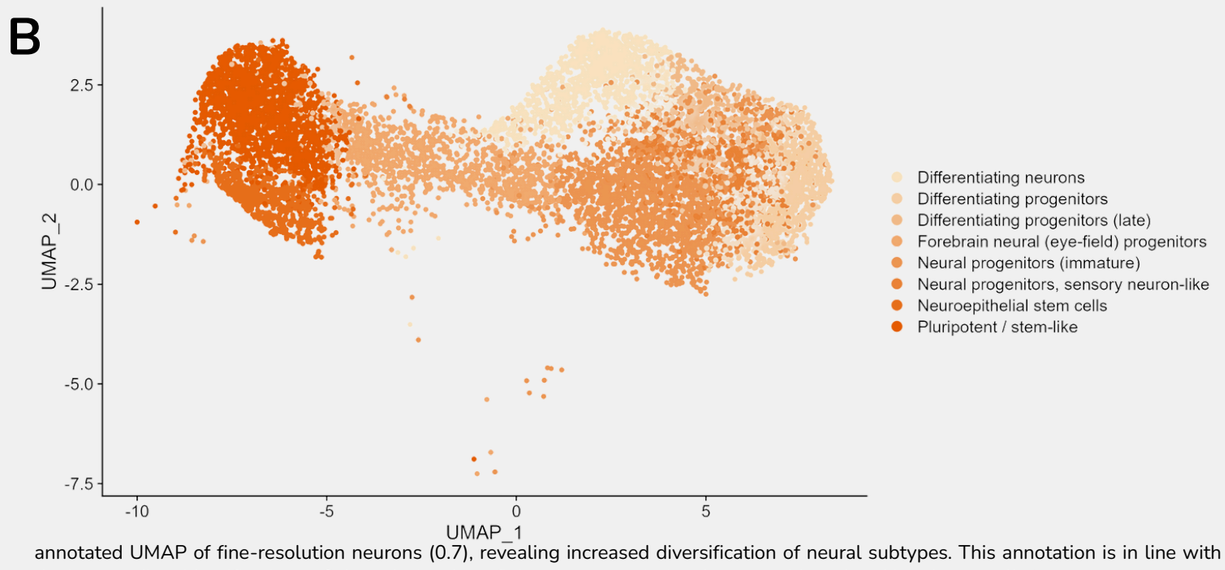
→ The differences in nFeature_RNA confirm distinct transcriptional states, consistent with the degree of cell maturation and specialization.

2 Identification of neural subtypes



Stacked violin plots showing cluster-specific marker gene expression, allowing functional annotation of major neural subtypes (progenitors, immature neurons, differentiating neurons).

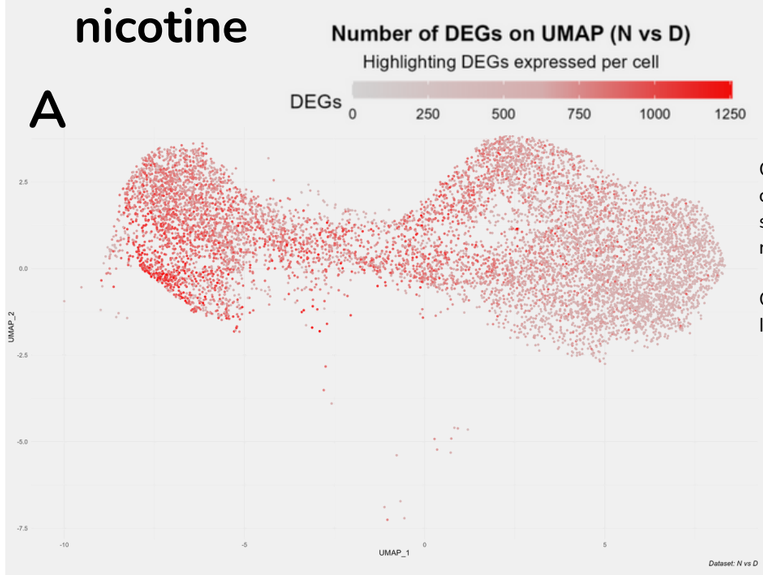
→ The differential expression confirms the biological identity of the subpopulations.
→ The stress brought by nicotine impacts all neural subtypes



annotated UMAP of fine-resolution neurons (0.7), revealing increased diversification of neural subtypes. This annotation is in line with the intermediate identification at 0.4 resolution, by refining the transcriptional states without breaking the overall structure.

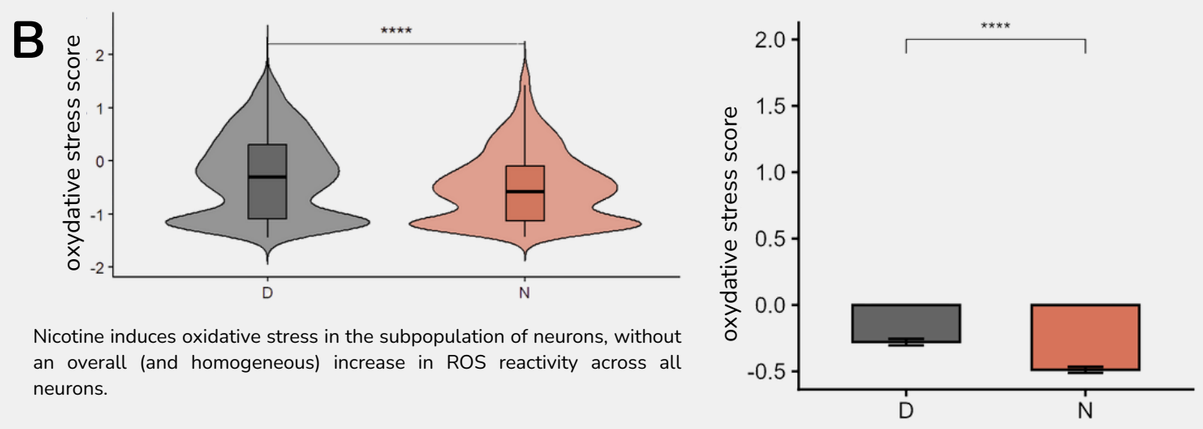
→ The increase in resolution reveals a progressive specialization of neurons.

3 Transcriptional modifications induced by the nicotine

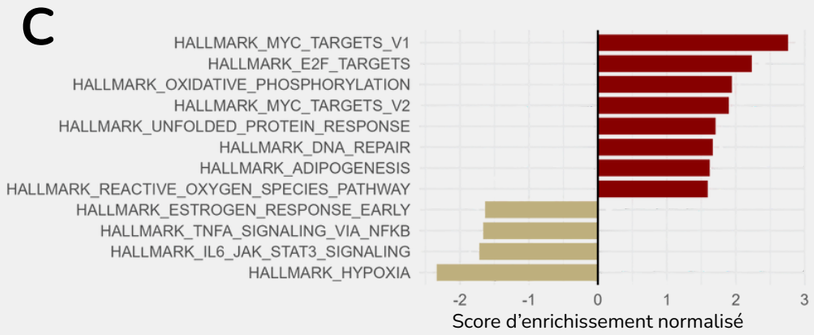


Cells with the highest scores (red) preferentially concentrate along the neural differentiation trajectory, suggesting a gradual activation of oxidative stress-related programs during maturation.

Oxidative stress is not homogeneous.
It follows the neural developmental trajectory.

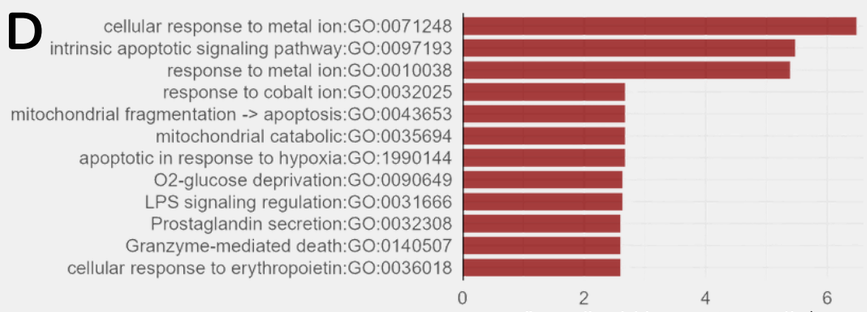


Nicotine induces oxidative stress in the subpopulation of neurons, without an overall (and homogeneous) increase in ROS reactivity across all neurons.



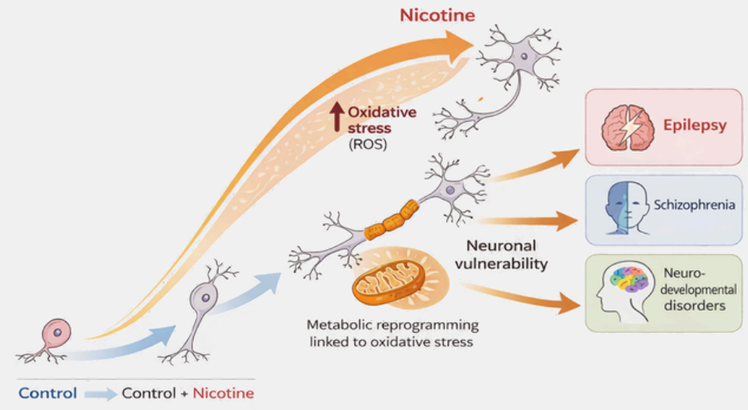
GSEA analysis reveals an enrichment of MYC and E2F signatures, as well as oxidative phosphorylation, misfolded protein response and ROS pathways in nicotine-exposed neurons, indicating increased metabolic and transcriptional activation.
In contrast, control neurons are relatively enriched for hypoxia and inflammatory signaling pathways (TNFα/NF-κ B, IL6/STAT3).

Nicotine exposure results in a marked activation of pathways associated with oxidative stress, cellular stress response and energy metabolism in neurons, suggesting metabolic reprogramming accompanying accelerated neuronal differentiation.



4 Conclusion

Exposure to nicotine disrupts human neurogenesis by altering neurodevelopmental trajectories and metabolic programs.



Multi-resolution analysis highlights increased diversification of neural subtypes, accompanied by differential activation of pathways related to oxidative stress.
These results suggest that nicotine influences neurodevelopment by acting on redox balance and maturation states, with potential implications for brain development.

1. **Single-Cell RNA Sequencing of Human Embryonic Stem Cell Differentiation Delineates Adverse Effects of Nicotine on Embryonic Development (H. Guo et al, 2019)**

Principaux packages :

- H. Wickham. ***ggplot2***: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.
- Hao et al. ***Seurat v4***: Dictionary learning for integrative, multimodal and scalable single-cell analysis. Nature Biotechnology (2023)
- Kassambara A (2025). ***_ggpubr***: 'ggplot2' Based Publication Ready Plots_. doi:10.32614/CRAN.package.ggpubr
- Carlson M (2025). ***_org.Hs.eg.db***: Genome wide annotation for Human_. R package version 3.22.0.