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Changes in DNA methylation and histone H3 acetylation induced by distinct psychotropic drugs on dopaminergic SH-SY5Y human neuroblastoma cells

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Abstract

Background: Misuse of psychotropic drugs raises concern due to their neurotoxic and addictive potential [1, 2]. Growing evidence correlates epigenetic effects with neurotoxicity and abuse potential, foreseeing epigenetic marks as predictive markers of drug-related neurotoxicity [3]. **Objective:** Identify a common epigenetic signature (based on global DNA methylation and histone H3 acetylation changes) induced by pharmacologically distinct psychotropic drugs. **Methods:** Dopaminergic-differentiated SH-SY5Y cells were exposed for 24h to 1 µM nicotine, 10 µM methamphetamine (METH), 10 µM 4-CMC (synthetic cathinone), 10 µM tapentadol, 1 µM heroin, and morphine (opioids), and 1 µM JWH-122 and THJ-2201 (synthetic cannabinoids), based on previously obtained cytotoxicity data, and considering physiological relevance. Cells were also exposed to two non-neurotoxic substances, 10 µM mannitol and 10 µM riluzole. Genomic DNA and histones were extracted, and global DNA methylation and histone H3 total acetylation were measured using commercially available colorimetric and fluorometric kits, respectively. Data were obtained from three independent experiments run in duplicate, expressed as % 5-methylcytosine (5-mC) and % H3 acetylation, compared to the untreated control. **Results:** Only METH, morphine, and THJ-2201 increased global DNA methylation (128%, 122%, 126%, respectively), a modification associated with gene transcription silencing. Decreased histone acetylation also leads to gene transcription repression, and was observed for heroin, morphine, and THJ-2201 (68%, 73%, and 82%, respectively). On the other hand, METH, 4-CMC, nicotine, tapentadol and JWH-122 increased histone H3 total acetylation (191%, 200%, 194 %, 244% and 165%, respectively), possibly promoting gene transcription. Non-neurotoxic riluzole reduced H3 histone total acetylation (43%), while mannitol was similar to control. **Conclusions:** Our preliminary data suggest that stimulants seem to increase histone H3 acetylation, while opioids and synthetic cannabinoids can be associated with either increased or decreased histone H3 acetylation. DNA methylation results were variable across drug classes. Large variability suggests limitations of the 24h exposure period and possible instability of the dopaminergic phenotype in SH-SY5Y cells. Future assays using hiPSC cells and longer exposure periods are needed for a more sensitive neurotoxicological epigenetic assessment.

Keywords: epigenetics; psychotropic drugs; neurotoxicity; abuse potential

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References

1. United Nations Office on Drugs and Crime. Online World Drug Report 2024 - Drug market patterns and trends. 2024 [cited 2025 May 14th]; Available from: https://www.unodc.org/unodc/en/data-and-analysis/wdr2024-drug-market-trends.html.
2. European Union Drugs Agency. European Drug Report 2025: Trends and Developments. 2025 [cited 2025 June 11th]; Available from: https://www.euda.europa.eu/publications/european-drug-report/2025_en.
3. Mazdai, L. et al., Epigenetic Studies for Evaluation of NPS Toxicity: Focus on Synthetic Cannabinoids and Cathinones. *Biomedicines* **2022**, *10(6)*, 1398, doi:10.3390/biomedicines10061398.

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