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Prolonged nicotine exposure, via electronic cigarette, selectively increases *Bdnf/TrkB* transcription, dynorphin peptide levels and OLIG2 in male rat VTA

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ABSTRACT

Different drugs of abuse affect the Central Nervous System (CNS) neuronal networks and reshape the expression of neuroplasticity-related genes in crucial parts of the mesocorticolimbic reward circuitry, such as the ventral tegmental area (VTA) and the nucleus accumbens (NAc). Recent evidence suggests that neuronal activity and life experience, including repeated drug exposure, can modulate oligodendrogenesis thus altering neuronal myelination. This study aimed to investigate whether the prolonged exposure to nicotine, via electronic cigarettes, affects oligodendrocyte differentiation. Results showed that exposure to nicotine mainstream enhances the expression of OLIG2, a transcription factor essential for oligodendrocyte differentiation, in male rat VTA. This effect was associated with increased mRNA levels of the epigenetic enzyme *Kdm6b*, which is involved in regulating OLIG2 expression and synaptic plasticity. In the same brain region, nicotine increased *Bdnf* and *TrkB* gene expression as well as dynorphin peptide levels, which are positive regulators for oligodendroglial differentiation. Noteworthy, these molecular changes occurred alongside a reduction in neurofilament light levels, suggesting potential axonal remodelling associated with enhanced oligodendrogenesis. No significant changes in investigated parameters were detected in the NAc, thus suggesting that the reported molecular alterations selectively occurred in the VTA. Protein correlation analysis revealed that prolonged nicotine exposure primarily affects neuroplasticity-related protein networks within this area. Overall, these findings suggest that prolonged nicotine exposure, through electronic cigarettes, induces alterations of oligodendrogenesis modulators in the VTA. These molecular changes may impact axonal conduction velocity and reward circuitry connectivity, promoting neuronal adaptations that could be relevant for the development of addictive behaviour.

1. Introduction

Nicotine addiction represents one of the major health concerns worldwide. Similar to other drugs of abuse (e.g., cocaine, opioids) (Volkow et al., 2019; Velasco et al., 2021), nicotine, the active alkaloid in tobacco, triggers several cellular and molecular adaptations in key brain regions associated with drug reward (Dani et al., 2001). Combustible tobacco has so far been the most common form of nicotine consumption, but the widespread use of electronic cigarette (EC), especially among young people (Salari et al., 2024), is now of great concern.

Numerous studies have highlighted nicotine's ability to produce lasting effects on cortico-limbic circuits and synapses (Placzek et al., 2009; Marguet et al., 2022). In particular, repeated exposure to nicotine has been shown to remodel the expression of neuroplasticity-related genes (Fan et al., 2023), affecting the neuronal networks of the ventral tegmental area (VTA) and nucleus accumbens (NAc) (Mount and Monje, 2017).

In this context, recent evidence suggests a crucial role for oligodendroglial lineage cells and myelin in reward circuit adaptation during addiction (Fletcher et al., 2021). Oligodendrocytes drive myelin production, promoting the formation of a protective sheath around axons and ensuring rapid and efficient neural communication (Martin et al., 2018). In the CNS, the myelination process requires the generation of functionally mature oligodendrocytes from oligodendrocyte precursor

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cells (OPCs); this process is essential for maintaining the integrity of brain circuits and can be strongly modulated by neuronal activity and experience throughout the lifespan (Osso and Hughes, 2024). Alterations in myelination can disrupt circuit dynamics and influence behaviour. Interestingly, maladaptive myelination has been implicated in the pathophysiology of neurological and psychiatric disorders (Fletcher et al., 2021), including addiction (Velasco et al., 2021). Notably, recent findings suggest that opioids can influence oligodendrogenesis, leading to maladaptive myelination of the brain's reward circuitry (Mount and Monje, 2017).

Brain-derived neurotrophic factor (BDNF) is a pivotal neurotrophin with a profound impact on synaptic plasticity, neuronal development, and circuit remodelling. It is widely expressed throughout the CNS, and growing evidence underscores its essential role in activity-dependent mechanisms that regulate oligodendrocyte development (Mount and Monje, 2017). Beyond its role in neural development (Rodríguez-Carrillo et al., 2023), BDNF is a central regulator of addiction-related neuroadaptations (Nikulina et al., 2014). Indeed, alterations of its expression have been extensively linked to drug craving, seeking, and relapse across various substance use disorders, including cocaine (Caputi et al., 2019a), alcohol (Caputi et al., 2021), and nicotine (Huang et al., 2020) addiction. Notably, the binding of BDNF with its cognate tropomyosin receptor kinase B (TrkB) has been shown to be essential for modulating the signalling cascade responsible for morphine-induced oligodendrogenesis in the VTA (Yalçın et al., 2024). In this respect, the broad involvement of BDNF in these processes further underscores its crucial role in neuroplastic changes underlying drug reward and dependence (Barker et al., 2015).

Furthermore, particular attention has been devoted to the dynorphin/κ-opioid receptor system (DYN/KOR), known to be involved in the modulation of stress responses, as well as of negative emotional states during chronic pain and drug addiction (Bruchas et al., 2010; Caputi et al., 2019a,b). Specifically, the DYN/KOR system is involved in the addictive effects of drugs and its activation has been linked to ethanol (Caputi et al., 2021) and nicotine self-administration (Gowrishankar et al., 2022). Interestingly, KOR signalling has been identified as a positive regulator for oligodendroglial differentiation and myelination (Mei et al., 2016). In this regard, recent evidence also highlighted the ability of dynorphin peptide to promote experience-dependent myelination following stress (Osso et al., 2021). However, little is known about OPC differentiation and myelination process in other experiences involving dynorphin release, such as following repeated drugs exposure (D'Addario et al., 2013; Caputi et al., 2014, 2016).

Based on this evidence, this study aimed to elucidate the molecular alterations underlying nicotine-induced changes in synaptic plasticity and neuronal connectivity. Specifically, we investigated the potential role of BDNFergic and DYN/KOR systems in modulating oligodendrogenesis within the VTA and NAc. To address this aim, we examined alterations in *Bdnf-TrkB* and DYN/KOR expression following prolonged nicotine exposure via electronic cigarettes (e-cigarettes; EC). These devices are mainly marketed as a putative means of quitting smoking and a less harmful replacement for traditional cigarette smoking (Chen et al., 2023).

Furthermore, we assessed changes in the expression of OLIG2, a well-established marker of oligodendrogenesis (Pepper et al., 2018), alongside the gene expression one of its epigenetic regulators, the histone demethylase enzyme *Kdm6b* (Sui et al., 2022). Finally, we examined the impact of prolonged nicotine exposure on neurofilaments (NFs) light, medium, and heavy chains (NF-L; NF-M; NF-H respectively), that represent critical cytoskeletal proteins essential for axonal integrity maintenance (Khalil et al., 2024). In this respect, previous studies showed a neurofilament decrease following chronic ethanol (Requena-Ocaña et al., 2023) and cocaine (Bavato et al., 2023), thus suggesting that neuroadaptation of the mesolimbic pathway may be linked to long-term changes in the expression of these axonal proteins which can influence axonal myelination and conduction velocity (Nowier et al., 2023).

2. Materials and methods

2.1. Animal model, nicotine exposure and tissue collection

The animal experiments were conducted following the guidelines of EU Directive (2010/63/EU) and were approved by the Committee on Animal Research and Ethics at the University of Bologna, as well as by the Italian Ministry of Health (permit number: 1072/2020-PR). Sample size (n) was based on our previous experiments and power analysis performed with the software G*Power 3.1. Fourteen male Sprague Dawley rats (7 weeks old) (ENVIGO RMS SRL, San Pietro al Natisone, Italy), were housed under standard conditions (12-h light–dark cycle, 22 ˚C, 60 % humidity) and fed ad libitum. After one week of acclimatization, the rats were divided into two groups: a control group (n = 7 rats; CTRL) and an exposed group (n = 7 rats; EC Nicotine). The rats in the exposed group were placed in an inhalation chamber (two animals per chamber), where they were exposed to nicotine delivered via a commercially available e-cigarette device (Eleaf joytech electronics 5000mAh battery 15 W power). Animals were exposed in order to consume 1 mL/day of e-liquid containing 18 mg/mL of nicotine for 28 days, for 3 h per day according to the Health Canada's smoking protocol guidelines The nicotine concentration was selected in accordance to the manufacturer indications and scientific literature (Gades et al., 20255). The control group spent the same time in the inhalation chamber as the exposed rats. The exposure chamber consisted of a propylene chamber (38 × 26.5 × 19 cm) with a capacity of 19 L. The pump (0.18 kW; 1.4/1.6 A; 230 V; 50/60 Hz) was connected on one side of the box, while the mainstream was puffed into the opposite side, generating the airflow into the chamber. The puff profile (5 s on, 15 s off, and 5 s on) with an airflow of 4 L/min was set as previously described (Canistro et al., 2017; Vivarelli et al., 2019). The exposure lasted for 20 min. The experiments were carried out in accordance with ARRIVE guidelines (Percie du Sert et al., 2020). At the conclusion of the treatment, the rats were euthanized, and the ventral tegmental area (VTA) and nucleus accumbens (NAc) were dissected from each rat according to the Rat Brain Atlas (Paxinos and Watson, 1982) and stored at −80 ˚C for subsequent molecular analysis.

2.2. Urine collection and cotinine levels determination

On day 20 of EC nicotine exposure, after the completion of the dosing session, rats were singly housed for 4 h in metabolic cages for urine collection. Access to water and food was ad libitum while in the metabolic cages (Budriesi et al., 2018). The urine samples were collected and stored at −80˚ until analysis. Cotinine levels were determined by Rat Cotinine ELISA Kit (Catalog No. MBS1609912, MyBioSource, USA) following the manufacturer's protocol.

2.3. RNA extraction and gene expression analysis

Total RNA was extracted using the method described by Chomczynski and Sacchi (2006). Each sample underwent DNAse treatment and converted into cDNA, as outlined in previous studies (Caputi et al., 2020; Rullo et al., 2021; Vivarelli et al., 2024). The relative abundance of each mRNA of interest was assessed by real-time qRT-PCR using the Sybr Green Gene Expression Master Mix (Life Technologies, USA) on a Step One Real-Time PCR System (Life Technologies). Gene expression levels were quantified using the delta-delta Ct (ΔΔCt) method and reported as relative expression ratios ($2^{-\Delta\Delta Ct}$). The average $2^{-\Delta\Delta Ct}$ values were then used for statistical analysis. All data were normalized to the housekeeping gene glyceraldehyde-3-phosphate dehy-

drogenase (GAPDH). The specificity of each PCR product was verified through melting curve analysis, conducted between 60 °C and 95 °C. The primers for PCR amplification were designed using Primer 3, and their sequences are provided below.:

– *Gapdh*
  Forward 5′-AGACAGCCGCATCTTCTTGT-3′;
  Reverse 5′-CTTGCCGTGGGTAGAGTCAT-3′;
– *Bdnf*
  Forward 5′- AAGTCTGCATTACATTCCTCGA -3′;
  Reverse 5′- GTTTTCTGAAAGAGGGACAGTTTAT -3′;
– *TrkB*
  Forward 5′- AAGTTCTACGGTGTCTGTGTG -3′;
  Reverse 5′- TTCTCTCCTACCAAGCAGTTC -3′;
– *Kdm6b*
  Forward 5′- ACCGCCTGCGTGCCTTAC -3′;
  Reverse 5′- GTGTTGCTGCTGCTGCTACTG -3′

2.4. SDS-page and immunoblotting

Protein extraction was performed using N-PER™ Neuronal Protein Extraction Reagent (Thermo Scientific, USA). The Halt protease and phosphatase inhibitor cocktail (Thermo Scientific) was added according to the manufacturer's guidelines. Protein concentration was determined with the Pierce BCA protein assay kit (Thermo Scientific). An equal amount of protein (40 μg) was loaded onto Bolt 4–12 % Bis-Tris Plus gels (Thermo Scientific) and transferred to 0.45 μm PVDF membranes (Thermo Scientific). After blocking for 45 min at room temperature with 5 % non-fat dry milk in TBS-T (Tris-buffered saline with 0.1 % Tween-20), the membranes were incubated overnight at 4 °C with the specified primary antibodies: Neurofilament-Heavy (NF-H) (1:500, cod. no. 2836, Cell Signaling, USA), Neurofilament Medium (NF-M) (1:1000, cod. no. ab254125, Abcam, UK), Neurofilament-Light (NF-L) (1:1000, cod. no. 2835, Cell Signaling, USA), OLIG2 (1:500, cod. no. sc-48817, SantaCruz Biotechnology, United States), and KOR (OPRK1 Polyclonal Antibody) (1:1000, cod. no. 44-302G, Invitrogen, USA). The results were standardized using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:1000; cod. no. MA5–15738, Invitrogen, USA) or β-tubulin (1:1000, cod. no. PA5-16863, Invitrogen, USA). The following peroxidase-linked secondary antibodies were used for the detection: Goat anti-rabbit (1:3000, cod. no. 65–6120, Invitrogen, USA) or Goat anti-mouse (1:3000, cod. no. 62–6520, Invitrogen, USA). Immunocomplexes were visualized via chemiluminescence using a Chemidoc MP Imaging System (Bio-Rad Laboratories, USA) and analysed with the software ImageLab 5.2.1. The gels were run in duplicate, and the data presented are the average from two separate Western blots. Full-size original immunoblots showing protein expression levels can be found in the Supplementary Materials.

2.5. Enzyme-linked immunosorbent assay (ELISA)

The proteins extracted as described above were measured for dynorphin peptide concentrations using a sandwich ELISA method and following the manufacturer's instructions (Catalog No. CSB-E13294r, Cusabio, USA). The obtained absorbance values were initially calculated as pg/mL, using a standard curve that was assayed in parallel to the test samples, and then converted into pg/μg of protein (Lorente et al., 2024; Boccella et al., 2025).

2.6. Statistical analysis

Data were evaluated using the Shapiro–Wilk test to confirm the normality of the distribution and Grubb's test to identify outliers. Statistical analysis was performed using the two-tailed unpaired *t*-test. All statistical analyses were performed using GraphPad Prism version 10.3.1 for Windows, (GraphPad Software, USA). The results are expressed as means ± standard error of the mean (SEM) (n = 5–7 animals/group). Pearson's correlation analysis was used to assess the associations between the normalized protein values in the VTA and NAc of animals. The regular correlation was computed using the rcorr function from the Hmisc package in R (10.32614/CRAN.package.Hmisc; https://rpubs.com/minhtri/968611). rcorr computes a matrix of Pearson's r-rank correlation coefficients for all possible pairs of genes of the matrix. The level of significance was set at $p < 0.05$.

3. Results

3.1. Cotinine levels

To confirm the effective exposure of animals to nicotine mainstream, urinary cotinine levels were measured in EC nicotine group, revealing a concentration of 151.17 ± 25.90 ng/mL (Fig. S1, see Supplementary file).

3.2. Ventral tegmental area

3.2.1. Prolonged nicotine inhalation led to an increase of Bdnf, TrkB and histone demethylase Kdm6b gene expression in the VTA

Rats exposed to e-cigarettes mainstream for 4 weeks showed an increase of neurotrophin *Bdnf* (1.31 ± 0.07 vs 1.02 ± 0.08, $p < 0.05$; Fig. 1A) and its cognate receptor *TrkB* (1.29 ± 0.01 vs 1.01 ± 0.07, $p < 0.01$; Fig. 1B) mRNA levels, in the VTA. Interestingly, this exposure also led to a significant increase in the mRNA levels of the histone demethylase *Kdm6b* (2.43 ± 0.37 vs 1.17 ± 0.28, $p < 0.05$; Fig. 1C), suggesting a link between nicotine exposure, epigenetic regulation, and neurotrophic signalling pathways in this brain region (Zhang et al., 2018; Swahari and West, 2019).

3.2.2. Exposure to nicotine via e-cigarettes increased the expression of the transcription factor OLIG2 and affected the DYN/KOR system in the VTA

To determine the onset of the oligodendrogenesis processes, the levels of transcription factor OLIG2 were evaluated, showing elevated levels following nicotine inhalation (120.85 ± 4.89 vs 100 ± 3.99, $p < 0.05$; Fig. 2A). This increase suggests adaptive/maladaptive alterations in oligodendrocyte regulation, potentially contributing to the neurobiological mechanisms underlying addiction (Yalçın et al., 2024).

Moreover, in the light of recent evidence highlighting the role of the DYN/KOR system in OPC differentiation and myelination process (Osso et al., 2021), we assessed the levels of dynorphin peptide and its kappa opioid receptor following prolonged nicotine exposure. Our analysis revealed a significant increase in dynorphin peptide levels in the smoking group compared to controls (0.81 ± 0.06 vs 0.60 ± 0.04, $p < 0.01$; Fig. 2B), without notable changes in KOR protein expression in the VTA (102.74 ± 5.26 vs 100 ± 4.71, $p > 0.05$; Fig. 2C).

3.2.3. Nicotine exposure resulted in a reduction in NF-L levels in the VTA

Given their critical role in maintaining axonal integrity, neurofilament levels were also assessed. While no significant changes were observed in NF-H (112.70 ± 18.81 vs 100 ± 12.25, $p > 0.05$; Fig. 3A) and NF-M levels (79.93 ± 12.93 vs 100 ± 12.51, $p > 0.05$; Fig. 3B), a significant reduction in NF-L levels (76.55 ± 3.69 vs 100 ± 3.70, $p < 0.01$; Fig. 3C) was observed in the nicotine-exposed group compared to controls. This decrease in NF-L suggests that prolonged nicotine inhalation, similar to other drugs of abuse (Bavato et al., 2023; Requena-Ocaña et al., 2023), may impair axon structure and function.

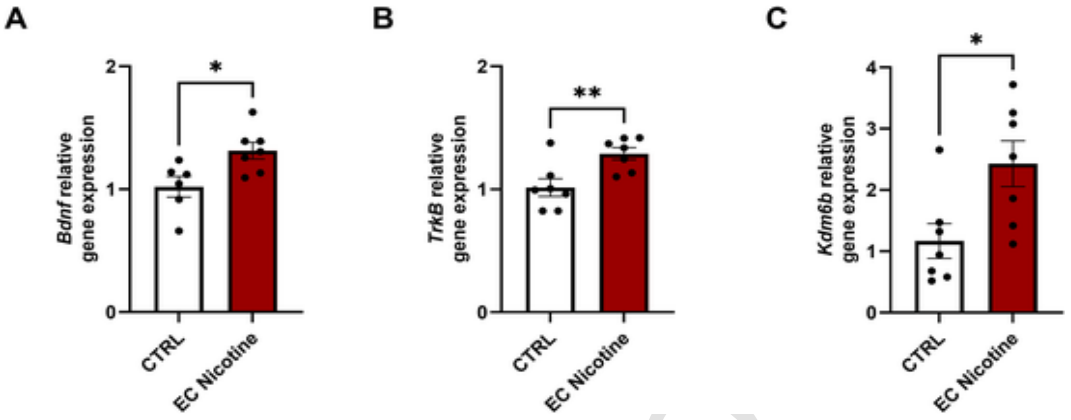

Fig. 1. Gene expression levels of *Bdnf, TrkB* (A, B) and of histone demethylase *Kdm6b* (C), measured by RT-PCR. Statistical significance was assessed using two-tailed unpaired *t*-test. Data are presented as $2^{-\Delta\Delta CT}$ values and expressed as the means ± SEM, n = 6–7/group, *p < 0.05, **p < 0.01. One outlier in CTRL group (A) was not included in data analysis.

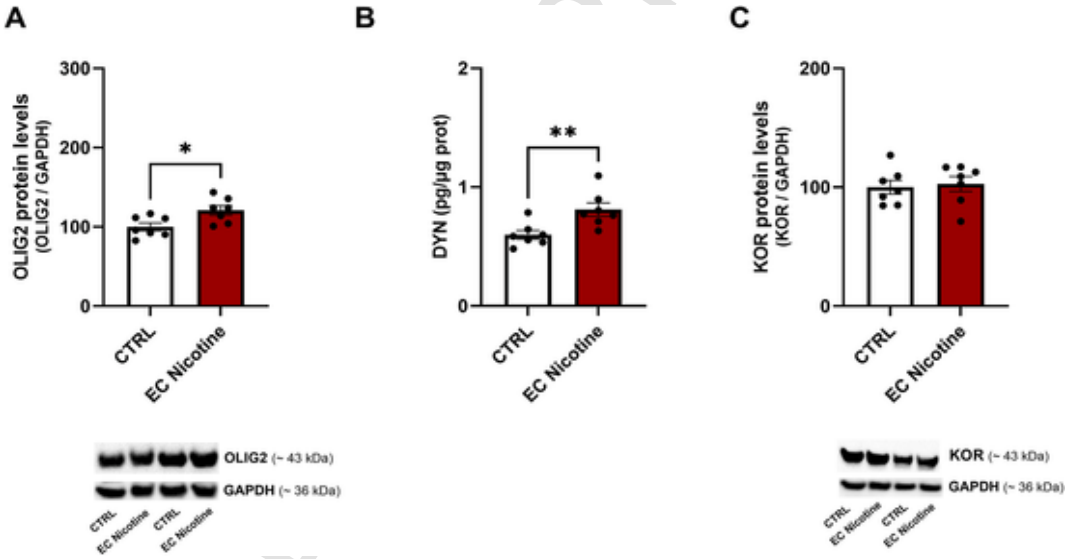

Fig. 2. Protein levels of transcription factor OLIG2 (~30/40 kDa) (A), dynorphin levels (µg/tissue; ELISA) (B) and protein levels of the KOR receptor (~43 kDa) (C). OLIG2 and KOR were normalized to GAPDH (~36 kDa). A representative blot is reported alongside the histogram. Statistical significance was assessed using a two-tailed unpaired *t*-test. Data are presented as means ± SEM, n = 7/group, p* <0.05, p** <0.01.

3.3. Nucleus accumbens

3.3.1. Nicotine delivered by e-cigarettes did not affect Bdnf, TrkB and Kdm6b mRNA levels in the NAc

Based on the strong relationships between VTA and NAc within the mesolimbic reward circuitry, the effect of prolonged nicotine inhalation was explored in accumbal brain area. Our results showed no significant differences in the expression of *Bdnf* (0.79 ± 0.15 vs. 1.13 ± 0.21, p > 0.05; Fig. 4A) or its receptor *TrkB* (0.98 ± 0.40 vs. 1.04 ± 0.39, p > 0.05; Fig. 4B) following nicotine exposure by e-cigarettes. Similarly, the histone demethylase *Kdm6b* mRNA levels were not affected by the treatment (1.12 ± 0.18 vs 1.11 ± 0.21, p > 0.05, Fig. 4C). These results indicate that nicotine exposure does not affect the BDNF/TrkB system or *Kdm6b* gene expression of this brain region.

3.3.2. Exposure to nicotine via e-cigarettes did not alter the protein expression of the transcription factor OLIG2, DYN and KOR in the NAc

Interestingly, exposure to nicotine did not result in any significant alterations in OLIG2 levels in this brain area (99.79 ± 7.24 vs 100 ± 6.08, p > 0.05, Fig. 5A). Moreover, no significant changes in the dynorphin peptide (0.55 ± 0.06 vs 0.51 ± 0.07, p > 0.05; Fig. 5B) and KOR protein (100.94 ± 10.92 vs 100 ± 9.20, p > 0.05; Fig. 5C) levels were detected. These findings suggest that different to VTA, nicotine exposure via e-cigarettes does not seem to affect OPC differentiation within the NAc.

3.3.3. Prolonged nicotine inhalation did not alter neurofilaments levels in NAc

Axonal integrity in the NAc, was assessed by measuring the protein levels of the three neurofilament isoforms. Similar to what was observed for other investigated parameters in this brain region, no significant changes were detected in different neurofilament isoform expression (NF-H: 80.27 ± 16.17 vs 100 ± 18.84, p > 0.05; Fig. 6A), (NF-M:

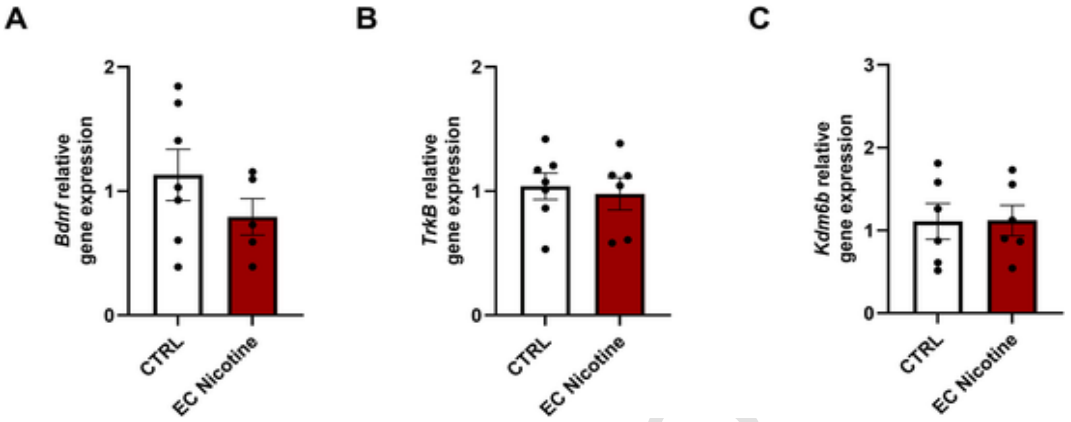

Fig. 4. Gene expression levels of *Bdnf*, *TrkB* (A, B) and of histone demethylase *Kdm6b* (C), measured by RT-PCR. Statistical significance was assessed using two-tailed unpaired *t*-test. Data are presented as $2^{-\Delta\Delta CT}$ values and expressed as the means ± SEM, n = 5–7/group. One outlier in CTRL group (C) and one/two outliers in EC nicotine group (A, B, C) were not included in data analysis.

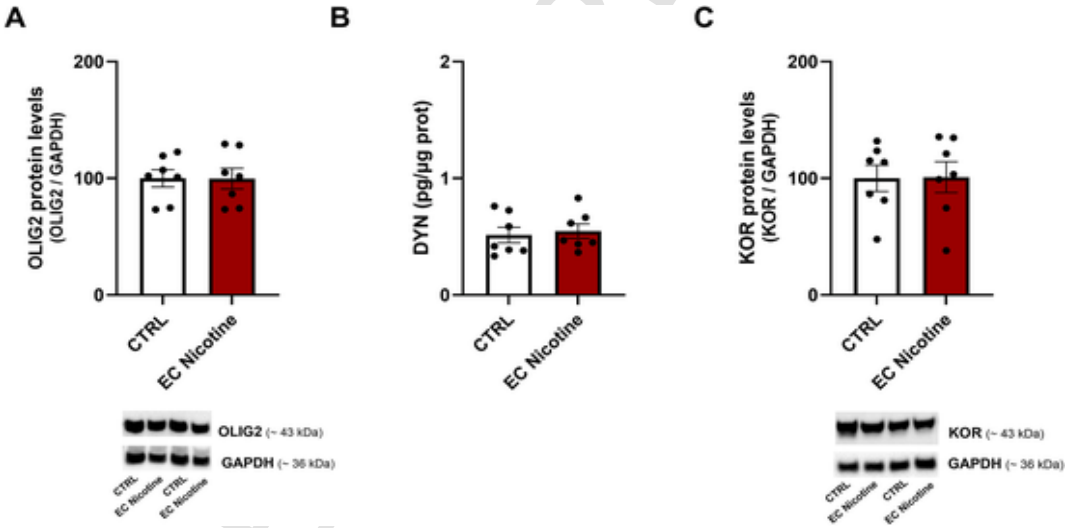

Fig. 5. Protein levels of transcription factor OLIG2 (~30/40 kDa) (A), dynorphin levels (µg/tissue; ELISA) (B) and protein levels of the KOR receptor (~43 kDa) (C). OLIG2 and KOR were normalized to GAPDH (~36 kDa). A representative blot is reported alongside the histogram. Statistical significance was assessed using a two-tailed unpaired *t*-test. Data are presented as means ± SEM, n = 7/group.

92.39 ± 21.35 vs 100 ± 17.76, p > 0.05, Fig. 6B), (NF- L: 72.32 ± 14.44 vs 100 ± 16.47, p > 0.05, Fig. 6C) thus suggesting that prolonged nicotine exposure does not impact axonal integrity in the NAc.

3.4. Prolonged nicotine exposure differently affects the of protein network dynamics in VTA and NAc

In light of the molecular alteration observed, we constructed Pearson's correlation matrix to investigate relationships among proteins potentially involved in oligodendrogenesis in the VTA and NAc, after prolonged nicotine exposure. The matrix illustrates Pearson's correlation coefficients, computed and plotted to show the relationships between the above-mentioned protein levels in the selected brain region. Interestingly, results showed that nicotine exposure via e-cigarettes induced area-specific alterations in protein networks of the two brain regions (Fig. 7A and B). Values for each Pearson's correlation coefficient and p-value are presented in Tables S1 and S2 (see Supplementary file).

4. Discussion

To shed new light on mechanisms contributing to neuronal adaptation events involved in nicotine addiction, this study assessed the impact of prolonged nicotine inhalation on oligodendrogenesis and some related neuropeptidergic system in the rat VTA and NAc, two crucial components of the reward circuitry. In particular, our findings suggest that nicotine exposure selectively alters the investigated parameters within the VTA, without affecting the same parameters in the NAc. This dysregulation may affect axonal conduction velocity and neurotransmitter release, so inducing long-lasting changes in the reward circuitry.

Interestingly, the data presented here indicate that nicotine, like other addictive substances, upregulates the neurotrophin *Bdnf* and its receptor *TrkB* within the VTA. This finding aligns with previous preclinical and clinical studies showing that smoking can influence BDNF levels both peripherally (Shafiee et al., 2024) and centrally (Huang et al., 2020). Nicotine-induced BDNF expression in the VTA may play a fundamental role in the synaptic plasticity underlying nicotine addiction. In this regard, recent findings suggest that chronic nicotine exposure

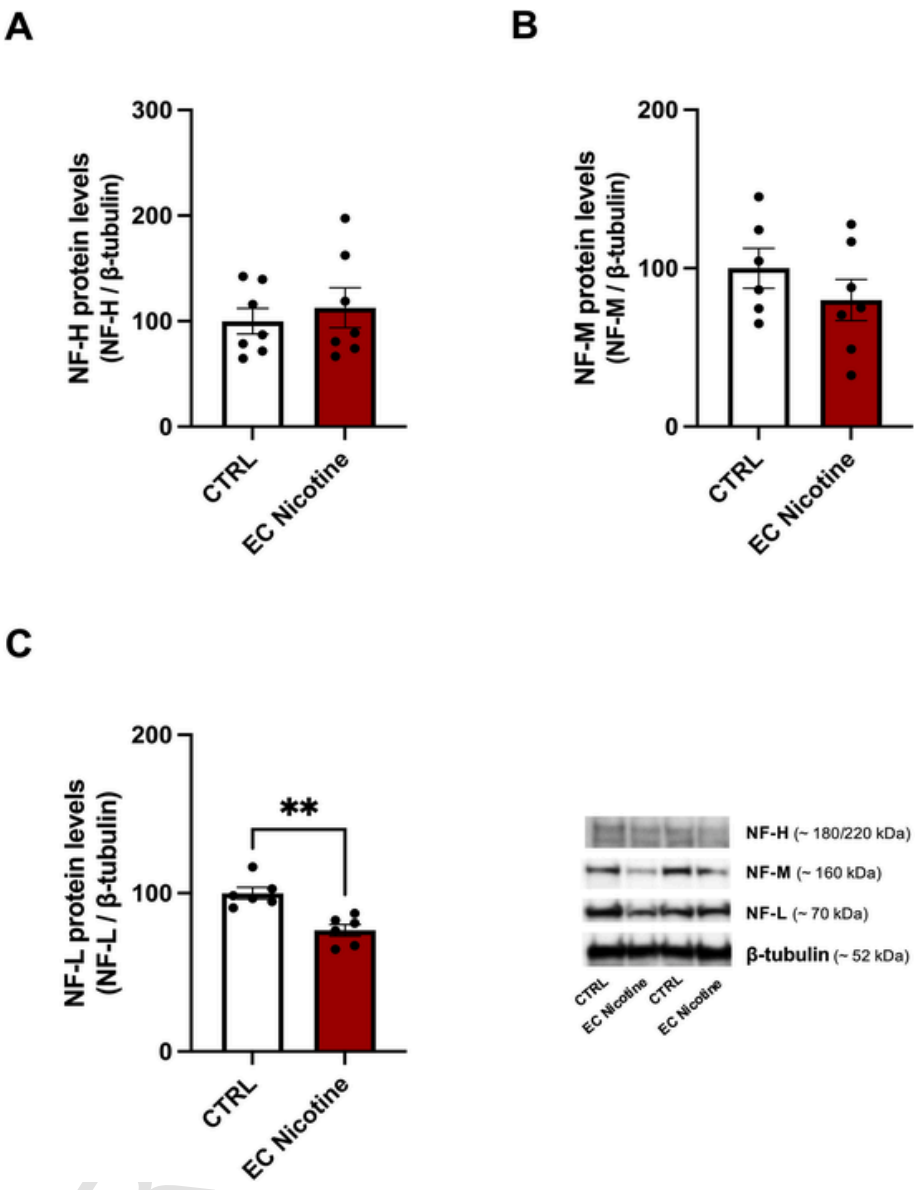

Fig. 3. Protein levels of NF-H (~180/220 kDa) (A), NF-M (~160 kDa) (B) and NF-L (~70 kDa) (C), normalized to β-tubulin (~52 kDa). A representative blot is reported alongside the histogram. Statistical significance was assessed using a two-tailed unpaired *t*-test. Data are presented as means ± SEM, n = 6–7/group, p* <0.05. One outlier in CTRL group (B, C) and EC nicotine group (C) were not included in data analysis.

heightens BDNF levels in the VTA and activates specific kinases which, in turn, remodel the expression of synaptic plasticity-related genes. These molecular changes lead to adaptive modifications in neuronal structure and function, ultimately facilitating the development of nicotine addiction (Bastianini et al., 2021; Fan et al., 2023). Furthermore, BDNF-TrkB signalling has been suggested to modulate oligodendrogenesis and myelination by promoting the proliferation and differentiation of OPCs into mature oligodendrocytes (Van't Veer et al., 2009). In this context, the increase in BDNF gene expression observed here may thus be involved in new myelin sheaths formation which in turn could promote the adaptive neuroplastic changes associated with prolonged nicotine exposure. In this regard, Yalçın et al. (2024) (Yalçın et al., 2024) recently highlighted the critical role of BDNFergic signalling in modulating the oligodendrogenesis process related to reward learning. The same authors also identified the dopaminergic neuronal activity-regulated myelin plasticity as a key circuit modification required for opioid reward.

Along with *Bdnf* and *TrkB* gene expression alterations, our results also showed an increase in DYN peptide levels within the VTA following nicotine exposure. Alterations in the dynorphin-KOR system have been reported to contribute to the development or expression of depressive, anxious, and addictive behaviours to different drugs of abuse (Caputi et al., 2014, 2016; D'Addario et al., 2013), including nicotine (Isola et al., 2009; Nygard et al., 2016). Interestingly, recent evidence identified dynorphin as a neuronal signal responsible for experience-dependent OPC differentiation and myelination. Specifically, it has been reported that stress stimuli can increase dynorphin release which in turn promotes oligodendrogenesis by activating oligodendroglia KORs (Osso et al., 2021). In this light, it is conceivable that besides BDNFergic role, the increase in DYN release within the VTA observed here could influence and promote drug-dependent myelin remodelling following prolonged nicotine exposure.

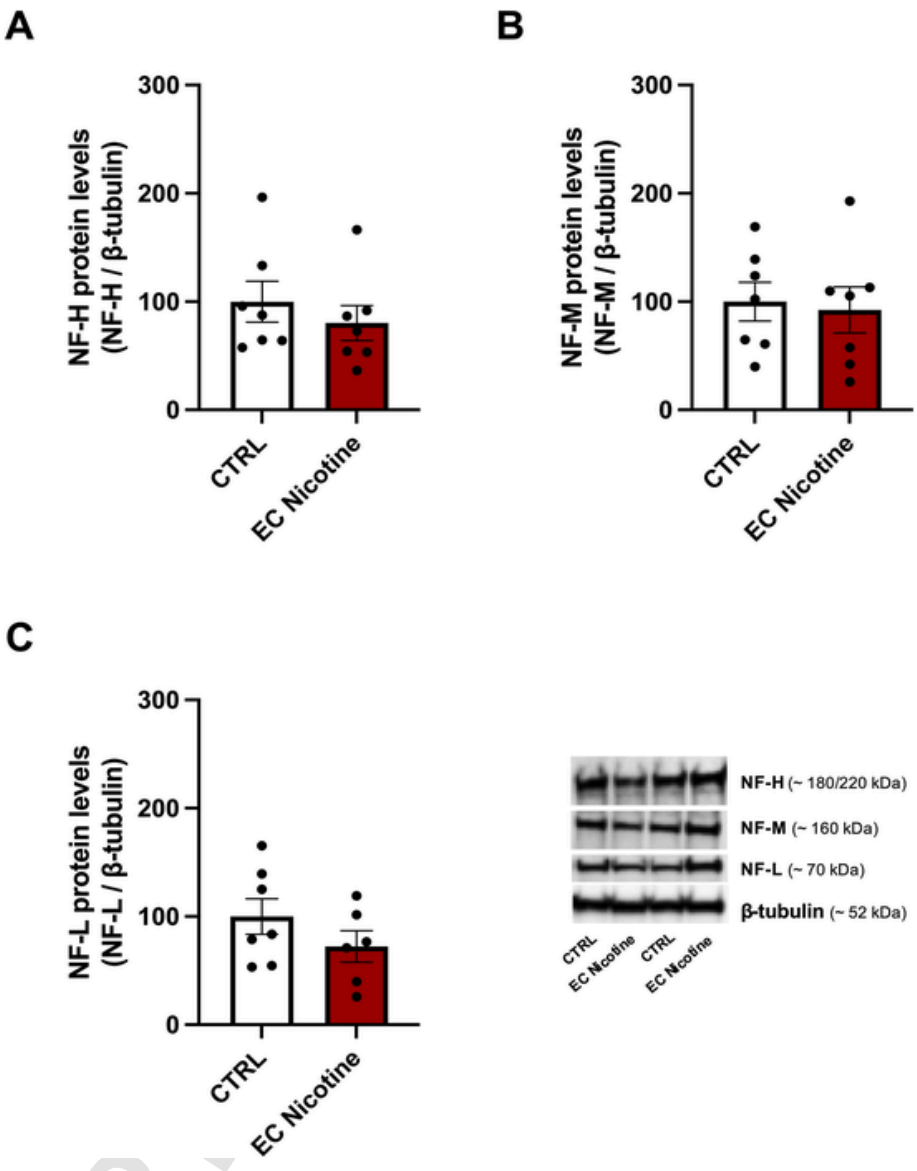

Fig. 6. Protein levels of NF-H (~180/220 kDa) (A), NF-M (~160 kDa) (B) and NF-L (~70 kDa) (C), normalized to β-tubulin (~52 kDa). A representative blot is reported alongside the histogram. Statistical significance was assessed using a two-tailed unpaired *t*-test. Data are presented as means ± SEM, n = 6–7/group. One outlier in EC nicotine group (C) was not included in data analysis.

In line with this hypothesis, nicotine inhalation from e-cigarettes might promote OPCs proliferation. In fact, an increase of OLIG2 protein levels was observed in the VTA of nicotine-exposed rats. OLIG2 is a transcription factor driving the expression of myelin-associated genes in oligodendrocytes and playing a crucial role in regulating key stages of early oligodendrocyte development (K. Zhang et al., 2022). It has been demonstrated that OLIG2 expression is epigenetically modulated by histone demethylase enzyme KDM6B (Sui et al., 2022). In this regard, our results showing an increase of *Kdm6b* gene expression may suggest the potential involvement of this epigenetic mechanism in the nicotine-induced OLIG2 increase. Notably, KDM6B also contributes to affecting activity-dependent neuronal adaptation and synaptic plasticity by modulating the release of glutamate and BDNF (Zhang et al., 2018; Swahari and West, 2019), which are both involved in the myelination process. In this respect, recent evidence highlighted that BDNF signalling, causes molecular switch to a drug-dependent-like motivational state by modifying the firing pattern of VTA dopamine neurons (Grieder et al., 2022). Moreover, nicotine administration, similar to other drugs of abuse, increases glutamate release onto DA neurons in the VTA, and induces glutamatergic synaptic plasticity which may be an important, early neuronal adaptation in nicotine reward and reinforcement (Gao et al., 2010).

Interestingly, our results also revealed a significant reduction in NF-L protein levels, despite no alterations in NF-M and NF-H proteins detected. Neurofilaments are critical cytoskeletal proteins essential for maintaining axonal integrity (Khalil et al., 2024) and are identified as a promising biomarker for substance use disorder (Requena-Ocaña et al., 2023; Zhu et al., 2024). In agreement with our finding, previous data highlighted nicotine's ability to alter the axonal distribution of NF-L within the VTA (Sbarbati et al., 2002). Thus, it is possible that alterations in the expression of proteins responsible for maintaining the neuronal cytoskeleton could participate in neuroadaptation of the mesolimbic dopaminergic pathway following prolonged nicotine exposure (Kovacs et al., 2010). In this light, it is possible that prolonged nicotine inhalation could compromise axonal integrity and induce adaptive plasticity by enhancing glutamate transmission. Indeed, nico-

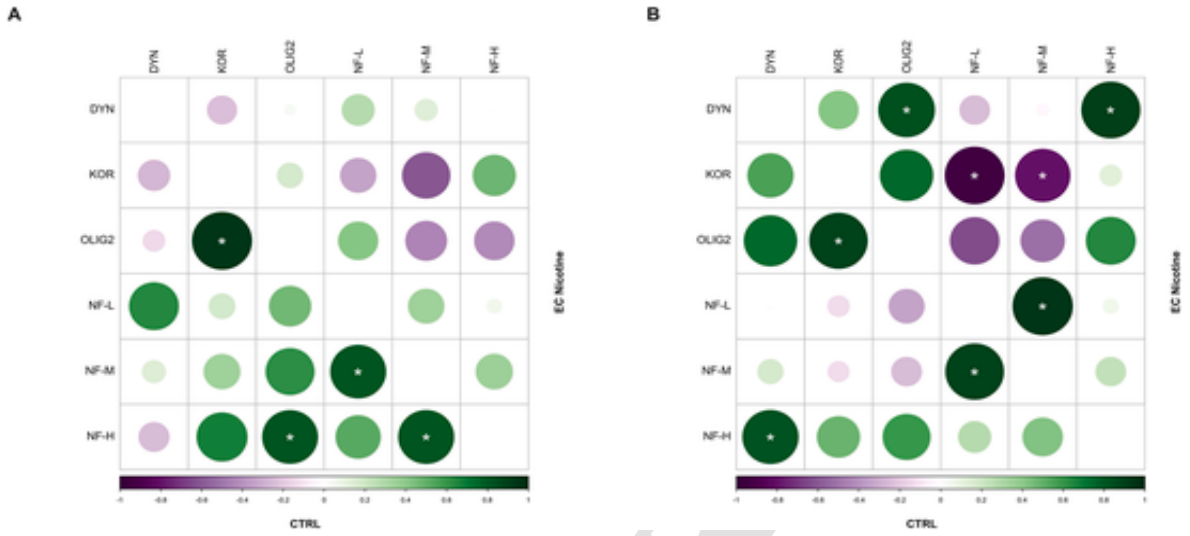

Fig. 7. Correlation matrix of DYN/KOR, OLIG2, and NFs protein levels in the VTA (A) and NAc (B) of rats. CTRL: bottom-left; EC Nicotine: top-right. Each pairwise comparison includes the correlation coefficient and p-value (n = 6–7 rats per group). Statistical significance was set at $p < 0.05$ (*). Correlation ranges from −1 to +1. Positive values (green) and negative values (violet) suggest positive and negative correlations, respectively. Color intensity and the size of the circle are indicative of the strength of the correlation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

tine exposure has been suggested to promote changes in glutamatergic signalling in brain mesocorticolimbic areas (D'Souza and Markou, 2013; Alasmari et al., 2016). This hypothesis is further supported by evidence showing that overactivation of glutamate receptors triggers rapid cytoskeletal changes, including the disruption of neurofilament proteins (Chung et al., 2005).

Unlike what was observed in the VTA, no significant changes in the expression of *Bdnf-TrkB* or DYN-KOR were detected in the NAc. Furthermore, nicotine exposure did not alter OLIG2, or its epigenetic regulatory modulator, *Kdm6b*, in this brain region, suggesting that the oligodendrogenesis process selectively occurs in the VTA. These findings are not surprising, since greater alterations in synaptic plasticity-related genes following chronic nicotine exposure (Fan et al., 2023; Morosini et al., 2024) have been reported in the VTA. Moreover, recent evidence demonstrates that morphine selectively promotes maladaptive myelination in the VTA (Yalçın et al., 2024). Similarly, nicotine may enhance dopaminergic neuronal activity and drive myelination within this area of the reward circuitry, ultimately facilitating reward learning and promoting nicotine dependence. Notably, the major occurrence of nicotine-induced oligodendrogenesis in VTA is further supported by protein correlation analysis, which clearly showed that prolonged nicotine exposure primarily affects the dynamics of protein networks involved in neuroplasticity within this specific brain region.

Given the higher prevalence of tobacco and nicotine product use among men (Higgins et al., 2015; Irvine et al., 2022; Vahratian et al., 2025), our molecular investigation primarily focused on male rats. However, numerous preclinical and clinical studies have demonstrated sex differences in various aspects of nicotine addiction, including distinct molecular and behavioural alterations (Flores et al., 2019; Lee et al., 2021; Pogun et al., 2017). Therefore, it is difficult to generalize our findings across sexes. Further investigations specifically addressing sex differences are still needed. In addition, it should be noted that the inclusion of immunohistochemical analyses could have provided additional support for our hypothesis and strengthened the interpretation of the findings. Further studies are warranted to better understand the relevance of the observed molecular results in the mechanisms underlying nicotine addiction.

In summary, this study provides evidence that prolonged nicotine exposure from e-cigarettes modulates oligodendrogenesis, likely by enhancing *Bdnf-TrkB*, DYN-KOR and *Kdm6b* expression in the VTA. Moreover, results highlight a decrease in NF-L protein levels in the same area. Overall, these changes underline the ability of nicotine to induce adaptive/maladaptive neuroplasticity that can influence axonal myelination and conduction velocity (Nowier et al., 2023) in selected regions of the mesolimbic reward circuitry.

Uncited references

; ; Osso and Hughes, 2024.

CRediT authorship contribution statement

Laura Rullo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Camilla Morosini:** Writing – review & editing, Writing – original draft, Methodology, Data curation. **Loredana Maria Losapio:** Writing – review & editing, Writing – original draft, Methodology, Data curation. **Fabio Vivarelli:** Writing – review & editing, Methodology, Funding acquisition. **Moreno Paolini:** Writing – review & editing. **Lucy C. Fairclough:** Writing – review & editing. **Donatella Canistro:** Writing – review & editing, Funding acquisition. **Patrizia Romualdi:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Sanzio Candeletti:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

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Declaration of competing interest

Authors declare that they have **no conflict of interest**.

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The Graphical Abstract was created in BioRender. https://BioRender.com/rstv0vu.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuropharm.2025.110540.

References

Alasmari, F., Al-Rejaie, S.S., AlSharari, S.D., Sari, Y., 2016. Targeting glutamate homeostasis for potential treatment of nicotine dependence. Brain Res. Bull. 121, 1–8. https://doi.org/10.1016/j.brainresbull.2015.11.010.

Barker, J.M., Taylor, J.R., De Vries, T.J., Peters, J., 2015. Brain-derived neurotrophic factor and addiction: pathological versus therapeutic effects on drug seeking. Brain Res. 1628 (Pt A), 68–81. https://doi.org/10.1016/j.brainres.2014.10.058.

Bastianini, S., Lo Martire, V., Alvente, S., Berteotti, C., Matteoli, G., Rullo, L., Stamatakos, S., Silvani, A., Candeletti, S., Romualdi, P., Cohen, G., Zoccoli, G., 2021. Early-life nicotine or cotinine exposure produces long-lasting sleep alterations and downregulation of hippocampal corticosteroid receptors in adult mice. Sci. Rep. 11 (1), 23897. https://doi.org/10.1038/s41598-021-03468-5.

Bavato, F., Kexel, A.-K., Kluwe-Schiavon, B., Maceski, A., Baumgartner, M.R., Seifritz, E., Kuhle, J., Quednow, B.B., 2023. A longitudinal investigation of blood neurofilament light chain levels in chronic cocaine users. Mol. Neurobiol. 60 (7), 3935–3944. https://doi.org/10.1007/s12035-023-03327-6.

Boccella, S., Fusco, A., Ricciardi, F., Morace, A.M., Bonsale, R., Perrone, M., Marabese, I., De Gregorio, D., Belardo, C., Posa, L., Rullo, L., Piscitelli, F., di Marzo, V., Nicois, A., Marfella, B., Cristino, L., Luongo, L., Guida, F., Candeletti, S., et al., 2025. Acute kappa opioid receptor blocking disrupts the pro-cognitive effect of cannabidiol in neuropathic rats. Neuropharmacology 266, 110265. https://doi.org/10.1016/j.neuropharm.2024.110265.

Budriesi, R., Vivarelli, F., Canistro, D., Aldini, R., Babot Marquillas, C., Corazza, I., Fato, R., Cirillo, S., Bergamini, C., D'Errico, A., Bolchi, C., Cevenini, M., Degiovanni, A., Frosini, M., Camarda, L., Chiarini, A., Micucci, M., 2018. Liver and intestinal protective effects of Castanea sativa Mill. bark extract in high-fat diet rats. PLoS One 13 (8), e0201540. https://doi.org/10.1371/journal.pone.0201540.

Bruchas, M.R., Land, B.B., Chavkin, C., 2010. The dynorphin/kappa opioid system as a modulator of stress-induced and pro-addictive behaviors. Brain Res. 1314, 44–55. https://doi.org/10.1016/j.brainres.2009.08.062.

Canistro, D., Vivarelli, F., Cirillo, S., Babot Marquillas, C., Buschini, A., Lazzaretti, M., Marchi, L., Cardenia, V., Rodriguez-Estrada, M.T., Lodovici, M., Cipriani, C., Lorenzini, A., Croco, E., Marchionni, S., Franchi, P., Lucarini, M., Longo, V., Della Croce, C.M., Vornoli, A., et al., 2017. E-cigarettes induce toxicological effects that can raise the cancer risk. Sci. Rep. 7 (1), 2028. https://doi.org/10.1038/s41598-017-02317-8.

Caputi, F.F., Caffino, L., Candeletti, S., Fumagalli, F., Romualdi, P., 2019a. Short-term withdrawal from repeated exposure to cocaine during adolescence modulates dynorphin mRNA levels and BDNF signaling in the rat nucleus accumbens. Drug Alcohol Depend. 197, 127–133. https://doi.org/10.1016/j.drugalcdep.2019.01.006.

Caputi, F.F., Di Benedetto, M., Carretta, D., Bastias del Carmen Candia, S., D'Addario, C., Cavina, C., Candeletti, S., Romualdi, P., 2014. Dynorphin/KOP and nociceptin/NOP gene expression and epigenetic changes by cocaine in rat striatum and nucleus accumbens. Prog. Neuropsychopharmacol. Biol. Psychiatry 49, 36–46. https://doi.org/10.1016/j.pnpbp.2013.10.016.

Caputi, F.F., Di Cesare Mannelli, L., Rullo, L., Micheli, L., Stamatakos, S., Posa, L., Ghelardini, C., Romualdi, P., Candeletti, S., 2020. The active second-generation proteasome inhibitor oprozomib reverts the oxaliplatin-induced neuropathy symptoms. Biochem. Pharmacol. 182, 114255. https://doi.org/10.1016/j.bcp.2020.114255.

Caputi, F.F., Palmisano, M., Carboni, L., Candeletti, S., Romualdi, P., 2016. Opioid gene expression changes and post-translational histone modifications at promoter regions in the rat nucleus accumbens after acute and repeated 3,4-methylenedioxy-methamphetamine (MDMA) exposure. Pharmacol. Res. 114, 209–218. https://doi.org/10.1016/j.phrs.2016.10.023.

Caputi, F.F., Rullo, L., Stamatakos, S., Candeletti, S., Romualdi, P., 2019b. Modulation of the negative affective dimension of pain: focus on selected neuropeptidergic system contributions. Int. J. Mol. Sci. 20 (16), 4010. https://doi.org/10.3390/ijms20164010.

Caputi, F.F., Stopponi, S., Rullo, L., Palmisano, M., Ubaldi, M., Candeletti, S., Ciccocioppo, R., Romualdi, P., 2021. Dysregulation of nociceptin/orphanin FQ and dynorphin systems in the extended amygdala of alcohol preferring marchigian Sardinian (msP) rats. Int. J. Mol. Sci. 22 (5), 2448. https://doi.org/10.3390/ijms22052448.

Chen, G., Rahman, S., Lutfy, K., 2023. E-cigarettes may serve as a gateway to conventional cigarettes and other addictive drugs. Adv. Drug Alcohol Res. 3, 11345. https://doi.org/10.3389/adar.2023.11345.

Chomczynski, P., Sacchi, N., 2006. The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. Nat. Protoc. 1 (2), 581–585. https://doi.org/10.1038/nprot.2006.83.

Chung, R.S., McCormack, G.H., King, A.E., West, A.K., Vickers, J.C., 2005. Glutamate induces rapid loss of axonal neurofilament proteins from cortical neurons in vitro. Exp. Neurol. 193 (2), 481–488. https://doi.org/10.1016/j.expneurol.2005.01.005.

D'Addario, C., Caputi, F.F., Rimondini, R., Gandolfi, O., Del Borrello, E., Candeletti, S., Romualdi, P., 2013. Different alcohol exposures induce selective alterations on the expression of dynorphin and nociceptin systems related genes in rat brain. Addict. Biol. 18 (3), 425–433. https://doi.org/10.1111/j.1369-1600.2011.00326.x.

Dani, J.A., Ji, D., Zhou, F.M., 2001. Synaptic plasticity and nicotine addiction. Neuron (Camb., Mass.) 31 (3), 349–352. https://doi.org/10.1016/s0896-6273(01)00379-8.

D'Souza, M.S., Markou, A., 2013. The «stop» and «go» of nicotine dependence: role of GABA and glutamate. Cold Spring Harb. Perspect. Med. 3 (6), a012146. https://doi.org/10.1101/cshperspect.a012146.

Fan, L., Chen, H., Liu, Y., Hou, H., Hu, Q., 2023. ERK signaling is required for nicotine-induced conditional place preference by regulating neuroplasticity genes expression in male mice. Pharmacol. Biochem. Behav. 222, 173510. https://doi.org/10.1016/j.pbb.2022.173510.

Fletcher, J.L., Makowiecki, K., Cullen, C.L., Young, K.M., 2021. Oligodendrogenesis and myelination regulate cortical development, plasticity and circuit function. Semin. Cell Dev. Biol. 118, 14–23. https://doi.org/10.1016/j.semcdb.2021.03.017.

Flores, R.J., Uribe, K.P., Swalve, N., O'Dell, L.E., 2019. Sex differences in nicotine intravenous self-administration: a meta-analytic review. Physiol. Behav. 203, 42–50. https://doi.org/10.1016/j.physbeh.2017.11.017. . 2019 May 1.

Gao, M., Jin, Y., Yang, K., Zhang, D., Lukas, R.J., Wu, J., 2010. Mechanisms involved in systemic nicotine-induced glutamatergic synaptic plasticity on dopamine neurons in the ventral tegmental area. J. Neurosci. 30 (41), 13814–13825. https://doi.org/10.1523/JNEUROSCI.1943-10.2010.

Gowrishankar, R., Gomez, A., Waliki, M., Bruchas, M.R., 2022. Kappa-opioid receptor activation reinstates nicotine self-administration in mice. Addict. Neurosci. 2, 100017. https://doi.org/10.1016/j.addicn.2022.100017.

Grieder, T.E., Yee, M., Vargas-Perez, H., Maal-Bared, G., George, S., Ting-A-Kee, R., George, O., van der Kooy, D., 2022. Administration of BDNF in the ventral tegmental area produces a switch from a nicotine-non-dependent D1R-mediated motivational state to a nicotine-dependent-like D2R-mediated motivational state. Eur. J. Neurosci. 55 (3), 714–724. https://doi.org/10.1111/ejn.15579.

Higgins, S.T., Kurti, A.N., Redner, R., White, T.J., Gaalema, D.E., Roberts, M.E., Doogan, N.J., Tidey, J.W., Miller, M.E., Stanton, C.A., Henningfield, J.E., Atwood, G.S., 2015. A literature review on prevalence of gender differences and intersections with other vulnerabilities to tobacco use in the United States, 2004-2014. Prev. Med. 80, 89–100. https://doi.org/10.1016/j.ypmed.2015.06.009. . 2015 Nov.

Huang, Z., Wu, D., Qu, X., Li, M., Zou, J., Tan, S., 2020. BDNF and nicotine dependence: associations and potential mechanisms. Rev. Neurosci. https://doi.org/10.1515/revneuro-2020-0044.

Irvine, D.S., Lee, E.Y., Janssen, I., Leatherdale, S.T., 2022. Gendered associations between e-cigarette use, cigarette smoking, physical activity, and sedentary behaviour in a sample of Canadian adolescents. Dialogues Health 1, 100029. https://doi.org/10.1016/j.dialog.2022.100029. . 2022 Jul 11.

Isola, R., Zhang, H., Tejwani, G.A., Neff, N.H., Hadjiconstantinou, M., 2009. Acute nicotine changes dynorphin and prodynorphin mRNA in the striatum. Psychopharmacology 201 (4), 507–516. https://doi.org/10.1007/s00213-008-1315-4.

Khalil, M., Teunissen, C.E., Lehmann, S., Otto, M., Piehl, F., Ziemssen, T., Bittner, S., Sormani, M.P., Gattringer, T., Abu-Rumeileh, S., Thebault, S., Abdelhak, A., Green, A., Benkert, P., Kappos, L., Comabella, M., Tumani, H., Freedman, M.S., Petzold, A., et al., 2024. Neurofilaments as biomarkers in neurological disorders—towards clinical application. Nat. Rev. Neurol. 20 (5), 269–287. https://doi.org/10.1038/s41582-024-00955-x.

Kovacs, K., Lajtha, A., Sershen, H., 2010. Effect of nicotine and cocaine on neurofilaments and receptors in whole brain tissue and synaptoneurosome preparations. Brain Res. Bull. 82 (1–2), 109–117. https://doi.org/10.1016/j.brainresbull.2010.02.008.

Lee, A.M., Mansuri, M.S., Wilson, R.S., Lam, T.T., Nairn, A.C., Picciotto, M.R., 2021. Sex differences in the ventral tegmental area and nucleus accumbens proteome at baseline and following nicotine exposure. Front. Mol. Neurosci. 14, 657064. https://doi.org/10.3389/fnmol.2021.657064. . 2021 Jul 14.

Lorente, J.D., Cuitavi, J., Rullo, L., Candeletti, S., Romualdi, P., Hipólito, L., 2024. Sex-dependent effect of inflammatory pain on negative affective states is prevented by kappa opioid receptors blockade in the nucleus accumbens shell. Neuropharmacology 242, 109764. https://doi.org/10.1016/j.neuropharm.2023.109764.

Marguet, F., Brosolo, M., Friocourt, G., Sauvestre, F., Marcorelles, P., Lesueur, C., Marret, S., Gonzalez, B.J., Laquerrière, A., 2022. Oligodendrocyte lineage is severely affected in human alcohol-exposed foetuses. Acta Neuropathol. Commun. 10 (1), 74. https://doi.org/10.1186/s40478-022-01378-9.

Martin, J.A., Caccamise, A., Werner, C.T., Viswanathan, R., Polanco, J.J., Stewart, A.F., Thomas, S.A., Sim, F.J., Dietz, D.M., 2018. A novel role for oligodendrocyte precursor cells (OPCs) and Sox10 in mediating cellular and behavioral responses to heroin. Neuropsychopharmacology 43 (6), 1385–1394. https://doi.org/10.1038/npp.2017.303.

Mei, F., Mayoral, S.R., Nobuta, H., Wang, F., Desponts, C., Lorrain, D.S., Xiao, L., Green, A.J., Rowitch, D., Whistler, J., Chan, J.R., 2016. Identification of the kappa-opioid receptor as a therapeutic target for oligodendrocyte remyelination. J. Neurosci. 36 (30), 7925–7935. https://doi.org/10.1523/JNEUROSCI.1493-16.2016.

Morosini, C., Vivarelli, F., Rullo, L., Volino, E., Losapio, L.M., Paolini, M., Romualdi, P., Canistro, D., Candeletti, S., 2024. Unburned tobacco smoke affects neuroinflammation-related pathways in the rat mesolimbic system. Int. J. Mol. Sci. 25 (10), 5259. https://doi.org/10.3390/ijms25105259.

Mount, C.W., Monje, M., 2017. Wrapped to adapt: experience-dependent myelination. Neuron (Camb., Mass.) 95 (4), 743–756. https://doi.org/10.1016/j.neuron.2017.07.009.
Nikulina, E.M., Johnston, C.E., Wang, J., Hammer, R.P., 2014. Neurotrophins in the ventral tegmental area: role in social stress, mood disorders and drug abuse. Neuroscience 282, 122–138. https://doi.org/10.1016/j.neuroscience.2014.05.028.
Nowier, R.M., Friedman, A., Brown, A., Jung, P., 2023. The role of neurofilament transport in the radial growth of myelinated axons. Mol. Biol. Cell 34 (6), ar58. https://doi.org/10.1091/mbc.E22-12-0565.
Nygard, S.K., Hourguettes, N.J., Sobczak, G.G., Carlezon, W.A., Bruchas, M.R., 2016. Stress-induced reinstatement of nicotine preference requires dynorphin/kappa opioid activity in the basolateral amygdala. J. Neurosci. 36 (38), 9937–9948. https://doi.org/10.1523/JNEUROSCI.0953-16.2016.
Osso, L.A., Hughes, E.G., 2024. Dynamics of mature myelin. Nat. Neurosci. 27 (8), 1449–1461. https://doi.org/10.1038/s41593-024-01642-2.
Osso, L.A., Rankin, K.A., Chan, J.R., 2021. Experience-dependent myelination following stress is mediated by the neuropeptide dynorphin. Neuron (Camb., Mass.) 109 (22), 3619–3632.e5. https://doi.org/10.1016/j.neuron.2021.08.015.
Paxinos, G., Watson, C., 1982. The Rat Brain in Stereotaxic Coordinates. Academic Press, p. iii. https://doi.org/10.1016/B978-0-12-547620-1.50001-1.
Pepper, R.E., Pitman, K.A., Cullen, C.L., Young, K.M., 2018. How do cells of the oligodendrocyte lineage affect neuronal circuits to influence motor function, memory and mood? Front. Cell. Neurosci. 12, 399. https://doi.org/10.3389/fncel.2018.00399.
Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W.J., Clark, A., Cuthill, I.C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S.T., Howells, D.W., Karp, N.A., Lazic, S.E., Lidster, K., MacCallum, C.J., Macleod, M., et al., 2020. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. PLoS Biol. 18 (7), e3000410. https://doi.org/10.1371/journal.pbio.3000410.
Placzek, A.N., Zhang, T.A., Dani, J.A., 2009. Nicotinic mechanisms influencing synaptic plasticity in the hippocampus. Acta Pharmacol. Sin. 30 (6), 752–760. https://doi.org/10.1038/aps.2009.39.
Pogun, S., Yararbas, G., Nesil, T., Kanit, L., 2017. Sex differences in nicotine preference. J. Neurosci. Res. 95 (1–2), 148–162. https://doi.org/10.1002/jnr.23858. . 2017 Jan 2.
Requena-Ocaña, N., Araos, P., Serrano-Castro, P.J., Flores-López, M., García-Marchena, N., Oliver-Martos, B., Ruiz, J.J., Gavito, A., Pavón, F.J., Serrano, A., Mayoral, F., Suarez, J., Fonseca, F. R. de, 2023. Plasma concentrations of neurofilament light chain protein and brain-derived neurotrophic factor as consistent biomarkers of cognitive impairment in alcohol use disorder. nt. J. Mol. Sci. 24 (2). https://doi.org/10.3390/ijms24021183. . Articolo 2.
Rodríguez-Carrillo, A., Verheyen, V.J., Van Nuijs, A.L.N., Fernández, M.F., Remy, S., 2023. Brain-derived neurotrophic factor (BDNF): an effect biomarker of neurodevelopment in human biomonitoring programs. Front. Toxicol. 5, 1319788. https://doi.org/10.3389/ftox.2023.1319788.
Rullo, L., Franchi, S., Amodeo, G., Caputi, F.F., Verduci, B., Losapio, L.M., Sacerdote, P., Romualdi, P., Candeletti, S., 2021. Interplay between prokineticins and histone demethylase KDM6A in a murine model of bortezomib-induced neuropathy. Int. J. Mol. Sci. 22 (21), 11913. https://doi.org/10.3390/ijms222111913.
Salari, N., Rahimi, S., Darvishi, N., Abdolmaleki, A., Mohammadi, M., 2024. The global prevalence of E-cigarettes in youth: a comprehensive systematic review and meta-analysis. Public Health Pract (Oxf). 16 (7), 100506. https://doi.org/10.1016/j.puhip.2024.100506.
Sbarbati, A., Bunnemann, B., Cristofori, P., Terron, A., Chiamulera, C., Merigo, F., Benati, D., Bernardi, P., Osculati, F., 2002. Chronic nicotine treatment changes the axonal distribution of 68 kDa neurofilaments in the rat ventral tegmental area. Eur. J. Neurosci. 16 (5), 877–882. https://doi.org/10.1046/j.1460-9568.2002.02167.x.
Shafiee, A., Beiky, M., Mohammadi, I., Rajai, S., Jafarabady, K., Moradi, S., Beikmohamadi, M., Teixeira, A.L., 2024. Effect of smoking on Brain-Derived Neurotrophic Factor (BDNF) blood levels: a systematic review and meta-analysis. J. Affect. Disord. 349, 525–533. https://doi.org/10.1016/j.jad.2024.01.082.
Sui, A., Han, B., Ren, W., He, W., Gao, C., Han, X., Liu, S., Zhang, Y., Qi, X., Guo, X., 2022. The epigenetic regulation of OLIG2 by histone demethylase KDM6B in glioma cells. J. Mol. Neurosci. 72 (5), 939–946. https://doi.org/10.1007/s12031-022-01976-1.
Swahari, V., West, A.E., 2019. Histone demethylases in neuronal differentiation, plasticity, and disease. Curr. Opin. Neurobiol. 59, 9–15. https://doi.org/10.1016/j.conb.2019.02.009.
Vahratian, A., Briones, E.M., Jamal, A., Marynak, K.L., 2025. Electronic cigarette use among adults in the United States, 2019-2023. NCHS Data Brief 524 (2025 Jan), CS356607. https://doi.org/10.15620/cdc/174583.
Van't Veer, A., Du, Y., Fischer, T.Z., Boetig, D.R., Wood, M.R., Dreyfus, C.F., 2009. Brain-derived neurotrophic factor effects on oligodendrocyte progenitors of the basal forebrain are mediated through trkB and the MAP kinase pathway. J. Neurosci. Res. 87 (1), 69–78. https://doi.org/10.1002/jnr.21841.
Velasco, B., Mohamed, E., Sato-Bigbee, C., 2021. Endogenous and exogenous opioid effects on oligodendrocyte biology and developmental brain myelination. Neurotoxicol. Teratol. 86, 107002. https://doi.org/10.1016/j.ntt.2021.107002.
Vivarelli, F., Canistro, D., Cirillo, S., Cardenia, V., Rodriguez-Estrada, M.T., Paolini, M., 2019. Impairment of testicular function in electronic cigarette (e-cig, e-cigs) exposed rats under low-voltage and nicotine-free conditions. Life Sci. 228, 53–65. https://doi.org/10.1016/j.lfs.2019.04.059.
Vivarelli, F., Morosini, C., Rullo, L., Losapio, L.M., Lacorte, A., Sangiorgi, S., Ghini, S., Fagiolino, I., Franchi, P., Lucarini, M., Candeletti, S., Canistro, D., Romualdi, P., Paolini, M., 2024. Effects of unburned tobacco smoke on inflammatory and oxidative mediators in the rat prefrontal cortex. Front. Pharmacol. 15. https://doi.org/10.3389/fphar.2024.1328917.
Volkow, N.D., Michaelides, M., Baler, R., 2019. The neuroscience of drug reward and addiction. Physiol. Rev. 99 (4), 2115–2140. https://doi.org/10.1152/physrev.00014.2018.
Yalçın, B., Pomrenze, M.B., Malacon, K., Drexler, R., Rogers, A.E., Shamardani, K., Chau, I.J., Taylor, K.R., Ni, L., Contreras-Esquivel, D., Malenka, R.C., Monje, M., 2024. Myelin plasticity in the ventral tegmental area is required for opioid reward. Nature 630 (8017), 677–685. https://doi.org/10.1038/s41586-024-07525-7.
Zhang, K., Chen, S., Yang, Q., Guo, S., Chen, Q., Liu, Z., Li, L., Jiang, M., Li, H., Hu, J., Pan, X., Deng, W., Xiao, N., Wang, B., Wang, Z.-X., Zhang, L., Mo, W., 2022. The Oligodendrocyte Transcription Factor 2 OLIG2 regulates transcriptional repression during myelinogenesis in rodents. Nat. Commun. 13 (1), 1423. https://doi.org/10.1038/s41467-022-29068-z.
Zhang, Y.-X., Akumuo, R.C., España, R.A., Yan, C.-X., Gao, W.-J., Li, Y.-C., 2018. The histone demethylase KDM6B in the medial prefrontal cortex epigenetically regulates cocaine reward memory. Neuropharmacology 141, 113–125. https://doi.org/10.1016/j.neuropharm.2018.08.030.
Zhu, N., Zhu, J., Lin, S., Yu, H., Cao, C., 2024. Correlation analysis between smoke exposure and serum neurofilament light chain in adults: a cross-sectional study. BMC Public Health 24 (1), 353. https://doi.org/10.1186/s12889-024-17811-8.