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Frontal Cortex Proteome Perturbation after Juvenile Rat Secondhand Smoke Exposure

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Abstract

Secondhand smoke remains a global concern for children's health. Epidemiological studies implicate exposure to secondhand smoke as a major risk factor for behavioral disorders, yet biological causation remains unclear. Model studies have mainly focused on secondhand smoke impacts to prenatal neurodevelopment, yet juvenile exposure represent a separate risk. Using ion mobility-enhanced data-independent mass spectrometry we characterized the effect of juvenile secondhand smoke exposure on the prefrontal cortex, a principal part of the brain involved in behavioral control. The produced dataset includes 800 significantly responsive proteins at a FDR 5% within the juvenile orbital frontal cortex, with 716 showing an increase in abundance. The neuroproteomic response reflects a prominent perturbation within the glutamatergic synaptic system, suggesting aberrant, disorganized excitation as observed underlying psychiatric disorders. The neuroproteomic response also discloses impacts to GABAergic and dopaminergic systems. Overall, the dataset provides a wealth of detail, facilitating further targeted research into the causal mechanisms underlying behavioral disorders associated with juvenile exposure to secondhand smoke and other environmental pollutants. All MS data have been deposited to the ProteomeXchange consortium with identifier PXD007690.

Keywords

Environmental exposure; Neurodevelopment; Behavioral Disorders; Secondhand Smoke; Environmental Tobacco Smoke; SHS; ETS; Brain; Neurotransmission

Environmental tobacco smoke (ETS), also known as secondhand smoke, remains a prevalent developmental health concern with approximately 40% of children susceptible worldwide [1,2]. Children exposed to ETS demonstrate an increased incidence of neurobehavioral disorders with deficits in attention, impulsivity, antisocial behavior, and increased anxiety [2-4]. With mounting evidence establishing that early-life ETS exposure correlates with increase health burdens for children, modeled animal exposure studies under controlled

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Conflict of Interest

The authors have declared no conflict of interest.

Supporting Information

Supporting information is available from the Wiley Online Library or from the author.

conditions are needed explicate a causal link between passive ETS exposure and neurodevelopment manifestations [5,6].

Addressing this, we devised a rat model to assess the impact of ETS exposure on neurodevelopmental aspects occurring between birth and the preschool years in children [7]. After adjusting for altricial differences in development, we arrived at a postnatal day (PD) 8 through 22 exposure period in the rat. The model was refined from prior perinatal ETS exposure schemes [8,9] to differentiate ETS impacts on juvenile circuit refinement from earlier development predominated by cellular division and migration [10]. Daily ETS exposure was adjusted to a mean of 100 $\mu\text{g}/\text{m}^3$ total suspended particulate to fall in-line with children's bedroom measures in homes with a pack-a-day smoker [11]. The model produced behavior impairment reminiscent of deficits reported in children, including activity, impulse, and attentional control impairments. The observed ETS phenotype implicated a variety of neuroanatomical structures. Cerebellum had been suggested as a principal area of perturbation [8], with cerebellar abnormalities reported in ETS exposed children [12]. Thus, earlier we assessed the juvenile cerebellum with unbiased proteomics and identified a predominant ETS influence on mitochondrial energetics and biogenesis [7], implicating abnormal coalescent cerebellar output, and thus maladaptive juvenile activity-dependent learning and cortical refinement [13,14]. Yet, it was not known whether ETS-induced mitochondrial effects were selectively cerebellar-dependent.

Regions of prefrontal cortex (PFC) mediate cognitive control and value-based decision making [13] in addressing impulses and compulsions [15]. In particular, the orbital frontal cortex (OFC) has been associated with deficits in controlling behavior [16]. The OFC and other PFC domains receive regulatory feedback via a cerebello-thalamo-cortical loop [13] to refine attentional, activity and behavioral control [17]; thus, it is possible that abnormal transmission from cerebellum mediates OFC dysfunction without a direct ETS impact on cortical circuitry. A recent study supports a predominant impact on hindbrain structures like cerebellum and brainstem when assessing altered myelination following inflated ETS exposure [18]. Yet, the same group separately observed that ETS influenced synaptic organization within the hippocampus, an area with no direct cerebellar feedback [19]. Recently researchers at Duke reported that the PFC was vulnerable *in utero* to doses of ETS condensate, which they directly contrasted with nicotine alone [6,20]. They determined that ETS condensate was more bioactive than nicotine in driving synaptic abnormality in the PFC. Thus, in this study we aimed to determine whether ETS further alters postnatal neurodevelopment within the PFC at a household-relevant level of exposure, generating a proteomic dataset to guide targeted studies on the causal relationship between environmental exposure and juvenile deficits. Data-independent mass spectrometry was employed for an unbiased assessment of ETS's molecular impact on the OFC.

Mated Sprague-Dawley dams (Harlan, Indianapolis, IN) and their litters were used in this study in accordance with US Public Health Service and National Institutes of Health policies and guidelines. Litters were exposed to ETS or room air for 6 h/day from PD 8 to 22 in a Teague TE-10 smoking system (Teague Enterprises, Woodland, CA) as described previously [7]. Exposure was regulated to a mean daily average of 100 $\mu\text{g}/\text{m}^3$ total suspended particulate, producing 3.5 ppm carbon monoxide and 26 ± 7.5 ng/mL blood cotinine. Brains

[25–27]. Precisely 800 proteins (37.5%) were significantly responsive to juvenile ETS exposure at a 5% FDR (Table 1, Supporting Information), with 716 increased and 84 decreased (Figure 1A). Half of the increased proteins had an effect size of 50% change or greater (Figure 1B), denoting a substantial neuronal protein perturbation. Upregulated proteins were enriched in association with neurons and associated synapses, while a smaller cohort related to the myelin sheath which is only beginning to form at this age (Figure 1C). In contrast, downregulated proteins were predominantly mitochondrial associated (Figure 1D). Among the increased cohort, dendritic (110; GO:0030425; $p=6.5e-102$) and axonal (108; GO:0030424; $p=6.6e-101$) proteins were equally represented, along with an enriched number of growth cone related proteins (31; GO:0030426; $p=3.6e-26$). Distinctly, the data were significantly enriched with 48 proteins from the axon guidance pathway (Reactome database; ID 1270303; $p=2.5e-21$), implicating an ETS effect on juvenile OFC circuit formation. Further assessment showed a near equal representation of presynaptic (76; GO:0098793; $p=2.0e-87$) and postsynaptic (95; GO:0098794; $p=9.9e-110$) related proteins as responsive to ETS, indicating an increase in protein mass for whole synapses, and not just greater presynaptic activity. The breakdown of ETS-altered synaptic proteins showed the greatest enrichment in connection with glutamatergic synapses (26; KEGG ID hsa04724; $p=2.0e-32$), though the data were also enriched in proteins related to GABAergic (16; KEGG ID hsa04727; $p=1.4e-12$), cholinergic (16; KEGG ID hsa04725; $p=6.4e-11$), and dopaminergic (17; KEGG ID hsa04728; $p=6.7e-11$) synapses.

Results here indicate a significant shift in OFC excitation following juvenile ETS exposure. Torres *et al.* previously saw a shift in excitatory synaptic proteins in hippocampal sections of juvenile rats, though at blood cotinine levels 4-fold higher than modeled here and atypically high for ETS exposure [19]. Data here affirm that the glutamatergic system is perturbed at house-hold relevant levels of ETS within the OFC, a region directly associated with behavioral control. AMPA, NMDA and mGluR glutamatergic receptors were represented. AMPA receptors such as GluR2 are known to peak in abundance around PD 24, the time of tissue collection in this study, suggesting a particular vulnerability [28]. Assessing GluR2 immunostaining in OFC, we saw a significant 1.5-fold increase ($p=0.0055$) with ETS prominently in the lower laminae (IV-V) that receive cerebello-thalamic input (green staining, Figure 2A,C). We further observed an anatomically correlative 1.9-fold increase ($p=0.0086$) in Vglut1 immunostaining, a marker of synaptic vesicles within glutamatergic synapses and an indicator of heightened neurotransmission (red staining, Figure 2A,C). Immunochemical results agreed well with the OFC proteomic findings of increased GluR2 (1.7-fold; found as Gria2 in Table 1, Supporting Information) and Vglut1 (1.9-fold; found as Slc17a7 in Table 1, Supporting Information) with ETS exposure. These results contrasted with a marginal decrease in GluR2 and VGlut1 observed in the cerebellum, pointing to regional specificity among domains with differing contributions to higher-order control functions (Figure 2B,C). Enhanced, disorganized cortical excitation as reported here in the OFC is known to underlie psychiatric disorders and behavioral deficits [29].

Enrichment analysis further associated the PFC neuroproteomic response with an abnormal cognitive phenotype (Figure 2D). This fits with the PFC's role in processing cognitive behavioral control in connection with deficits seen in ETS exposed children [5,10], and reported previously in this animal model [7]. Results here substantiate PFC vulnerability to

ETS at a house-hold relevant levels during juvenile neurodevelopment. The impact of ETS as related to behavioral control thus extends beyond our earlier findings in the cerebellum [7], and speak to impacts directly tied to the OFC and possibly other domains of the PFC. A subsequent manuscript will report on additional findings in this model that demonstrate significant deficits in disinhibited behavior connected with the OFC along with decreased attention and socialization involving other PFC regions. Furthermore, the PFC neuroproteomic response to ETS was strikingly different than that in cerebellum, impacting principally an upregulation of the glutamatergic synaptic network, though accompanied by altered GABAergic and dopaminergic systems. Mitochondrial impacts that dominated in the cerebellum were less prominent in PFC and the cerebellar glutamatergic network was appreciably less impacted than what was observed in OFC. The present proteomic dataset thus serves to support further research to characterize the broader molecular and neurobiological implications of maladaptive neurodevelopment in the juvenile PFC in response to ETS and other environmental exposures.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ETS	environmental tobacco smoke
OFC	orbital frontal cortex
PD	postnatal day
PFC	prefrontal cortex

References

- [1]. Öberg M, Jaakkola MS, Woodward A, Peruga A, Prüss-Ustün A, Lancet 2011, 377, 139. [PubMed: 21112082]
- [2]. Hwang S-H, Hwang JH, Moon JS, Lee D-H, Korean J. Pediatr. 2012, 55, 35. [PubMed: 22375147]
- [3]. Chastang J, Baiz N, Cadwallader JS, Robert S, Dywer JL, Charpin DA, Caillaud D, de Blay F, Raherison C, Lavaud F, Annesi-Maesano I, PLoS One 2015, 10, e0138164. [PubMed: 26352140]
- [4]. Hamer M, Ford T, Stamatakis E, Dockray S, Batty GD, Arch. Pediatr. Adolesc. Med 2011, 165, 326. [PubMed: 21135317]
- [5]. Pagani LS, Neurosci. Biobehav. Rev 2014, 44, 195. [PubMed: 23545330]
- [6]. Hall BJ, Cauley M, Burke DA, Kiany A, Slotkin TA, Levin ED, Toxicol. Sci 2016, 151, 236. [PubMed: 26919958]
- [7]. Fuller BF, Cortes DF, Landis MK, Yohannes H, Griffin HE, Stafflinger JE, Bowers MS, Lewis MH, Fox MA, Ottens AK, Environ. Health Perspect 2012, 120, 1684. [PubMed: 23014793]
- [8]. Gospe SM, Zhou SS, Pinkerton KE, Pediatr. Res 1996, 39, 494. [PubMed: 8929871]

- [9]. Slotkin TA, Pinkerton KE, Garofolo MC, Auman JT, McCook EC, Seidler FJ, Brain Res. 2001, 898, 73. [PubMed: 11292450]
- [10]. Zhou S, Rosenthal DG, Sherman S, Zelikoff J, Gordon T, Weitzman M, Curr Probl Pediatr Adolesc Heal. Care 2014, 44, 219.
- [11]. McCormack MC, Breyse PN, Hansel NN, Matsui EC, Tonorezos ES, Curtin-Brosnan J, Williams AL, Buckley TJ, Eggleston PA, Diette GB, Environ. Res. 2008, 106, 148. [PubMed: 17927974]
- [12]. Stoodley CJ, Cerebellum 2016, 15, 34. [PubMed: 26298473]
- [13]. Wang SSH, Kloth AD, Badura A, Neuron 2014, 83, 518. [PubMed: 25102558]
- [14]. Miquel M, Toledo R, García LI, Coria-Avila GA, Manzo J, Curr. Drug Abuse Rev 2009, 2, 26. [PubMed: 19630735]
- [15]. Torregrossa MM, Quinn JJ, Taylor JR, Biol. Psychiatry 2008, 63, 253. [PubMed: 18194683]
- [16]. Koenigs M, Tranel D, J. Neurosci 2007, 27, 951. [PubMed: 17251437]
- [17]. Stoodley CJ, Cerebellum 2016, 15, 34. [PubMed: 26298473]
- [18]. Torres LH, Annoni R, Balestrin NT, Coletto PL, Duro SO, Garcia RCT, Pacheco-neto M, Mauad T, Camarini R, Britto LRG, Marcourakis T, Arch Toxicol 2015, 89, 2051. [PubMed: 25182420]
- [19]. Torres LH, Garcia RCT, Blois AMM, Dati LMM, Durão AC, Alves AS, Pacheco-Neto M, Mauad T, Britto LRG, Xavier GF, Camarini R, Marcourakis T, PLoS One 2015, 10, e0136399. [PubMed: 26305213]
- [20]. Slotkin TA, Stadler A, Skavicus S, Card J, Ruff J, Levin ED, Seidler FJ, Toxicol. Sci 2017, 155, 75. [PubMed: 27633979]
- [21]. Cortes DF, Landis MK, Ottens AK, Electrophoresis 2012, 33, 3712. [PubMed: 23160985]
- [22]. Distler U, Schmeisser MJ, Pelosi A, Reim D, Kuharev J, Weiczner R, Baumgart J, Boeckers TM, Nitsch R, Vogt J, Tenzer S, Proteomics 2014, 14, 2607. [PubMed: 25211037]
- [23]. Karpievitch Y, Stanley J, Taverner T, Huang J, Adkins JN, Ansong C, Heffron F, Metz TO, Qian W-J, Yoon H, Smith RD, Dabney AR, Bioinformatics 2009, 25, 2028. [PubMed: 19535538]
- [24]. Chen H, Chan DC, Hum. Mol. Genet 2005, 14, R283. [PubMed: 16244327]
- [25]. Farrelly L, Rosato-Siri MV, Föcking M, Codagnone M, Reines A, Dicker P, Wynne K, Farrell M, Cannon M, Cagney G, Pasquini JM, Cotter DR, Proteomics 2017, 17, 1.
- [26]. Franklin JL, Mirzaei M, Wearne TA, Homewood J, Goodchild AK, Haynes PA, Cornish JL, J. Proteome Res 2016, 15, 1455. [PubMed: 26941107]
- [27]. Swart PC, Russell VA, Vlok NM, Dimatellis JJ, J. Mol. Neurosci 2018, 65, 277. [PubMed: 29915983]
- [28]. Murphy KM, Tcharnaia L, Beshara SP, Jones DG, Front. Mol. Neurosci 2012, 5, DOI 10.3389/fnmol.2012.00065.
- [29]. Yizhar O, Fenno LE, Prigge M, Schneider F, Davidson TJ, Ogshea DJ, Sohal VS, Goshen I, Finkelstein J, Paz JT, Stehfest K, Fudim R, Ramakrishnan C, Huguenard JR, Hegemann P, Deisseroth K, Nature 2011, 477, 171. [PubMed: 21796121]

Figure 1.

The PFC neuroproteomic response to juvenile ETS exposure. (A) Volcano plot of the differential response to ETS vs air control within the OFC neuroproteome, representing 800 protein measures above an FDR of 5%. (B) Histogram showing the effect size distribution across the ETS-responsive OFC neuroproteome. (C) Chart of significantly enriched cellular component associations among ETS-increased proteins, along with the number of associated proteins. (D) Chart of significantly enriched cellular component associations among ETS-decreased proteins within the OFC neuroproteome.

Figure 2.

Anatomical and phenotypic relevance of ETS effect on the juvenile OFC. Representative immunofluorescence images from (A) OFC and (B) cerebellar tissues of ETS-exposed and air-control animals: DAPI-stained nuclei (blue), GluR2-stained glutamatergic synapses (green), and Vglut1-stained glutamatergic synaptic vesicles. (C) Immunofluorescence integrated density of GluR2 and Vglut1 staining; $n=4-5$ animals; 6 measures per animal within layers 4/5 of OFC and the granular layer (GL) of cerebellum. $*p<0.025$, two-way Students t-test. (D) Radar plot of the phenotypic association of the OFC neuroproteomic response to ETS, with the enrichment p -value given along the radial axis.