Effects of Pre- and Post-natal smoke exposure on airway neural responses

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ABSTRACT:

Abundant epidemiological data shows that maternal smoking during pregnancy or second-hand smoke exposure during neonatal life and infancy increases the incidence of respiratory illnesses later in life. However, underlying mechanisms initiated in early life but affecting adult disease remain undefined. Neurotrophins, including nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), are neurotrophic factors essential in promoting and maintaining differentiation, growth, and survival of central and peripheral nervous system. The goal of this proposal is to examine the potential role of neurotrophin release in mediating long-term effects of prenatal and early postnatal smoke exposure. Our studies showed that an initial exposure to smoke during prenatal and early postnatal periods significantly changed lung function, substance P (SP) innervation in trachea and the level of NGF in BAL fluid after re-exposure to smoke on adult (postnatal day (PD) 60). In controls, smoke exposure (PD 28) with a subsequent re-exposure at PD 60 did not affect lung function or SP innervation or NGF levels. PCR array revealed enhanced expression of multiple genes including NGF and its receptor by smoke exposure only during early prenatal periods. These data suggest that a critical period of exposure exists in early periods of development. Increased expression of NGF and its receptor in airway caused by smoke exposure may enhance susceptibility to respiratory illnesses in adult. These findings have obvious and significant implication both for smoking during pregnancy and the effects of second hand tobacco smoke exposure. The study provides evidence that prenatal and early postnatal life are periods of enhanced susceptibility to detrimental effects of cigarette smoke. These effects appear

to be mediated through the release of neurotrophic factors like NGF which regulate growth and maturation of the airway during these susceptible periods.

Epidemiological studies show that children of mothers who smoked cigarettes daily have increased probability of developing or exacerbating respiratory illnesses [1-3]. The nervous system, including the nerves supplying the airways, is highly susceptible to environmental influences during development. Airway innervation develops rapidly during fetal and early postnatal life, parallel to the development of the lungs [4]. Given the dynamic and vulnerable nature of developmental processes, this period of morphogenesis is likely to be exquisitely sensitive to environmental insults [5]. Substance P (SP), a member of the tachykinin family, has potent effect on airway smooth muscle tone, vascular permeability to protein and mucus secretion [6-8]. SP can trigger symptoms of bronchial asthma, including airway hyperresponsiveness (AHR) and acute inflammation [7-11]. Our recent studies demonstrated that increased SP levels in the airway was involved in smoke exposure-induced AHR[12] and that NGF released during irritant exposure, mediated the changes in phenotype and neuronal responses of airway neurons [13,14]. Joad and coworkers showed that rats exposed to tobacco smoke in prenatal and early postnatal periods had increased airway responsiveness as adults compared to controls [15]. However, their studies did not separate prenatal and early postnatal periods from the later postnatal periods. Thus, we hypothesized that a critical periods of sensitivity to smoke exposure exists within prenatal and early postnatal period, especially with respect to airway innervation. In this study, we examine changes SP innervation and neurotrophin expression in the airways and AHR after cigarette smoke exposures that occur during specific “critical period”.

Three different age groups of mice (embryonic days (ED) 7 by maternal exposure, postnatal days (PD) 2 and 21) were exposed either directly or indirectly to cigarette smoke or filtered air (sham smoke exposure). The ED7 group was exposed indirectly by maternal exposure and the two PD groups were exposed directly. Maternal or direct exposures lasted for 10 consecutive days (6 hours per day). After the exposure periods, the mice were raised in normal animal housing and allowed to mature to postnatal day 59. On PD 59 all groups (both smoke exposed and controls) were re-exposed to smoke once for 6 hrs. Lung function, SP innervation and NGF expression were measured at 24 hrs after re-exposure.

There was no significant difference in the average baseline of lung resistance (RL) and dynamic pulmonary compliance (Cdyn) prior to methacholine (MCh) challenge between filtered air (control) and smoke exposure during prenatal or postnatal periods in the three different groups (Table 1). The MCh dose-response curves for RL were significantly elevated and Cdyn was significantly decreased in ED7 and PD2 smoke exposure groups (Figure 1) after the smoke re-exposure on PD 60. However, there was no significant difference in the bronchomotor responses to MCh between PD21 air exposure and smoke exposure group after the smoke re-exposure. The percent area of SP-IR nerve fibers in tracheal smooth muscle was significantly increased in the ED7 and PD2 smoke exposure groups. SP nerve fiber density in trachea was significantly increased from 0.07% and 0.08% in ED7 and PD2 air exposure groups to 0.15% and 0.16%, respectively, in the ED7 and PD2 smoke exposure groups (Figure 2 and 3) after the smoke re-exposure. At the same time, SP nerve fiber density did not change significantly in PD21 groups, increasing only from 0.07% in PD21 air exposure animals

to 0.09% in PD21 smoke exposure animals (Figure 2 and 3). These findings suggest that the initial exposure to smoke during prenatal and early postnatal period significantly enhances the levels of SP in tracheal smooth muscle. Similar effects were found in NGF released into BAL fluid. The concentrations of NGF in the BAL fluid were significantly elevated in the ED7 and PD2 smoke exposure groups after the smoke re-exposure. At the same time, the level of NGF was not significantly changed in PD21 groups (Figure 4) after the smoke re-exposure.

Next, we test the effect of smoke during early postnatal period on changes of neurotrophins gene expression in lung. PD 2 mice were exposed to either smoke or filtered air for 10 consecutive days and neurotrophins gene expression was immediately measure after smoke exposure. PCR array showed increased gene expression for NGF and the NGF receptor gene expression after smoke exposure during early prenatal periods. A gene expression of second neurotrophin, BDNF, was not affected by smoke exposure (Figure 5). While direct cause and effect was not determined in this study, previous studies in our laboratory have verified the close relationship between NGF expression and increased SP innervation after irritant exposures [13,16].

Recent studies have shown that infants and children exposed to irritants in early life have both immediate and persistent detrimental effects on lung health [17,18]. Airway sensitivity and airway responsiveness changed with age [19,20]. But, most of these studies focus on immune mechanisms that contribute to the development of airway diseases. Innervation of the airways and lung are not fully developed in the early postnatal stage of life. The sensitivity of airway nerves in newborns is less than adult levels[21]and the distribution and density of peptidergic nerves in young children is less than adults [22].

Current results clearly show that prenatal and early postnatal life as a period of sensitivity to inhaled smoke. Further, the present study found that SP nerve fibers in tracheal smooth muscle and NGF expression was significantly elevated, indicating that increased SP and NGF were may contribute to alteration of airway function which associate with enhancing susceptibility to respiratory illnesses in later life.

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Table 1. Effect of smoke on the baseline of R_L (cm H_2O/ml/s) and C_{dyn} (ml/cm H_2O)

	Air		Smoke	
	R_L	C_{dyn}	R_L	C_{dyn}
ED7	1.12 ± 0.11	0.053±0.014	1.18 ± 0.19	0.046±0.016
PD 2	1.01 ± 0.09	0.065±0.011	1.09 ± 0.13	0.053±0.012
PD 21	1.08 ± 0.05	0.062±0.008	1.02 ± 0.10	0.069±0.011

Data are means±SE.

FIGURE LEGENDS

Figure 1. MCh Dose responses of RL and Cdyn in prenatal (A and D), early postnatal (B and E) and late postnatl (C and F) filtered air (control) or smoke exposed mice after the smoke re-exposure on PD 60. Values are means ± SE of 6 mice in each group. Peak response to each dose of MCh in each animal was averaged over 3 consecutive breaths. * Significant difference comparing corresponding data between control and smoke animals, P ≤0 0.05.

Figure 2. Fluorescence photomicrographs of substance P (SP)- -immunoreactive nerve fiber density within tracheal smooth muscle in prenatal filtered air (A) or smoke (B) exposure, early postnatal (C) and late postnatl (D) smoke exposure mice after the smoke re-exposure on PD 60. A (air exposure in prenatal period): negative SP-immunoreactive nerve in tracheal smooth muscle. B (smoke exposure in prenatal period): increased SP-immunoreactive nerve fibers in tracheal smooth muscle. C (smoke exposure in early postenatal period): increased SP-immunoreactive nerve fibers in tracheal smooth muscle . D (smoke exposure in late postnatal period): Few SP-immunoreactive nerve fibers are present in tracheal smooth muscle. Magnification: x285.

Figure 3. The changes of SP nerve fiber density in tracheal smooth muscle in prenatal (A), early postnatal (B) and late postnatal (C) filtered air (control) or smoke exposed mice after the smoke re-exposure on PD 60. Values are means ± SE; $n = 6$ in each group. *Significant difference between control and smoke exposed mice, $P \leq 0.05$.

Figure 4. The levels of NGF release in bronchoalveolar lavage fluid in prenatal (A), early postnatal (B) and late postnatal (C) filtered air (control) or smoke exposed mice after the smoke re-exposure on PD 60. Values are means ± SE; $n = 6$ in each group. NGF

was measured by ELISA. * Significant difference between control and smoke exposed mice, $P \leq 0.05$.

Figure 5. The effect of smoke during early postnatal period on changes of neurotrophins gene expression in lung.

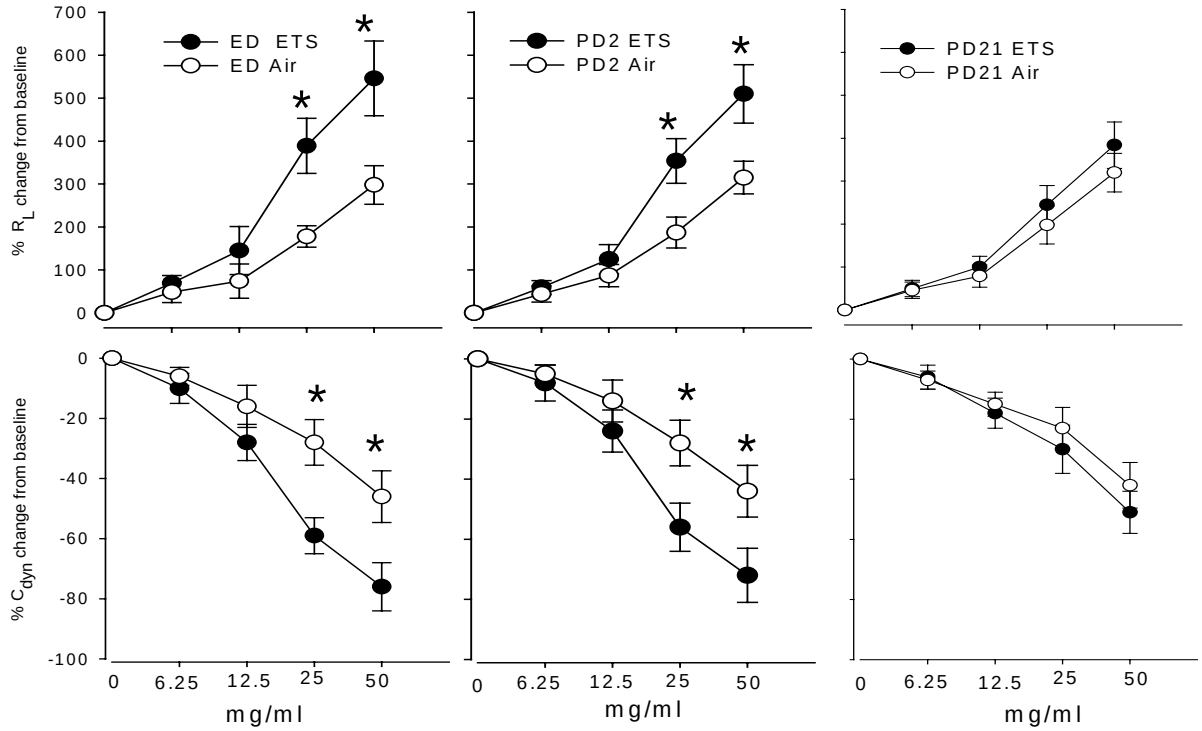


Figure 1.

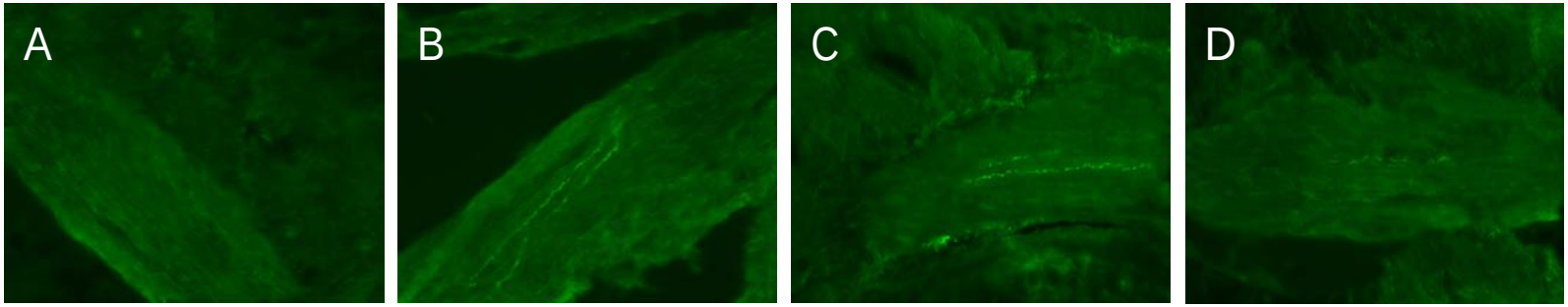
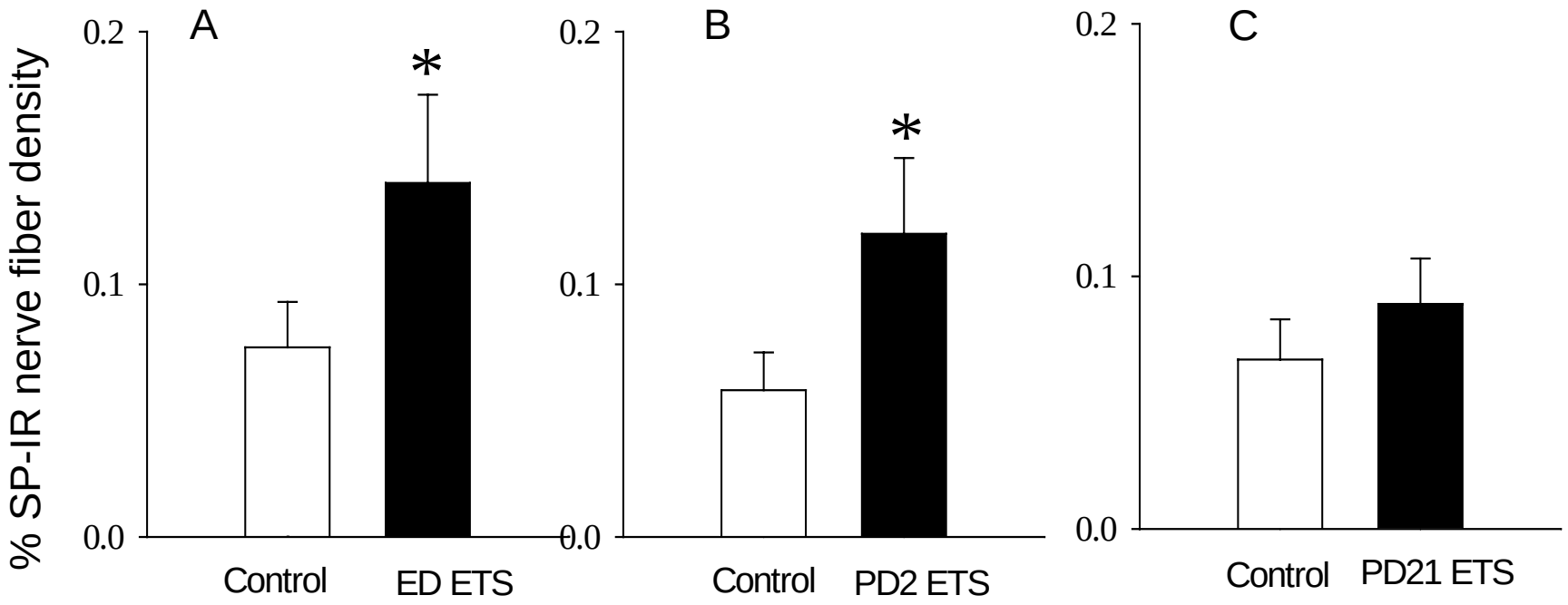


Figure 2.



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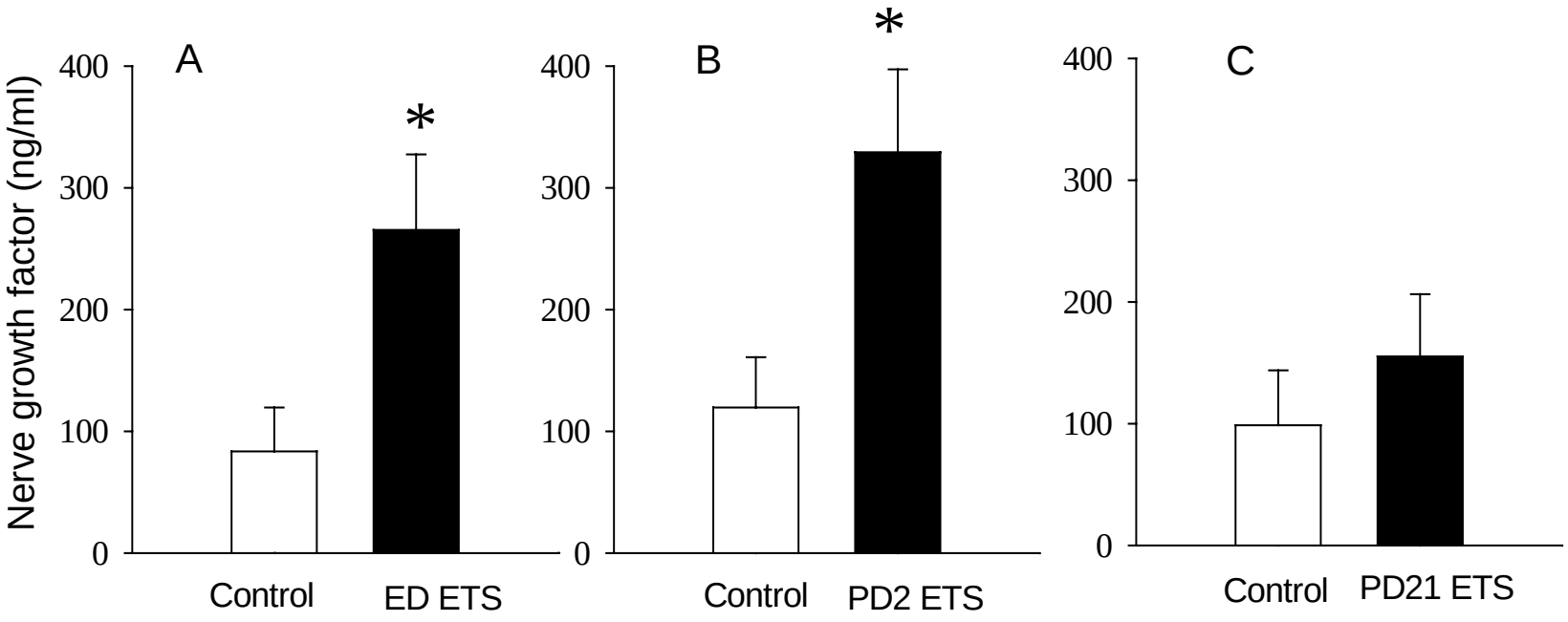

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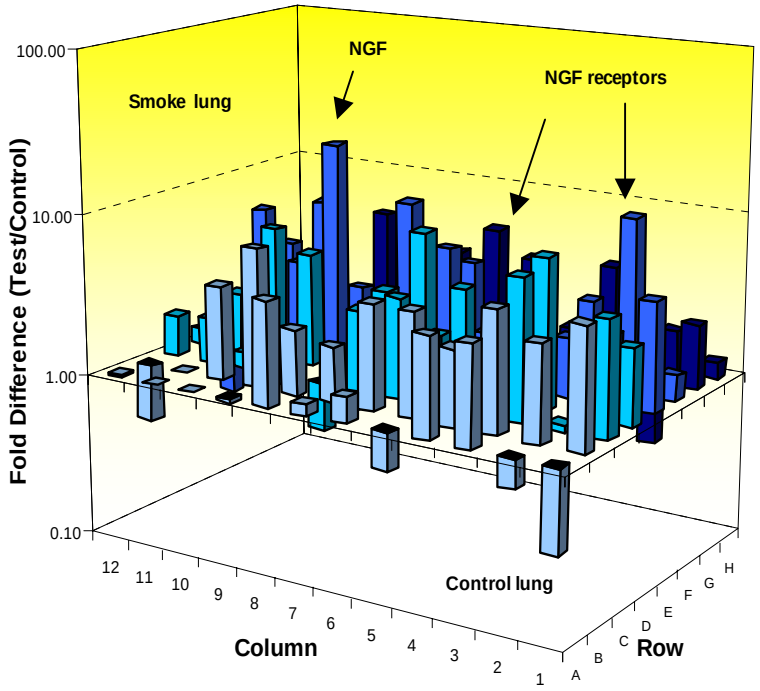

Figure 5.