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Research report

Low intensity, long term exposure to tobacco smoke inhibits hippocampal neurogenesis in adult mice

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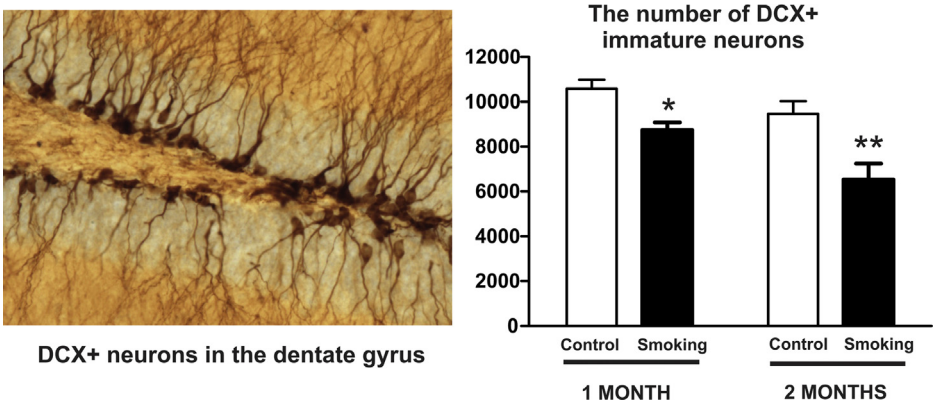
HIGHLIGHTS

- We studied the effect of low intensity tobacco smoke on newborn neurons in the dentate gyrus.
- Cigarette smoke exposure reduced the number of adult–born immature neurons.
- Absence of substantial changes in respiratory function.
- Histopathology of the lungs revealed marked inflammatory reactions.
- Even mild, but long-term tobacco smoke exposure affects adult–born neurons and inflammatory mediators may contribute to this effect.

GRAPHICAL ABSTRACT



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ABSTRACT

Previous data have shown that high dose of nicotine administration or tobacco smoke exposure can reduce cell formation and the survival rate of adult–born neurons in the dentate gyrus. Here, we subjected adult mice to low intensity cigarette smoke exposure over long time periods. We did a 2 × 30 min/day smoke exposure with two cigarettes per occasion over 1- or 2-months. Subsequently, we carried out a systematic quantitative histopathological analysis to assess the number of newborn neurons in the dentate gyrus. To investigate cell proliferation, the exogenous marker 5-bromo-2′-deoxyuridine (BrdU) was administered on the last experimental day and animals were sacrificed 2 h later. To investigate the effect of tobacco smoke on the population of immature neurons, we quantified the number of doublecortin-positive (DCX+) neurons in the same animals. We found that exposing animals to cigarette smoke for 1- or 2-months had no influence on cell proliferation rate, but significantly reduced the number of DCX-positive immature neurons. Our tobacco smoke exposure regimen caused no substantial changes in respiratory functions, but histopathological analysis of the pulmonary tissue revealed a marked perivascular/peribronchial edema formation after 1-month and signs of chronic pulmonary inflammation after 2-months of cigarette smoke exposure. These data demonstrate that even mild exposure to cigarette smoke, without significantly affecting respiratory functions, can have a negative effect on adult–born neurons in the dentate gyrus,

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when applied over longer time periods. Our data indicate that besides nicotine other factors, such as inflammatory mediators, may also contribute to this effect.

1. Introduction

Cigarette smoke contains approximately 8000 toxic chemicals including nicotine, carbon monoxide, heavy metals, hydrogen cyanide, and polycyclic aromatic hydrocarbons [1,2]. Smoking is a global health problem. In the US, approximately 42 million adults smoke cigarettes (see http://www.cdc.gov/) and in EU countries 15–55% of the adults smoke cigarettes depending on their gender and country of origin [3]. While self-reports suggest that smoking tobacco can alleviate negative affective states or cognitive impairments [4], others report abnormal hippocampal neurochemistry in smokers [5].

The hippocampal dentate gyrus has a unique regenerative capacity as it is one of the brain areas where a remarkable number of neurons are produced every day. It has been shown that these adult–born neurons of the dentate are essential for normal cognitive functioning and memory formation [6,7]. Deficits in the generation and function of adult–born neurons have been suggested to contribute to the pathophysiology of various neuropsychiatric disorders including drug seeking and addiction [8,9]. These ideas are based on the observations that different types of drugs of abuse, including nicotine, can influence the generation of neurons in the dentate.

The first reports investigating the effect of nicotine on adult hippocampal neurogenesis used intravenous self-administration in rats and demonstrated an inhibitory effect, but only for high doses [10]. This has been confirmed by others who used different application protocols for nicotine, but again only high doses had inhibitory effect [11]. However, the effects of nicotine injections might be significantly different compared to the situation when animals are exposed to tobacco smoke. In a well-designed study, Bruijnzeel et al. [12] exposed adolescent rats to tobacco smoke for 4 h/day for 14 days and investigated hippocampal cell proliferation as well as the survival rate of the newly formed neurons by 5-bromo-2′-deoxyuridine (BrdU) immunohistochemistry. They found that tobacco smoke exposure decreased both the number of dividing progenitor cells and the number of surviving new cells [12]. They used the whole body smoke exposure model, and they subjected the rats to a rather high dose of tobacco smoke, i.e., after a 3-day initiation period with increasing dosage, from experimental day-4 onwards, they exposed the animals to 15 cigarettes/h for 4 h every day [12]. This means that the animals were exposed to the smoke of 60 cigarettes per day, for 11 days.

Here we also employed the whole body smoke exposure model [13] that is ideal for investigating smoking intensity- and duration-dependent changes simultaneously in different organ systems. In this model, we subjected adult mice to a low intensity smoke exposure, but over long time periods. In this model, we analyzed cell proliferation in the dentate gyrus using the exogenous marker BrdU [14] and quantified the population of immature neurons after labeling them with doublecortin (DCX) for immunohistochemistry [15,16].

2. Material and methods

2.1. Animals

Adult male NMRI mice weighing 41 ± 4 g (8 weeks of age; $n = 28$; $n = 7$/experimental group) at the beginning of each experiment were used. The original breeding pairs were purchased from Innovo Ltd. (Gödöllő, Hungary), but experimental animals were bred and kept (group-housed) in a temperature and humidity-controlled animal facility and maintained on a standard 12-h light/dark cycle (lights on at 8 AM). Food and water were available ad libitum in the home cages.

2.2. Ethical considerations

All procedures were carried out according to the 1998/XXVIII Act of the Hungarian parliament on Animal Protection and Consideration Decree of Scientific Procedures of Animal Experiments (243/1988). They were approved by the Ethics Committee on Animal Research of University of Pécs according to the Ethical Codex of Animal Experiments (licence No.: BA02/2000-5/2011).

2.3. Drugs

BrdU and carbachol was purchased from Sigma (Sigma–Aldrich, St. Louis, MO, USA). BrdU was dissolved in sterile saline (0.9% sodium chloride). Research cigarettes (3R4F) were purchased from the University of Kentucky (College of Agriculture, Reference Cigarette Program, Lexington, KY, USA).

2.4. Tobacco smoke exposure

Mice were exposed to 3R4F research cigarette smoke with the help of a manual smoking system (TE2; Teague Enterprises, Woodland, CA). Whole-body smoke exposure was performed twice a day for 30 min in the closed chamber of the equipment using only two cigarettes per occasion (for six mice in the cage) for 1 or 2 months. Cigarettes burned down within 10 min; then the ventilator was switched off for 20 min, and the chamber was ventilated afterward for 10 min. The average concentration of the total suspended particles in the chamber was 70 mg/m^3.

2.5. Pulmonary function tests

Unrestrained pulmonary function measurements in conscious, spontaneously breathing animals were performed after 1 and 2 months of cigarette smoke exposure. Mice were placed in the chamber of a whole body plethysmograph (PLY 3211, Buxco Europe Ltd., Winchester, UK). The flow transducers (TRD5700, Buxco Europe Ltd., Winchester, UK) were connected to the preamplifier module, which digitized the signals via an analog-to-digital converter (MAX2270 Buxco Europe Ltd., Winchester, UK). We determined the enhanced pause (Penh) value, a calculated parameter ((expiratory time/relaxation time)-1)/(max. expiratory flow/max. inspiratory flow) indicating bronchoconstriction, which was induced by aerosolization of 22 mM of the muscarinic acetylcholine receptor agonist carbachol. Acquisitions were taken in a 2-min-long baseline period aerosolized with saline and then 15 minutes after the increasing carbachol concentrations. Ventilation parameters (*f*, TV, Ti, PIF, Te, PEF, RT and Penh) were measured every 10 s and averaged by the BioSystem XA Software for Windows (Buxco Research Systems).