A realistic innovative mouse model for studying the addictive effects of nicotine exposure via cigarette smoke

L. Ponzoni [1,2,3], D. Braida [2], R. Martucci [1,2], C. Gotti [2,4], M. Sala [2,4]
[1] Graduate School in Pharmacological Sciences, [2] Dipartimento di Biotecnologie Mediche
e Medicina Traslazionale, Università degli Studi di Milano; [3] Fondazione Fratelli Confalonieri Milano, [4] Istituto di Neuroscienze, CNR

Tobacco smoking is thought to be a form of drug dependence. The main addictive agent delivered via cigarette smoking is nicotine, which induces stimulation and pleasure, and reduces stress and anxiety. Many factors contributing to the development and maintenance of nicotine addiction have been investigated in human populations and animal models. A main limitation of the animal models developed so far for the study of smoking dependence, is that nicotine is administered in ways (e.g., intraperitoneal, subcutaneous or intravenous administrations) that are very different from the way used by humans (i.e., smoking). This makes the animal results less reliable and not completely superimposable with the human condition (1,2).The aim of the present study was to validate a rodent model of nicotine exposure using a non-invasive and high throughput technique that could mimic both the intermittent aspect and the route of administration used by humans. To this end, Balb/C mice were daily exposed, in groups of 30, to the smoke of seven Chesterfield Red cigarettes (3 times a day for 7 weeks). We used a mechanical ventilator to vaporize cigarette smoking in an air-controlled cage. Control mice were exposed to the same ventilator using the same schedule but received only air. After 3 and 7 weeks smoke-exposed animals were killed and brains removed. The level of neuronal nicotinic receptors (nAChRs) were then evaluated by binding studies. Our results demonstrated a significant upregulation of nAChRs, particularly $\alpha 4\beta 2$ receptor subtype, only after seven weeks. Thus, this time was chosen for the behavioural analysis.The behavioural tests were conducted from 24 hours to 60 days after exposure to smoke or air. During this period, the body weight was monitored and found significantly reduced by exposure to cigarette smoke. Immediately after smoking cessation, withdrawal syndrome (WDW) was precipitated by mecamylamine injection (1mg/kg). Signs (sniffing, wet dog shakes, forepaw tremors, digging) and symptoms of abstinence (ptosis, piloerection, teeth chattering, genital licks), scored for 30 min, were significantly increased in the smoke-exposed group. Spontaneous motor activity, recorded for 15 minutes in a activity cage, was decreased only 24 hours after smoking cessation. The presence of cognitive deficits was evaluated by the spatial object recognition test. A significant difficulty in recognizing the new spatial location of a familiar object, at least up to 30 days of abstinence, was found.Emotional profile was measured using elevated plus maze test for anxiety-like behaviour, and tail suspension and anhedonia for depressive-like behaviour. The animals exposed to cigarette smoke, showed after 48 hours an anxious state and, after 30 days, a depressive like behaviour. In humans tobacco smoke is frequently associated with marijuana smoke (3). Thus, after smoking cessation, in another group of animals, we verified, using the conditioned place preference (CPP) test, whether mice were cross-sensitized to the reinforcing effect of ?9-tetrahydrocannabinol (?9-THC). ?9-THC (0.01-0.3 mg/kg) induced a greater CPP in nicotine pre-exposed animals compared to control group. In conclusion, we propose an innovative cigarette smoke procedure in mice as a valid model for nicotine addiction studies and for nicotine and other drugs of abuse interaction.

References

1. Athina, Phil. Trans. R. Soc., 2008
2. Oliver *et al*., Pharmacol Biochem Behav, 2010
3. Degenhardt *et al*., Addiction, 2001