

Short term exposure to cigarette smoke on general activity and anxiety

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OBJECTIVE: Sidestream cigarette smoke differs from mainstream smoke because it presents more intense effects. We aimed to evaluate the performance of rats exposed to short term sidestream cigarette smoke during open field and elevated plus-maze tests.

METHOD: Cigarette exposure was carried out during 5 days. The rats were exposed to the cigarette smoke during one hour. Elevated plus-maze and open field tests were applied according to previous studies. During the open field test we measured the time spent stopped, raising and the number of times that the rat intercepted each cross (locomotion). We examined the number of entries in the open and closed arms and the time spent in the closed and open arms during elevated plus-maze test.

RESULTS: Control group presented significantly higher values regarding locomotion and raising during the open field test. No difference was noted between the groups regarding the elevated plus-maze test.

CONCLUSION: Sidestream cigarette smoke exposure for short term on rats affects their performance during the open field test.

KEYWORDS: tobacco; anxiety; rats.

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INTRODUCTION

Smoking is associated with increased morbidity and with overall mortality causes¹⁻⁴. Cigarette smoke can be classified into two categories, one being the mainstream smoke usually inhaled by active smokers, and the other being the sidestream smoke emitted from a cigarette and inhaled by so-called "passive smokers". Sidestream smoke contains a larger variety of oxidants and other harmful compounds than that contained in mainstream smoke⁴. Passive smokers are thus exposed to almost the same chemicals in cigarette smoke as active smokers are.

In addition to the physical dependence derived from the continuous administration of nicotine, smokers develop behavioral dependence – also called psychosocial or psychological dependence – when they use cigarette smoking to cope with situations of varying degrees of stress and when they associate smoking with certain social situations.⁵ All components of dependence are interrelated so that smoking behavior continues due to both the physical effects of nicotine use and the psychological effects of smoking.⁶ The assessment of behavioral dependence, however, is not as well established, and although some authors have studied the behavioral dependence on

smoking⁶, its association with smoking cessation treatment outcomes and its influence on treatment selection have not yet been determined.

Over the past 15 years, the elevated plus-maze test has become the most widely used animal model for the study of drug effects on anxiety. In the conventional form of the test, anxiety is routinely assessed by measurements of open arm avoidance while locomotor activity is most reliably measured by the frequency of closed arm entries.⁷ The open field test is widely used as a standard screening procedure to measure psychomotor activity and exploration.⁸ Furthermore, behavioral changes due to cigarette smoke exposure can be measured by the analysis of social interactions⁹.

In view of the above considerations, our investigation was undertaken to evaluate the performance of rats exposed to sidestream cigarette smoke during short term on the Open Field and Elevated Plus-maze tests.

METHOD

Animals

Male Wistar rats (320-370 g) were kept in the Animal Care Unit of our University. Rats were housed individually in plastic cages under standard laboratory conditions. They were kept under a 12 h light/dark cycle (lights on at 07:00 h) and had free access to food and water. The Institution's Animal Ethics Committee approved the housing conditions

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and authorized the experimental procedures (number 259/07). Efforts were made to minimize the number of animals used.

Sidestream cigarette smoke exposure

The method used was that proposed by de Paiva et al.¹⁰, implemented by Chen et al.¹¹ and standardized in our laboratory to expose the animals to smoke in a modified incubator. The rats were placed in the transparent chamber, with a volume of approximately 95 cm × 80 cm × 65 cm, where fifteen rats remained. During the first day, the smoke was released at a rate of one cigarette, once a day, during one hour. From the second day on, smoke was released at a rate of two cigarettes, once a day, also during one hour. The total duration of these experiments was five days and all the exposures were during the afternoon, between 2 p.m. and 6 p.m. The cigarette used was a commercial brand with the following composition: 1.1 mg of nicotine and 14 mg of tar.

Behavioral tests

The behavioral tests were performed in the following order: (i) 5 min exposures to Open Field test and (ii) 5 min exposures to the Elevated Plus-maze test. Each behavioral test was performed in a sound-isolated room with lighting. During the tests the experimenter stayed in the room adjacent to the one in which the experiments were performed. All the tests were performed by only one experimenter so as not to induce differences of interpretation of data. The open field apparatus, and the elevated plus-maze device were cleaned using 5% ethanol before introducing each animal, to preclude the possible cueing effects of odors left by previous subjects. To avoid influences of circadian rhythms on performance of the animals the behavioral tests were carried out between 9 p.m. and 10 p.m. The behavior of rats during exposure to the Open Field and Elevated Plus-maze was videotaped and then analyzed. All the tests were performed between four and five hours after the last cigarette smoke exposure.

Open field test

The open field apparatus is similar to that described by Broadhurst.¹² The number of crossings (locomotion), the number of rearing and the total immobility time were recorded during 5 min. To minimize possible influences of circadian changes on rat open field behavior, control and experimental animals were intermixed.

Elevated plus-maze test

The elevated plus-maze was made of wood and had two open arms (50 × 10 cm) and two enclosed arms of the same size with 40 cm high walls; it was elevated 50 cm above the ground. Each rat was placed in the center square (10 × 10 cm) and observed for the number of entries into each type of arm (all four paws defining an entry) and the time in the open and closed arms. These parameters were recorded for 5 min. Control and experimental rats were intermixed, and the observations were made between 2:00 and 5:00 p.m. as prescribed by File¹³.

Statistical analysis

Values are reported as the means ± standard error. The Student "t" test was used to compare groups for parametric data. For nonparametric data, the Mann-Whitney U test was

employed. In all cases, results were considered significant for p < 0.05.

RESULTS

Significant differences between Control (n = 7) and Smoke (n = 7) groups were observed for some aspects during the open field test. The control group showed higher values regarding locomotion and raising, while the smoking group remained stopped longer; however, this last difference did not reach statistical significance; these results are shown in Table 1.

There was no significant difference between the two groups during the Elevated plus-maze test. Table 2 shows a great variability among animals of Smoke group in relation to number of entries in the closed arm, time spent in the closed arm and time spent in the open arm.

DISCUSSION

This study aimed to evaluate the performance of rats exposed to sidestream cigarette smoke for short term on open field and elevated plus-maze tests.

During the open field test rats exposed to cigarette smoke spent less time moving and raising and remained stopped longer than the control group. Thus, anxiety was observed to be increased in the group exposed to sidestream cigarette smoke. Behavior in rodents is determined by the conflict between the drive to explore the unknown area/object and the motivation to avoid potential danger. Exploration behavior in rodents summarizes a broad spectrum of behavioral patterns such as risk assessment behaviors, walking, rearing, climbing, sniffing, and manipulating objects.¹⁴ Exploration is gradually inhibited by anxiety, thereby representing an indirect measurement of anxiety.¹⁴⁻¹⁶ Because pharmacological treatment acts fundamentally on the abstinence syndrome, of which anxiety is one of the main symptoms, it is likely that the reduction in anxiety in ex-smokers was due at least in part to their treatment.¹⁷ The fact that a gradual decrease in anxiety in ex-smokers has been described in other studies would seem to indicate that nicotine increases rather than diminishes anxiety⁷, an observation which is confirmed in our study.

Table 1 - Behavioral evaluation of smoking (n = 7) and control (n = 7) groups during open field test.

Parameters	Control	Smoking	p value
Locomotion	36.5	17.7	0.0072
Raising	11.2	5.2	0.0221
Time stopped (s)	145.7	174.3	0.2886

Table 2 - Behavioral evaluation of smoking (n = 7) and control (n = 7) groups during elevated plus-maze test.

Group	Closed arm (s)	Closed arm (number of entries)	Open arm (s)	Open arm (number of entries)
Control	0.5 ± 0.2	0.167 ± 0.1	295.83 ± 2.53	2.5 ± 0.846
Smoking	1.67 ± 1.08	0.33 ± 0.21	292.5 ± 4.59	1.5 ± 0.5
P value	0.0571	0.3093	0.1089	0.1363

Nicotine dependence is a complex condition involving pharmacological and nonpharmacological factors. The determinant among the former is the addictive capacity of nicotine, a psychoactive substance which produces positive reinforcement (improving concentration and the feeling of wellbeing) and negative reinforcement (removing withdrawal syndrome symptoms)^{18,19}.

According to Pellow et al.¹⁶, regarding the elevated plus-maze test, the preference shown for the closed arms reflects an aversion toward the open arms, caused by fear or anxiety induced by the open space. The elevated plus-maze test is the most popular test to search for new anxiolytic agents^{7,16}. Our findings show that the exposure to sidestream cigarette smoke during five days is not active in this test of fear or anxiety, as indicated by no differences between the two groups with respect to the time spent and the number of entries into the open vs. closed arms. In previous studies, anxiogenic effects of chronic nicotine on this test have been reported^{9,20}. Under our conditions, no anxiogenic behavior was observed in rats exposed to smoke, whereas the opposite was verified on the open field test. It is known that nicotine treatment acts as an anxiolytic²¹. Although its underlying mechanisms have not been sufficiently elucidated, some contributions of temporary modifications of the endogenous serotonin have been suggested for the occurrence of anxiolytic effects^{9,22}. Time-dependent modifications in the stress-related endocrine system²³ also seem to correlate with the temporary occurrence of nicotine-induced anxiolytic effects. Nevertheless, based on animal models of nicotine treatment in which anxiolytic and antidepressant effects were predominantly observed^{9,20}, the appearance of contrary behavioral symptoms, for example anxiogenic vs. anxiolytic symptoms, seemed to be controlled closely by the treatment conditions (i.e. dose, time after use).

While in the open field test we noted significant differences in some variables, during the elevated plus-maze test there was no significant difference between the two groups. In contrast to other methods used to study anxious behavior in rodents, the open field test allows a comprehensive description of the animal's behavior, since more behaviors can be readily observed and quantified^{7,24}. Locomotion, a behavior that can be interpreted as an adaptation to a stressful situation, was affected by the short term exposure to cigarette smoke according to our findings. Perhaps, an exposure during a longer period (more than 1 month) may cause stress adaptation and present similar findings compared to control.

To the best of our knowledge, this is the first research to demonstrate that exposure to sidestream cigarette smoke during five days affects the performance of rats in open field test. This knowledge may contribute to a better understanding of the mechanism involved in behavioral changes affected by secondhand smoke. These findings may possibly open new perspectives for more research and may benefit experimental and clinical investigations. This study has some limitations. First, we did not evaluate behavioral tests everyday; we believe that information of daily values of these tests would be more informative to our study. On the other hand, rats might have adapted to the ambient and this could influence our data. Second, dependence is normally measured with the Fagerström test alone²⁵; in the case of our study we aimed to verify whether the exposure to cigarette smoke could affect the performance of rats during open field and elevated plus-maze tests.

CONCLUSION

Our data indicate that rats exposed to sidestream cigarette smoke during a short period present altered performance in two aspects, locomotion and raising in open field test without significance change regarding the elevated plus-maze test.

AUTHORS' CONTRIBUTIONS

All authors participated in the design of the study and writing the manuscript as well as approving the final manuscript.

RESUMO

OBJETIVO: Exposição à fumaça de cigarro em "sidestream" difere da exposição ativa, pois apresenta efeitos mais intensos. Nosso objetivo foi avaliar o desempenho de ratos expostos à fumaça do cigarro em "sidestream", a curto prazo, em testes em campo aberto e em labirinto elevado em cruz.

MÉTODO: A exposição do cigarro foi realizada durante 5 dias. Os ratos foram expostos à fumaça de cigarros durante uma hora. Testes em campo aberto e em labirinto elevado em cruz foram aplicados de acordo com estudos anteriores. Durante o teste de campo aberto, medimos o tempo gasto parado, o tempo em ereção e o número de vezes que o rato interceptou cada cruz (locomoção). Avaliamos o número de entradas e o tempo gasto nos braços fechados e abertos durante o teste de labirinto elevado em cruz.

RESULTADOS: O grupo controle apresentou valores significativamente mais elevados em relação a locomoção e ereção durante o teste de campo aberto. Não houve diferença entre os grupos em relação ao teste labirinto em cruz elevado.

CONCLUSÃO: A exposição à fumaça do cigarro em "sidestream" a curto prazo em ratos afeta o seu desempenho durante o teste de campo aberto.

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