The impact of chronic stress on behavior in rats with ligature-induced periodontal disease

O impacto do estresse crônico no comportamento de ratos com doença periodontal induzida por ligadura

Abstract

Purpose: To evaluate the impact of chronic stress on behavior at different time points (7, 15 and 30 days) in rats with ligature-induced periodontal disease.

Methods: We selected 60 rats that were randomly divided into 2 groups: a stress group subjected to chronic stress by physical restraint one day after periodontitis induction (GE) and a control group (CG). After periods 10 animals from the GE group and 10 from the CG were analyzed in the open field and elevated plus-maze models.

Results: The results show that at 7 days, increased walk in the central segment of the apparatus and peripheral open field ($P<0.05$) were observed in the GE group as well as increased instances of standing on two legs and self-cleaning ($P<0.05$). In the elevated plus-maze, no statistical differences were observed ($P<0.05$). At the 15-day time point, increased instances of standing on two legs and self-cleaning ($P<0.05$) were observed in the GE group. At 30 days, no behavioral differences were observed between the groups ($P<0.05$).

Conclusion: We conclude that stress affect the behavior of animals subjected to 7 and 15 days of chronic stress after the induction of periodontal disease by ligation.

Key words: Stress; rats; periodontitis

Resumo

Objetivo: Avaliar o impacto do estresse crônico em diferentes tempos (7, 15 e 30 dias) sobre o comportamento de ratos com doença periodontal induzida por ligadura.

Metodologia: Selecionaram-se 60 ratos, divididos aleatoriamente em 2 grupos: GE – submetidos a estresse crônico um dia após a indução de periodontite e grupo controle – GC. Decorrido o prazo de 7, 15 e 30 dias sortearam-se 20 animais por cada período, sendo 10 do GE e outros 10 do GC, em ato contínuo realizaram-se a análise em campo aberto e labirinto em cruz elevado.

Resultados: Os resultados demonstram que aos 7 dias o GE demonstrou maior caminhada no segmento central e periférico do aparato campo aberto ($P<0,05$), além de ficar em duas patas e realizar a autolimpeza um maior número de vezes ($P<0,05$). No tempo experimental de 15 dias para o GE houve uma maior movimentação do número de vezes em duas patas e do ato de autolimpeza ($P<0,05$). Aos 30 dias de estresse nenhuma mudança comportamental foi encontrada na comparação entre os grupos ($P<0,05$).

Conclusão: Conclui-se que o estresse interferiu no comportamento aos 7 dias e aos 15 dias para os animais submetidos ao estresse crônico e doença periodontal induzida por ligadura.

Palavras-chaves: Estresse; rato; periodontite

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Introduction

It has been currently known that the immune, endocrine and the central nervous systems are highly interconnected (1). A interplaying factor related to these three systems is inflammation and its products (2-4). In this respect, periodontitis is a disease whose onset is related to the formation of biofilm by microorganisms potentially able to cause inflammatory systemic repercussions, particularly in generalized and advanced stages (5).

Given the broadening of emotional disorders in the society (6,7), their epidemiological relationship with periodontitis has been found to show strong correlations (8). Nevertheless, further clinical trials are needed as an attempt to better establish common risk factors – such as the relationship between tobacco and diabetes with periodontitis – in addition to the strong impact on the quality of life (2,9).

It seems to be clear that studies in rats have showed a relationship between stress and induced periodontitis (8). That corroborates with the impact of the CNS on the inflammatory response to augment bone loss. Accordingly, Györfi et al. (10) induced experimental periodontitis in two groups. One of the groups received a drug that scrambled the periodontum nervous fibers, and the other one served as control. The findings demonstrated that the group receiving the medication was conditioned to a higher progression of disease when compared to the control. Thus, it was raised the doubt on whether ligature-induced periodontitis associated with stress would be able to modify not only biological variables but also animal behavior.

In this line of research, Breivik et al. (11) injected microorganisms into newborn animals during early childhood and induced periodontitis in adulthood. The data revealed that besides the fact that the animals subjected to the stressing challenge developed greater bone loss and quantity of inflammatory products, there were systemic repercussions in the animals' behavior. Another study by this same group of authors (12), using medication, demonstrated a relationship between the progression of bone loss and animal behavior. Authors from different fields of knowledge have use the locomotor-behavioral tool of the animal to assess the relationships between diseases (13,14).

The number of days of stress associated with the induction of disease has varied in the literature (8). Hence, this research group published a paper addressing this theme, demonstrating that the stress model for 7 and 15 days would be enough to assess the progression of induced periodontitis associated with stress (15). Importantly, one must take into account the time of protocol of animal subjection to stress and research costs. In order to find a model for assessing CNS behavior in rats, associated with the induction of experimental periodontitis, this paper is expected to generate information on the variables related to animal behavior in the experimental periods of 7, 15 and 30 days with ligature-induced periodontitis associated or not with stress.

The present study aimed to evaluate the impact of chronic stress on behavior at different time points (7, 15 and 30 days) in rats with ligature-induced periodontal disease.

Methodology

Sixty animals of the Rattus Novergicus species, weight average of 250 g, two months old were selected for the study. The animals were acclimatized to the housing conditions during 10 days. The rats were maintained in boxes (16×40×30 polyethylene) housing three rats each and they all received standard rat feed and water ad libitum, under a light/dark cycle of 12 hours with a controlled temperature of 23°C and + 40% humidity. This project was submitted to and approved by the ethics and research committee and at University Estadual Paulista (UNESP) N°74-05.

Experimental Groups

Initially, a research assistant randomly divided the animals in two experimental groups:

- Group GE – Chronic Stress + ligation (n=30)
- Group CG – Control (n=30)

After the division, the animals in the GE group were subjected to chronic physical stress one day after the induction of experimental periodontal disease. Twenty animals (10 from each group) were randomly selected at each time point (7 days, 15 days and 30 days) after periodontitis induction. The animals were analyzed in both the elevated plus-maze and in the open field.

Stress Induction

The model of physical chronic stress induction chosen for this study was the immobilization during, seven times a week for 30 days in different times of the day Immobilization (1,13). The animals were exposed (GE) to an average temperature of 24 °C by placing them in polyvinil chloride tubes (PVC) compatible with their size. Then, the tubes were stopped up on both sides with metallic wire, enabling the animals to breathe while they were immobilized for 12 hours.

Experimental Periodontal Disease

For the induction of periodontal disease, GE animals received general anesthesia via an intramuscular injection of 0.1 ml of ketamine (Dopalen, Agribrands, Animal Health, Southeastern Brazil, Brazil) combined with 0.05 ml of xylazine hydrochloride (Rompun, Bayer, Animal Health, São Paulo, Brazil. Brazil) for every 100 grams of body weight. After anesthesia, a sterile number 4 silk suture (Ethicon, Johnson & Johnson, São Paulo, Brazil) was placed around the upper right second molar (16,17).

Behavioral Test

For the motor and psychological performance assessments, behavioral tests were used in the elevated plus-maze (Fig. 1) and the open field (Fig. 2). The open field apparatus consisted of a circular arena with a 50-cm radius. Two circular line segments formed smaller circles within the

area that were divided by eight equally spaced lines in the central circle and sixteen equally spaced lines in the outer circle. The plus-maze apparatus consisted of four arms (two open and two closed) 53 cm in length and 13 cm in width, with walls 30 cm high.

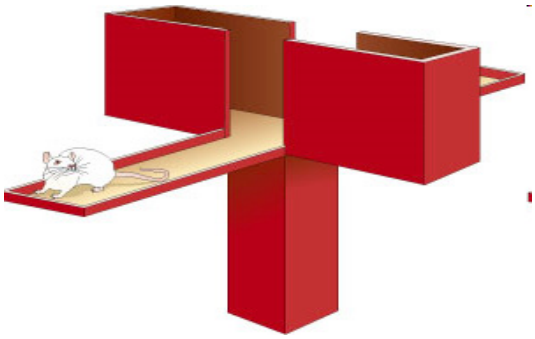

Fig. 1. Illustration elevated plus-maze.

Fig. 2. Illustration open field.

The open field recordings assess fear through animals' exploratory activity and motor activity assessment. Stressed rats do not attempt to venture from the walls of the open field and avoid staying on their hind legs (18).

In a quiet, well-lit environment with a temperature of 24 °C, the animals were placed in the central portion of the open field and observed for 5 minutes. They were then transferred to the elevated plus-maze and observed for another 5 minutes. After each observation, the apparatuses were cleaned with distilled water.

For the open field test, the number of central and peripheral segments covered and instances of the animal standing on two legs without much support (up) or grooming (nose scratching) were recorded. For the elevated plus-maze, the time spent in the open arms and in the closed arms was recorded. The elevated plus-maze is one of the most efficient and commonly used animal models of anxiety (19,20).

Analysis of Results

For the parameters of the open field (the number of central and peripheral segments crossed and the number of instances of up and self-cleaning) and the plus-maze (the number of entries into the arms and the time spent in each respective local), an inter-group analysis was performed at each experimental time point of the study (7, 15 and 30 days), and a separate analysis was conducted for each parameter.

The data normality was assessed by adherence Liliefors. A Student's t test for independent samples was used for statistical analysis, and a *p*-value less than 0.05 was considered significant.

Results

The results (shown in Table 1) show behavioral changes at 7 days of stress (GE), including higher values for the center and periphery variables (number of segments traveled), self-cleaning and the number of times an animal was on two feet without support (all *P*-values <0.05).

Regarding the elevated plus-maze, no statistical differences between groups were observed ($P<0.05$). In the group exposed to 15 days of stress, variables were statistics that have changed the parameters of self-cleaning and up ($P<0.05$). At 30 days of any change behavior found when comparing groups ($P<0.05$).

Table 1. Results show behavioral changes at 7, 15 and 30 days of stress.

	7 days		15 days		30 days	
	Control	Stress	Control	Stress	Control	Stress
			Open Field			
Center Variables	3.1±1.9*	4.5±3.04*	2.4±2.6	1,1±0,3	3,1±1,1	4,5±3,0
Periphery Variables	23.1±8.9*	34.4±9.1*	18.6±7.9	26,0±10,3	23,1±8,6	34,4±11,8
Two feet without support	3.8±3.1*	8.0±5.0*	3.6±2.3*	6,5±3,2*	3,8±3,1	8,0±5,0
Self-Cleaning	1.5±0.7*	3.4±1.87*	1.8±0.9*	3,2±1,3*	1,5±0,7	3,4±1,8
			Elevated Plus-Maze			
Open arms	43.0±83.7	31.6±74.1	20.8±13.5	39,4±28,9	61,0±43,3	85,6±37,6
Close arms	256.9±83.7	268.3±74.1	279.1±13.5	260,5±28,9	236,1±45,7	225,5±45,4

Discussion

PNI research involving this paradigm and attempts to elucidate the possible associations of the central nervous system with the endocrine and immune systems began three decades ago. Currently, it is known that a major factor in this interaction is stress because stress triggers specific reactions by both the immune system and the endocrine system (21,22).

Stress may be defined as a response state of the organism to forces acting simultaneously on the body, wich, if excessive, i.e., straining the capacity of adaptive processes beyond their limits, lead to diseases of adaptation and eventually to diseases of exhaustion and death (23).

Several studies have shown a correlation between PNI and induced periodontitis in rats by stimulating the hypothalamic-pituitary-adrenal (HPA) axis of the neuroendocrine response to the stressor stimulus. Though this relationship exists, it is still unclear whether periodontitis really has the ability to stimulate the HPA axis (13,23). We sought to establish this relationship in this research by using an experimental model of chronic physical stress.

One of the difficulties of this research is the complexity of human behavior. Currently, animal studies have raised auxiliary hypotheses regarding behavioral studies related to the biological understanding of the body. These hypotheses help in understanding these diseases and lead to clinical responses that help patients (3,7,13).

The interference of stress and the induction of periodontitis has been the subject of studies since the 1960 (24). The interference of stress in the progression of periodontitis has been demonstrated in several studies. Gyorfi et al. (10), administered a drug that destroyed parts of nerve fibers to highlight the relationship between the CNS and periodontitis in young rats. After inducing periodontitis and evaluating the major component of neurogenic inflammation, vascular permeability, the authors found statistical differences further destruction of the periodontium group of animals undergoing the stress. These findings demonstrate the communication between the nerve pathways of the periodontium, given its immune-inflammatory response to the induction of periodontitis.

Several studies have associated stress with induced periodontitis, but few have attempted to associate the effect of these factors (stress and periodontitis) on the CNS (25,26,16).

The open field and elevated plus-maze models are widely used by researchers in neuroscience (16,19). Several other experimental possibilities could be explored within this methodology, including applying stress for only a short time period. The stress model was chosen for this study was physical restraint for 12 hours, which is widely used in medical research (23).

Breivik et al. (11) subjected animals to a microbial challenge at an early age and assessed the impact of this stress on the behavior and progression of periodontitis in adults. The results showed increased motor activity in the animals subjected to stress, consistent with the findings of this study.

Besides the investigation herein reported, there is another one which evaluated brain surgeries and antidepressant medication, demonstrating that same relationship – there are behavioral changes in rats related to the progression of induced periodontitis.

These studies have showed a behavioral change associated with ligature-induced periodontitis and chronic stress. In this context, some factors might be outlined. The first one is related to the fact of periodontitis be considered a disease with systemic repercussions, but the pathways of such manifestations still need a clearer method. That is, there are systemic inflammatory markers sensitive to this pattern of infection, such as fibrinogen and C-reactive protein (27), but still with indicators of mild alterations in the body, differently from what is seen, for instance, in the cardiology field (28).

One point that could be refined in this research is related to the use of digital reading of animal movements. Despite this shortcoming and operators to become more susceptible to reading errors, they were well trained in pilot studies prior to the execution of this work. It is highlighted that the exams were simple, inexpensive and always undertaken by two operators. Another limitation of this study was the lack of laboratory tests that limited some answers. However, it is noteworthy that these tests have already been exhaustively repeated with the same model of stressor used in this study.

The study of periodontics is approached with increasing frequency in the basic sciences. It is hoped that this work will minimize stress in animal studies and motivate future studies on the CNS and induced periodontal disease in rats.

Conclusion

It was observed that chronic physical stress associated with induced periodontal disease was able to alter the CNS at 7 and 15 days after the induction of periondontitis.

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