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Transmission of stress between cagemates: A study in rats

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HIGHLIGHTS

- We investigated the interaction between stressed and unstressed rats housed together.
- Our results provide an evidence for transmission of stress between cagemates.
- We have found an inverted U-shaped relationship between stress level and anxiolytic effectiveness.

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ABSTRACT

The neuroendocrine responses triggered by stressors cause significant behavioral changes in animals. Considering the continuous behavioral interaction between social animals, it would be reasonable to suggest that the aforementioned behavioral changes can lead to transmission of stress between individuals. In the present study the aim is to investigate the outcomes of the behavioral interaction between stressed and unstressed animals housed together. A total of 28 adult male Wistar rats were used in the study. The animals were randomly allocated to four groups. Two of the groups were exposed to white noise stress in a period of 15 days, while the other two groups remained unstressed. One of the stress exposed groups served as the stress control (SC) group and one of the non-stressed groups served as the reference value (RV) group. The remaining two groups were transmission groups. Every two animals of the non-stressed transmission group (TC) have been housed with two other animals of the stress exposed transmission group (TS) during the experimental period. After the stress exposure period, six animals from each group were subjected to behavioral assessment in an elevated plus maze (EPM), and subsequently, their cortisol levels were determined.

White noise exposure of animals in the SC group induced a stress response indicated by an 1.8 fold increase of plasma cortisol level compared to the RV group (2.11 ± 0.43 and 1.16 ± 0,02, respectively). The transmission groups (TS and TC) entered the open arms more frequently and spent more time in open arms compared to the RV group. White noise exposure caused a stress response characterized by an elevation of cortisol level in rats. The gradual decrease of cortisol level from the SC towards the RV group may be interpreted as an evidence supporting the hypothesis of stress-transmission between cagemates. The moderate stress levels of the transmission groups, but not low and high levels of the SC and RV groups, decreased the anxiety-like behavior, which indicates an inverted U-shaped relationship between stress levels and anxiolytic effectiveness.

1. Introduction

The term “stress” was initially suggested by Hans Selye (1936) about 75 years ago [1], and refers to the physiological response (stress response) elicited when an individual perceives a threat (stressor) to its homeostasis [2]. The stress response triggered by the stressor begins with the activation of hypothalamic–pituitary–adrenal (HPA) axis and proceeds with a cascade of alterations related to endocrine, nervous and immune systems [3–5]. In spite of the fact that stress response is a physiological protective phenomenon, like inflammatory response, it can also have detrimental effects on the organism [6]. Stress response has been found to be able to generate a wide range of problems ranging from impairment of wound healing to even cancer [6,7]. The World Health Organization (WHO) estimated that mental diseases, including stress-related disorders, will be the second leading cause of disabilities by the year 2020 [8]. In previous years stress-related disorders (i.e. depression and anxiety) have been found to cause an economic loss of around $100 billion, because of work days lost, increased impairment at work, and a high use of health care services in the US [9,10]. Furthermore, in terms of agricultural production, stress is an important cause of economic losses impairing the health and productivity of livestock and poultry [11,12]. Because of its aforementioned detrimental health effects, its role in economic losses and the importance for the animal

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welfare, stress has been a major research focus for a wide range of disciplines, since it had been initially defined [13].

The neuroendocrine responses triggered by stressors cause significant behavioral changes in animals [14]. Considering the continuous behavioral interaction between social animals, it would be reasonable to suggest that the aforementioned behavioral changes can lead to transmission of stress between individuals. The behavioral changes caused by stress have been investigated intensively and various animal models have been used for this purpose [15]. However, to the best of our knowledge, there are no behavioral studies focusing on the transmission of stress between animals housed together. In the present study the aim is, therefore, to investigate the outcomes of the behavioral interaction between stressed and unstressed animals that are housed together.

2. Methods

2.1. Animals and grouping

A total of 28 adult male Wistar rats (weighing 340–420 g) were used in the study. They were housed in polycarbonate cages (4 rats/cage) with wood chip bedding. All animals were fed with standard laboratory chow and tap water ad libitum. The cages were maintained in a climate-controlled animal room (temperature: 22 ± 3 °C/relative humidity: 60 ± 5%) with 12/12 h light/dark cycle.

All experimental procedures were approved by the local ethics committee of the Istanbul University. The animals were randomly allocated to four groups as shown in Table 1. All animals were marked with bars on the tail using permanent markers for identification. The animals in groups 2 and 4 were exposed to white noise stress, while the untreated group (group 1) served as the reference value (RV) group. Each of the two animals from the non-stressed group (group 3) has been housed with two other animals from the stressed group (group 4) during the experimental period with the aim to investigate the stress transmission.

2.2. Stress exposure

Animals in groups 2 and 4 were exposed to white noise stress between 11:00 and 15:00 p.m. on alternate days during a period of 15 days (8 stress sessions in total). White noise was produced by a white noise generator, amplified electronically and emitted by loudspeakers installed into a sound-isolated cabinet. Loudspeakers (one speaker per each cage) were fixed above the shelves of the cabinet where the cages were placed. The noise intensity in the bottom of cages was measured with a sound meter, adjusted to 100 dB, which confirmed that the sound intensity was equal (± 1 dB) in all cages. Another cabinet with same specifications with unplugged loudspeakers served as the control cabinet. The background noise level in the cabinets was measured at 50 (±5) dB.

Prior to the stress sessions animals were transferred into the new cages (2 animals per cage), which then were placed into the cabinets. Animals from groups 2 and 4 were exposed to white noise in the stress cabinet for 4 h in every stress exposure session, while in the same period the animals of groups 1 and 3 were kept in the control cabinet. After this four-hour session every animal was returned to its initial cage and was housed there with the same cage-mates during the experimental period.

2.3. Behavioral testing and scoring

After the eight stress sessions in 15 days, six animals from each group (that is, all of the six animals from group 3 and group 4, and six randomly selected animals from group 1 and group 2 each) were subjected to behavioral assessment in an elevated plus maze (EPM).

EPM apparatus consisted of a plus-shaped open roofed construction with two totally open and two trilateral enclosed opposite arms. Each arm is 50 cm long and 10 cm wide. The arms are joined to a central square platform (10 cm × 10 cm). The walls of closed arms are 40 cm high. The maze is elevated at 80 cm from the floor.

At the beginning of the test, each animal was placed on the central square platform facing the open arm. Then the free movements of animals have been video recorded during the 5 min-long trial period. The entire maze was cleaned using ethanol solution (5% v/v) and wiped dry between trials.

At the end of the behavioral testing, video records have been processed using the computer software EthoLog 2.2 [16]. Total time spent in open arms (open arm time — OAT), total time spent in closed arms (closed arm time — CAT), open arm entries (OAE) and closed arm entries (CAE) made have been scored. The frequency of head dips, rears and stretch–attend postures was determined [17].

2.4. Blood tests

Animals were anesthetized (xylazine/ketamine — 10/75 mg/kg) after the behavioral testing and exsanguinated via cardiac puncture. Blood samples were collected into EDTA-coated tubes, and plasma samples were separated via centrifugation. Cortisol levels of the plasma samples were determined using an automated analyzer (Siemens Immulite 2000 XPi, Siemens Medical Solutions USA Inc., Malvern, PA).

2.5. Statistical analysis

Statistical analysis was performed using the SPSS-software package (ver. 11.5.2.1, SPSS Inc., Chicago, IL, USA). Data were first tested for normality using the Shapiro–Wilk test. Data of parameters found to be normally distributed were then compared using one-way analysis of variance (ANOVA). If the normality assumption was found to be violated, non-parametric Kruskal–Wallis test was used for statistical analysis. Tukey HSD and Mann–Whitney U tests were used for pairwise comparisons following parametric and nonparametric tests, respectively. Results are expressed as mean ± SEM.

3. Results

White noise exposure of animals in the SC group induced a stress response indicated by a 1.8 fold increase of plasma cortisol level compared to the RV group (2.11 ± 0.43 and 1.16 ± 0.02, respectively). Even though a gradual numerical increase in the cortisol levels of experimental groups (RV, TC, TS and SC groups, respectively) was observed (Fig. 1), only the difference between the SC and RV groups was found to be statistically significant ($P = 0.012$).

Table 1
Grouping and experimental design.

	n	Group name	Abbreviation	Treatment	Housing
Group 1	8	Reference value	RV	No treatment	Divided into two cages — (2 × 4)
Group 2	8	Stress control	SC	White noise stress	Divided into two cages — (2 × 4)
Group 3	6	Transmission/control	TC	No treatment	2 animals from group 3 (non-stressed) have been housed
Group 4	6	Transmission/stressed	TS	White noise stress	with 2 animals from group 4 (stressed) — (3 × 4)