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EFFECTS OF COLD STRESS ON SPONTANEOUS BEHAVIOR OF RATS

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ABSTRACT
PURPOSE: Activation of the hypothalamic–pituitary–adrenal axis by stress is thought to promote physiological and behavioral changes so that an organism can deal with stress. A number of studies have revealed that various stressors produce differential effects, which are frequently referred to as stressor specific response. The purpose of our study was to investigate the effects of cold stress on spontaneous behavior of male Wistar rats using an open field test.
METHODS: Stress procedure includes 1 hour cold exposure (4oC) in refrigerating chamber. The behavioural responses were assessed during a 5-min observation in open field test which enables the determination of spontaneous motor activity and may allow the interpretation of the registered motor patterns in terms of exploratory motivation and emotional reaction.
RESULTS: The obtained results show that low temperature as inescapable physical stressor significantly reduced ambulation and rearing of rats and significantly increased their freezing behavior compared to the control group.
CONCLUSIONS: One hour cold stress procedure showed well expressed inhibitory effect on spontaneous behavior of male Wistar rats in the open field test.

Key words: physical stress, open field, locomotor activity, immobility

INTRODUCTION

Stress can be described as an organism's response to any influence exerted by environmental or endogenous factors that disrupt homeostatic mechanisms within the organism (1). An integral mediator of the stress response is a set of structures in the brain and peripheral nervous system known as hypothalamic–pituitary–adrenal (HPA) axis. It promotes physiological and behavioral changes so that an organism can deal with stress (2).

Individual differences in the responsiveness of the stress pathways including the sympathetic nervous system and HPA axis have been studied in animals and humans (3-6). The animal's response to stress (and the individual variation it displays) depends not only upon the state and conditions of the animal but also upon the nature (or type) of the stressor itself (3, 7). Stressors can be physical (immobilization, cold or hot exposure) or psychological (emotional) in nature, and have wide-ranging effects on neuroendocrine, autonomic, immune, and hormonal function (8, 9).

A wide variety of animal stress models can be used to understand still unknown mechanism of stress-induced behavioral changes. It is known that temperature fluctuation induced stress. Acute change in temperature leads to stressful conditions by activation of temperature regulatory centre in the hypothalamus and subsequently HPA axis. It leads to acute release of adrenocortical hormones in the blood stream responsible for acute stress. A sharp decrease in temperature using either cold water or freezer has been used frequently to induce acute stress (9).

Taking into account all this background, the aim of the present study was to investigate the effect of cold stress (low temperature

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exposure) on spontaneous behavior of male Wistar rats using an open field test.

MATERIALS AND METHODS

Animals and housing. Twelve experimentally naive male Wistar rats (180-200g) served as subjects. They were kept under normal conditions at ambient room temperature (22 ± 2°C) with a constant day/night cycle (light on from 07:00 to 19:00 h). Rats had free access to standard food and water. All experiments were performed during the light period between 09:00 and 13:00 h.

The experimental procedures were carried out in accordance with the institutional guidance and general recommendations on the use of animals for scientific purposes.

Acute model of cold stress: The animals were placed in a refrigerating chamber at 4oC. The control group was not submitted to 1 hour stress procedure.

Open Field Test: The behavioural responses to novelty were assessed during a 5-min observation session using an open field. The open field is an open-topped, glass box (height 60 cm and square base 100 x 100 cm), which was placed on a white plastic board divided into 25 squares 20 x 20 cm. Each animal was placed in the center of the field in the same position and the field was cleaned after each rat with 70% alcohol. Behavioral observation in the open field involved measurement of the following variables: ambulation, rearing and body posture.

Statistical Analysis: Data collected in the open field were analyzed by Student's t-test. Differences between the experimental groups were considered to be significant if $P \leq 0.05$.

RESULTS

The rats were randomly divided in two experimental groups: a cold stress (CS) group and a control group (CON). Each group included 6 rats. The observation in open filed for CS group was performed immediately after stress procedure. The animal was placed in the center of the apparatus and the following behavioral items are recorded for a 5 min period: horizontal locomotion or ambulation (number of crossings of the lines marked on the floor – horizontal activity), rearing or leaning (animal lifted both front paws simultaneously from the floor of the open field - vertical activity), freezing or immobility (total amount of time an animal was completely motionless, with the exception of breathing).

Our observations during 5-min period showed highly significant changes between experimental groups on all measures. **Figures 1A and 1B** depict significant reduction in ambulation (approximately 76%; $P < 0.001$) and rearing (approximately 57%; $P < 0.05$) of CS animals compared to CON group. The opposite reaction was observed in freezing behavior where immobility was significantly increased in CS group **(Fig. 1C)**.

DISCUSSION

The stress is generally considered as the functional adaptation of the organism in order to cope with a changing and challenging environment. Thus, exposure to a stressor is immediately followed by somatic and neuro-physiological reactions involving peripheral organs (such as adrenals, cardiovascular and respiratory systems, metabolism), and brain areas. It has been established that when animals are subjected to acute stress, a wide range of physiological alterations take place, and a short single intense exposure to stress can produce profound changes in an animal's stress response and behavior (10).

In the present study, we examined 1 hour CS effects on several behavior patterns of male Wistar rats. In several respects, the general patterns replicate what has been reported with rodents, though some notable differences were obtained. In our experiments, the single exposure of 1 h CS (acute, physical stress) to rats resulted in significant behavioral alterations including the decrease in the total line crossings and rearing and an increase in freezing behavior.

According literature data the animal's response to stress depends not only upon the state and conditions of the animal but also upon the nature of the stressor itself (3, 7). Literature data revealed that stress can be physical (cold exposure) or psychological (emotional) in nature and have wide-ranging effects on neuroendocrine, autonomic, immune, and hormonal function. Physical stress has both a physical and an emotional component, but emotional stress does not include a physical component. Previous research using the same paradigm has established that physical stress caused inactivity in a small open field while emotional stress caused naloxone-reversible hyperactivity (8, 9, 11).

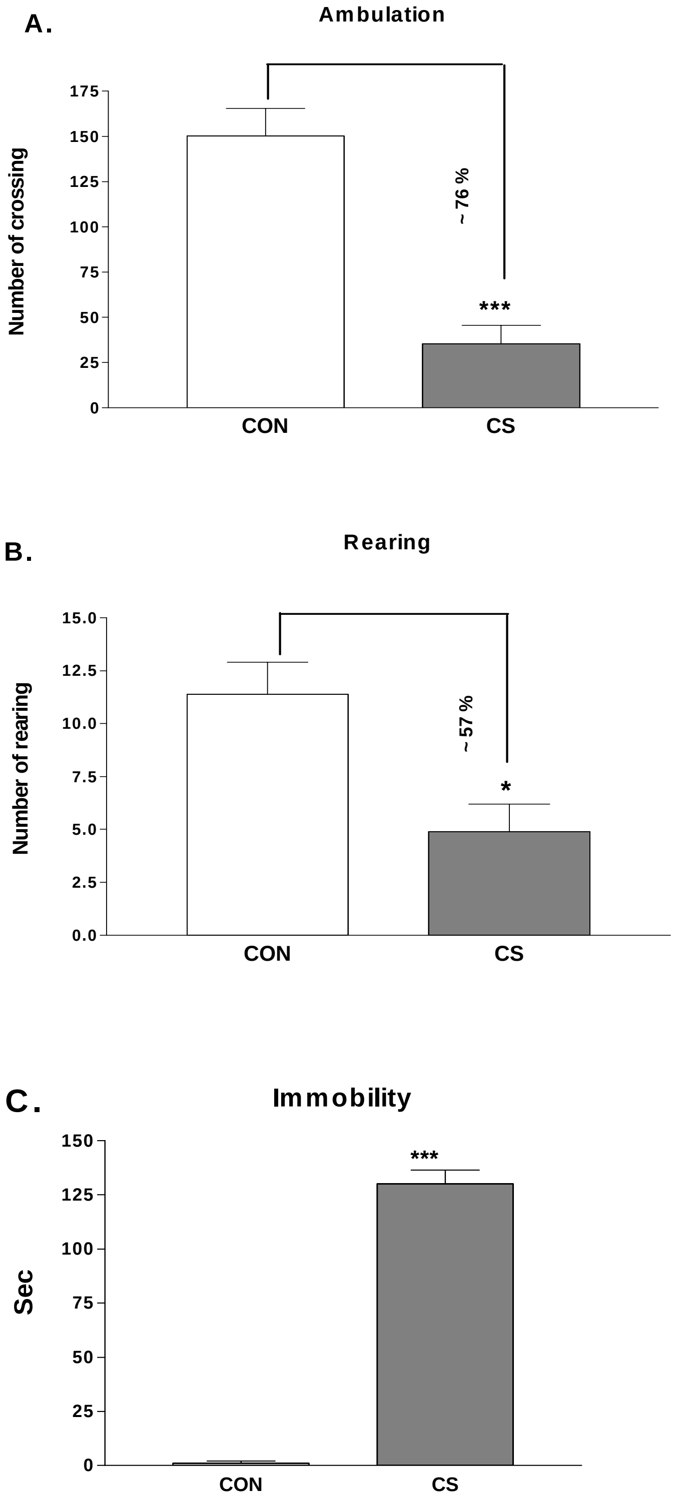

Fig. 1 Behavior during open field test. Mean and SEM are depicted for ambulation (A), rearing (B) and immobility duration (C). Male Wistar rats were previously subjected to cold stress (CS) or control treatment (CON). N = 6 animals; *P<0.05, ***P<0.001 vs. control.

According to classification mention above, cold stress is physical stressor with emotional component. It is one of the most commonly employed animal models for studying different aspects related to stress. Different research groups have shown induction of acute physical stress by low temperature exposure and immobilization which have been reported to impair motor activity, modulate pain perception, anxiety and depression-like behaviors in the animals (12, 13). Our findings corroborate previous reports describing behavioral changes evoked by acute physical stress known as passive coping strategies. These strategies are characterised by reduced somatomotor activity (i.e. quiescence or immobility), decreased vigilance, decreased reactivity, and often hypotension and bradycardia. As well, as a secondary response, a period of passive coping to promote recovery and healing may follow an episode of active coping which has successfully dealt with a stressor (8, 10, 14, 15). Besides, according to Hearn (2005), in the open field test an increase in freezing behavior was regarded as evidence of anxiogenesis while increases in line crosses and rearing were considered anxiolytic effects (16).

In conclusion, one hour cold stress procedure showed well expressed inhibitory effect on spontaneous behavior of male Wistar rats in the open field test. These results confirm anxiogenic effects of physical stressors. Future research should be directed at elucidating the brain areas or peripheral structures that mediate relevant stress-induced behavior. It may be necessary to selectively target brain areas of interest such as the PVN, PAG or amygdaloid region of the brain involved in a wide range of normal behavioral functions and psychiatric conditions.

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