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Research report

Estrogen and voluntary exercise interact to attenuate stress-induced corticosterone release but not anxiety-like behaviors in female rats

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HIGHLIGHTS

- Ovariectomized rats underwent restraint stress and 1 or 3 d access to running wheels.
- Voluntary running did not alter anxiety-like behaviors, with or without restraint.
- Voluntary running reduced corticosterone (CORT) levels in rats with estrogen.
- Exercise more effectively reduces CORT with estrogen, regardless of stress.
- Duration of exercise or distance run may mediate this stress hormone reduction.

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ABSTRACT

The beneficial effects of physical exercise to reduce anxiety and depression and to alleviate stress are increasingly supported in research studies. The role of ovarian hormones in interactions between exercise and anxiety/stress has important implications for women's health, given that women are at increased risk of developing anxiety-related disorders, particularly during and after the menopausal transition. In these experiments, we tested the hypothesis that estrogen enhances the positive impact of exercise on stress responses by investigating the combined effects of exercise and estrogen on anxiety-like behaviors and stress hormone levels in female rats after an acute stressor. Ovariectomized female rats with or without estrogen were given access to running wheels for one or three days of voluntary running immediately after or two days prior to being subjected to restraint stress. We found that voluntary running was not effective at reducing anxiety-like behaviors, whether or not rats were subjected to restraint stress. In contrast, stress-induced elevations of stress hormone levels were attenuated by exercise experience in estrogen-treated rats, but were increased in rats without estrogen. These results suggest that voluntary exercise may be more effective at reducing stress hormone levels if estrogen is present. Additionally, exercise experience, or the distance run, may be important in reducing stress.

1. Introduction

Anxiety disorders and depression are the most common mental illnesses in the United States, affecting 40 million adults age 18 or older, and the economic burden is substantial [1,2]. The chronic and often debilitating nature of these disorders results in costly medical therapies, as well as impaired workplace productivity [2]. These costs could be reduced or avoided with more widespread awareness, recognition, and appropriate early intervention. Accumulating evidence is converging on the concept that lifestyle factors such as exercise can play a role in improving the quality of life. The potential of physical exercise to combat cardiovascular disease, decrease body weight, and delay the physiological effects of aging has been well supported by research studies [3–5]. Moreover, it is becoming increasingly clear that participation in physical activity has beneficial effects for psychological and/or neurological health, as exercise has been shown to play a role in alleviating anxiety and depression [6,7]. However, the underlying mechanisms of the beneficial effects of exercise on anxiety and depression remain unresolved.

Although animal studies have been useful in elucidating some of these mechanisms, including the contribution of central nervous system (CNS) pathways and neurotransmitter systems that may play a role (e.g., [41,43]), there is still much controversy and disagreement. The controversy seems to be attributable to three major issues. First, the type and duration of long-term physical

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activity are highly variable among studies that have investigated the effects of exercise on anxiety [8–18]. Second, such studies typically employ high intensity exercise, which may itself be stressful, and this stress may, in turn, contribute to anxiety and depression [10,14,17]. Finally, most animal studies of exercise are done in males, and usually in male rats [9,12–18]. The latter is particularly perplexing, as exercise may be especially beneficial to women, who suffer disproportionately from anxiety disorders and major depressive disorder (MDD; [22]).

Importantly, the likelihood of developing MDD and anxiety disorders increases in women during perimenopause and the menopausal transition [22], suggesting that premenopausal women are protected from anxiety/depression by ovarian hormones. In fact, numerous studies have shown that ovarian hormones influence physiological and behavioral responses to stressful stimuli [19–21]. Although many exercise studies have focused on the stressful aspects of prolonged and/or endurance exercise for women's health (for review see [33]; see also [35]), at present it remains unknown whether shorter duration, moderate-intensity exercise may have beneficial effects on stress responses in the presence of ovarian hormones such as estrogen. Better understanding of this issue may be particularly relevant in addressing the difficulties of adhering to an exercise program. Accordingly, in this study we investigated the combined effects of moderate-intensity voluntary exercise and estrogen on behavioral and hormonal responses to a stressor in female rats. Our specific goals were to determine whether (1) voluntary running influenced behavioral responses associated with stress/anxiety as well as plasma levels of the stress hormone, corticosterone (CORT), in response to acute restraint stress; (2) whether estrogen modulated the influence of voluntary running on these responses; and (3) whether prior experience with running was a necessary for any beneficial effects.

2. General methods

2.1. Animals, ovariectomy, and estrogen replacement

Adult female Sprague-Dawley rats weighing between 250 and 350 g were used in these experiments. Rats were individually housed in plastic cages, and given *ad libitum* access to Harlan rodent diet (no. 2018) and water. Rats were maintained in a temperature-controlled room (21–25 °C) on a 12:12 light:dark cycle with lights on at 7:00 AM. All procedures were approved by the Oklahoma State University Center for Health Sciences Animal Care and Use Committee.

Under sodium pentobarbital anesthesia (50 mg/kg body weight IP; Sigma–Aldrich), rats were bilaterally ovariectomized (OVX) using a ventral approach and given 7–10 days to recover. After recovery from ovariectomy, rats were given subcutaneous injections of 17-β-estradiol-3-benzoate (EB, Fisher Scientific; 10 μg/0.1 mL sesame oil) or the vehicle (OIL; 0.1 mL sesame oil) on a 4-day protocol that mimics patterns of estrogen fluctuations during the estrous cycle. Specifically, rats were given EB or OIL daily for two consecutive days (Day 1 and Day 2), and were tested 48 h after the second injection (i.e., on Day 4; see Section 2.2, below). Previous work [23] showed that this estradiol replacement protocol produces circulating plasma levels of estradiol on Day 4 that are comparable to those at estrus, as we also showed in our recent publication [24]. All rats were weighed on all four days of the protocol.

2.2. Experimental design and conditions

Approximately one hour before lights out on Day 3 of the 4-day protocol, groups of EB- and OIL-treated OVX rats were weighed and then placed in a clear acrylic rodent restraint tube (Harvard Apparatus; restrained) for 45 min [30]. Other groups of EB- and OIL-treated rats were weighed but were not placed in the restraint tube (not restrained).

To assess the influence of exercise on stress responses, some EB- and OIL-treated OVX rats were given free access to running wheels (Lafayette Instruments) that were attached to the home cages. An electronic counter (Lafayette Instruments) monitored the number of revolutions to determine the distance run. To assess the influence of exercise *experience*, some rats were given access to running wheels only on Day 3 immediately after the restraint stress. Testing was timed to ~coincide with the dark phase of the cycle when rats are most active; thus, rats had approximately 14 h access to the running wheels (1 day exercise), primarily during the dark period. Other rats had access to running wheels beginning immediately after EB/OIL injections on Day 1, were subjected to restraint stress on Day 3, and then were given approximately 14 additional hours of access to the running wheels immediately after restraint (3 days exercise). Control groups of EB- and OIL-treated rats had no access to running wheels during the experiment (no exercise).

Thus, groups of EB- and OIL-treated OVX rats were tested in one of six conditions; conditions and numbers of animals in each condition are shown in Table 1.

Table 1
Experimental conditions and numbers of oil vehicle (OIL) or estradiol benzoate (EB) treated ovariectomized rats with (restrained) or without (not restrained) restraint stress. Some rats were sedentary (no exercise), others had access to running wheels only for ~14 h after restraint (1 day exercise), others had access to running wheels for 2 days prior to restraint and for 14 h after restraint (3 days exercise).

	OIL-treated		EB-treated	
	Not restrained	Restrained	Not restrained	Restrained
No exercise	N = 5	N = 10	N = 6	N = 11
1 day exercise	N = 6	N = 6	N = 5	N = 6
3 days exercise	N = 8	N = 8	N = 7	N = 7

2.3. Anxiety-like behaviors: testing apparatus and procedures

The Elevated Plus Maze (EPM) consists of an elevated (~50 cm higher than ground level), plus-shaped platform with two open arms (50 × 10 cm) and two enclosed arms (50 × 10 cm). Rats show a reliable preference for the closed arms that is positively related to anxiety [31]. Thus, EPM testing is a widely used behavioral assay for anxiety in rodents that has been employed to assess anti-anxiety effects of pharmacological agents and steroid hormones [31], as indicated by increased time spent on the open arms.

All rats were tested on the EPM on Day 4 of the 4-day protocol, a point that corresponds with reliable and replicable behavioral effects of EB in numerous studies [24–26,28], as well as with EB effects on central neural activation and neurotransmitter systems, as shown in our studies [27,29]. Rats with 1 or 3 days of exercise experience were tested after overnight access to running wheels; rats that did not exercise were tested at approximately the same time of day (9:00–11:00 AM). Each rat was placed at the junction of the four arms of the EPM facing an open arm and allowed to freely explore the maze for 10 min. This test was recorded using a video camera placed vertically above the EPM. Videos of the behavioral tests were evaluated using a software program (Noldus Information Technology, Ethovision XT, The Netherlands), which assessed the duration of time spent in center, open, and closed arms. The number of times the rat crossed the center of the EPM (center crossings) also was monitored to ensure that all animals demonstrated normal locomotor and exploratory activity.