Attenuation of the Immunosuppressive and Adrenal Responses to Noise Stress in Rats Via Exercise

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Abstract: The ability of exercise to attenuate the stress response in rats was studied. Forty male rats with varied genetic backgrounds were randomly divided into stress and non-stress groups. Both stress and non-stress groups were then divided into exercise and non-exercise groups (n=10). The stressed rats were exposed to 105-110 decibel noise, duration of ten seconds, at three separate times between 11:00 p.m. and 6:00 a.m. After 27 days, all rats were injected with sheep erythrocytes. One week following the injection, animals were sacrificed. Blood was collected and agglutination tests were performed to determine immunological response. The immunological data were transformed by expressing the reciprocal of the data as powers of the $base_2$. Adrenal glands were removed and their mass was determined. Stressed rats that exercised demonstrated greater than 300% increase in antibody production as compared to stressed rats denied exercise. Non-stressed rats allowed access to exercise exhibited a slight rise in antibody production as compared to non-stressed non-exercising rats, however this increase was not significant. The results from the adrenal data were inconclusive. The immunological results, therefore, support the hypothesis that exercise attenuates the stress response.

Stress is something everyone seems to understand without much explanation, and yet in actuality it is a rather poorly defined phenomenon. In general, stress involves an organism encountering and reacting to a situation and the physiological and mental tension or strain this causes the organism (Burchfield, 1979). Although different types and intensities of stress elicit somewhat different responses in different organisms, a relatively common and consistent physiological response has been identified for most vertebrates. Stressful stimuli activate the sympathetic nervous system and cause an increase in heart rate and blood pressure (Cohen and Obrist, 1975). Increases in plasma levels of corticosteriods, catecholamines, and glucose have also been shown in various studies (Griffiths, 1981; Sinyor et al., 1983; Hull et al., 1984; Sothman et al., 1987). These physiological changes are commonly referred to as the "fight or flight" response, which prepares the body for action in the case of danger.

Stress has been associated with an even greater array of physiological changes, though. Studies done in Japan by Akira Tsuada (1981) have shown an increase in adrenal weight due to stress in rats. Dunn (1988) reviews the literature noting immunosuppressive affects of stress on an organism. Interestingly, Dunn suggests a link between the adrenal gland and the immunosuppressive effects of stress on an organism. It is noted, however, that for immunosuppressive effects to be observed, the stress should be "relatively acute or

chronic" (Dunn, 1988. p. 594).

Burchfield (1979) describes three distinct categories of acute, chronic, and chronic intermittent stress, and holds that an organism's reaction to these types of stressor states is different. Acute stress arises when a situation occurs for a relatively short time period and then does not occur frequently, or perhaps not at all. Chronic stress occurs when an individual is exposed to the stressful event continuously. Chronic intermittent stress is described as a situation in which an organism is repeatedly exposed to a stimulus for a given amount of time.

Noise stress (acute, chronic, and chronic intermittent) has been shown to be a sufficient environmental stressor to bring about many physiological changes in rats. Overton et al. (1991) found that chronic intermittent noise stress of 110 db caused cardiovascular and sympathoadrenal responses in the rat. De Boer et al. (1989) found that both acute and chronic noise stress of rats caused changes in plasma levels of catecholamines and corticosterone; the two types of stress differed in the response observed. Chronic noise stress has also been shown to affect insulin secretion in male rats (Armario et al., 1985). Review of the literature did not reveal a study using noise stress to affect immune response.

It is not difficult then to find a significant environmental stressor for rats. It may be more difficult to find ways to alleviate or attenuate the stress response, however.

Physical exercise has commonly been promoted as a means of managing psychophysiological responses to stress (Heywood, 1978; Folkins and Sime, 1981; Mills and Ward, 1985). Tharp and Carson (1975) found evidence supporting their hypothesis that chronic physical exercise functions to reduce emotionality in rats. Sinyor et al. (1983) measured reactivity to psychological stress using physiological, biochemical, and subjective measures in trained and untrained individuals; evidence pointed toward aerobic fitness influencing reactivity to stress in a laboratory setting. A review by Crews and Landers (1987) showed that aerobically fit subjects had reduced psychosocial stress response to either control groups or baseline values.

Gordon and Scott (1991, p. 683-684) hold that in current research exercise is emerging as "one of the few potentially effective and physiologically desirable nonpharmacologic approaches" for treating mild hypertension. Reduced incidence of coronary heart disease is correlated with regular physical activity (Overton et al., 1991). Results of Sime (1977) suggest that brief mild exercise reduces some autonomic responses to an acute stressor. The findings of Sothman et al. (1987) indicate that individuals with high cardiovascular fitness may have an attenuated norepinephrine response to psychological stress. Trained rats exposed to acute noise stress show less of an elevation in arterial pressure and heart rate than due untrained rats. There are a number of further examples in which physical training has been seen to lessen the severity of the cardiovascular response to stress (Birrell and Roscoe, 1978; Hull et al., 1984; Claytor et al., 1988). There seems to have been little if any previous study, however, concerning the effects of exercise on the immunosuppressive stress response.

The purpose of this study was to extend the investigation of attenuation of the stress response through exercise, using immune and adrenal measures. Rats were used as subjects. The stressor chosen for this study was chronic intermittent noise stress. There were three main reasons for this: its ease of availability; Dunn's (1989) suggestion that stress must be acute or chronic to affect the immune system; and it has been shown in various studies to bring about other physiological changes in rats (Armario et al., 1985; De Boer et

al., 1988; Overton et al., 1991). My hypothesis was that the immune response would be suppressed in the stressed rats but that this immunosuppression would not be present in the stressed rats which had exercise available. Similarly, the hypothesis was that the adrenal size would be larger in the stress group, but not for the stress group with exercise available.

MATERIALS AND METHODS

Forty male rats of varied genetic backgrounds, and approximately three months in age were randomly divided into four groups: Stress No Exercise; Stress Exercise; Non-Stress No Exercise; Non-Stress Exercise. They were provided with food and water *ad libitum*. The rats were housed individually in 26 by 26 by 26 cm wire cages with a wooden back. Half of these had exercise wheels attached, and half did not. The number of revolutions per day was recorded for the exercise group.

The stressed group was exposed to 105-110 db noise (a horn blast), duration of ten seconds, at three different times between the hours of 11:00 pm and 6:00 am (using a variation of the method described by Overton et al., 1991). This was done on a random schedule between these hours to prevent habituation to the noise stress. After 27 days, all rats were injected IP with antigen [2 mL of a 1% suspension of sheep red blood cells (SRBC)] (Ader and Cohen, 1975). They were sacrificed one week following and blood was removed from the heart.

Adrenals were removed, washed in saline, and weighed. Values were converted to a ratio of adrenal weight to body weight and multiplied by a factor of 10,000.

Serum was isolated from the blood samples through centrifugation. Antibody titrations were performed using standard procedures in microtiter plates (Sigma, 1989) and hemagglutination was assessed. The end point dilutions were recorded, and then reciprocals of the end point values were expressed as powers of the $base_2$ (Ader and Cohen, 1975) and used in statistical analyses. A factorial analysis of variance and the Tukey honestly significant difference test were performed using SYSTAT.

RESULTS

Weights of adrenal glands varied among the treatment groups (see Appendix), but the only significant difference occurred between the Stress Exercise and the Stress No Exercise groups ($p = 0.026$) (Table 1). There were no other significant differences among the adrenal measures.

Immune response showed little variation except in the Stress No Exercise Group (see Appendix). The mean immunosuppressive index for the stress group was significantly lower ($p < 0.001$) than the indices for the other groups (Table 1). There was a significant interaction effect for stress and exercise ($p = 0.002$). The main affect was for the Stress No Exercise group only.

A difference in revolutions per day between the two exercise groups was found to be marginally significant (t-test, $p = 0.073$) with the stressed rats running somewhat more than the non-stressed rats. It should be taken into consideration, however, that there was great variation in the ranges and that no outliers were dropped for this analysis.

Table 1. Mean adrenal responses and transformed and non-transformed mean anti-SRBC titers for rats under all experimental conditions.

Group	Mean Adrenal Index (± SD)	Mean Immune Response	
		Transformed	Non-Transformed
No Stress No Exercise	1.595 (0.258)	19.5	741,455
No Stress Exercise	1.609 (0.263)	19.7	834,180
Stress No Exercise	1.354 (0.216)	17.7	212,927
Stress Exercise	1.742 (0.345)	19.4	691,802

DISCUSSION

It was found that immunosuppression was in the Stress No Exercise group and not in the other three groups, supporting the original hypothesis. Stressed rats that exercised demonstrated greater than a 300% increase in antibody production as compared to stressed rats denied exercise. The interaction affect between stress and exercise can be interpreted to mean that the exercise effect modulated the stress effect, but the main effect was for the stress group only. In other words, under normal no stress conditions, an exercise wheel made no difference in the immune response, but under stress conditions exercise appeared to prevent the immune suppression effect of stress. Such findings support the first hypothesis of this paper.

The second hypothesis concerned the adrenal measure and was not supported by the evidence. The only significant difference in the adrenal measures was that the Stress Exercise and Stress No Exercise groups were different from one another (but not from the other groups). An explanation for these findings is illusive. Before considering it too carefully, however, I would suggest a refinement of the procedures for removing and weighing adrenals.

The finding that the amount run by the two exercise groups differed from one another was not significant at the $p = 0.05$ level, should perhaps be considered because there was a wide degree of variance and no outliers were dropped from the analysis. Not surprisingly, the stressed rats ran more than the non-stressed rats ($p = 0.073$). These data do not specifically pertain to the hypotheses, but might be useful in future studies.

The findings in this study that support the hypothesis that exercise attenuates the immunosuppressive response to stress are exciting and full of possibilities. It would be best to first see this study replicated. Further areas of interest concerning this finding could then be explored. It would be curious to see if the effect of exercise on immunosuppression in a stressful condition generalizes to other animals, e.g., humans. Another area to investigate would be the generalization of these findings to other stressors. The noise stress aspect also has future possibilities. Would loud noises, say from busy streets, airports, or busy factories,

cause an immunosuppressive response and could this be modified by exercise? The research possibilities are numerous and exciting.

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APPENDIX

Table 2. Body weights and adrenal weights for rats exposed to stress or no stress and allowed to exercise.

Rat Number	Body Weight (g)	Adrenal Weight (g)
Stress		
1.0	228.0	0.0391
2.0	261.0	0.0386
3.0	330.0	0.0388
4.0	226.5	0.0334
5.0	214.0	0.0361
6.0	238.0	0.0349
7.0	257.0	0.0427
8.0	238.0	0.0428
9.0	229.0	0.0412
10.0	217.0	0.0576
No Stress		
11.0	275.0	0.0425
12.0	193.0	0.0379
13.0	260.0	0.0358
14.0	251.0	0.0400
15.0	263.0	0.0427
16.0	260.0	0.0349
17.0	232.0	0.0499
18.0	234.4	0.0353
19.0	245.0	0.0329
20.0	279.0	0.0430

Table 3. Body weights and adrenal weights for rats exposed to stress or no stress and not allowed to exercise.

Rat Number	Body Weight (g)	Adrenal Weight (g)
Stress		
0.5	260.0	0.0357
1.5	240.0	0.0402
2.5	280.0	0.0351
3.5	261.0	0.0370
4.5	267.0	0.0361
5.5	299.0	0.0406
6.5	293.5	0.0329
7.5	266.0	0.0256
8.5	247.0	0.0338
9.5	281.0	0.0466
No Stress		
10.5	257.0	0.0425
11.5	265.5	0.0507
12.5	252.5	0.0421
13.5	229.0	0.0296
14.5	202.0	0.0375
15.5	235.0	0.0344
16.5	233.5	0.0339
17.5	272.0	0.0484
18.5	257.0	0.0286
19.5	227.0	0.0400

Table 4. Anti-SRBC titers for rats exposed to stress or not exposed to stress.

Exercise Group		Non-exercise Group	
Rat Number	Immune Response	Rat Number	Immune Response
Stress		Stress	
1.0	19.0	0.5	18.0
2.0	20.0	1.5	19.0
3.0	19.0	2.5	16.0
4.0	19.0	3.5	17.0
5.0	20.0	4.5	18.0
6.0	19.0	5.5	18.0
7.0	20.0	6.5	17.0
8.0	19.0	7.5	18.0
9.0	19.0	8.5	18.0
10.0	20.0	9.5	19.0
No Stress		No Stress	
11.0	20.0	10.5	20.0
12.0	20.0	11.5	19.0
13.0	20.0	12.5	20.0
14.0	18.0	13.5	19.0
15.0	19.0	14.5	19.0
16.0	20.0	15.5	19.0
17.0	20.0	16.5	20.0
18.0	19.7	17.5	19.0
19.0	20.0	18.5	20.0
20.0	20.0	19.5	20.0