Pharmacology Biochemistry & Behavior, Vol. 32, pp. 913–917. Maxwell Pergamon Macmillan plc, 1989. Printed in the U.S.A. 0091-3057/89 $3.00 + .00

Individual Behavioral and Neuroendocrine Differences in Responsiveness to Audiogenic Stress

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Received 12 August 1988

IRWIN, M. R., D. S. SEGAL, R. L. HAUGER AND T. L. SMITH. *Individual behavioral and neuroendocrine differences in responsiveness to audiogenic stress.* PHARMACOL BIOCHEM BEHAV **32**(4) 913–917, 1989.—A relatively wide range of individual differences in neuroendocrine, immune and behavioral components of the audiogenic stress response has been found. In this study, an analysis of the association between the physiological and behavioral measures revealed that the degree of noise-induced suppression of both general activity and ingestive behaviors was significantly correlated with activation of adrenal steroid secretion following both acute and repeated noise exposures. Splenic natural killer cytotoxicity was not correlated with the behavioral measures of the stress response. Characterization of individual behavioral response profiles may be needed to evaluate accurately the neuroendocrine effects of stress.

Stress Adrenal activity Immune function Natural killer cells Depression Neuroendocrinology

RESPONSIVENESS to inescapable, aversive stimulation has been used as an animal model to investigate clinical depression. Similar to the neuroendocrine and immune changes associated with depressive illness (9, 13, 16, 22), the acute administration of stressors such as sound exposure, rotation, and intermittent shock to rats produces an elevation in plasma corticosterone levels and a reduction in cell-mediated immune responses including natural killer (NK) cytotoxicity (18, 20, 21). NK cells are thought to be important in host resistance to some viral challenges and metastatic spread of tumors (6).

Despite efforts to minimize experimental variation, neuroendocrine and immunological responses to aversive stressors often show a relatively wide range of individual differences in rats. Likewise in clinical research, considerable variation in neuroendocrine and immunological values has been found among individuals undergoing severe life distress (7, 8, 10). In this regard, psychologic factors have been suggested as at least potentially responsible for mediating these physiological changes in clinical populations. For example, during bereavement, the severity of depressive symptoms, and not simply the occurrence of the event, is associated with a reduction in NK activity (12) and an increase in adrenocortical activity (7,8). Demonstration of a similar relationship in animals would facilitate the study of possible mechanisms underlying stress-induced changes in these variables.

Noise stimulation was selected for use in the present studies because, in contrast with most other paradigms used in animal studies, this stressor allows for characterization of the behavioral response profile during, as well as after, stimulus exposure (23). We have recently characterized the audiogenic stress response with respect to a variety of different locomotor and ingestive behaviors and have found that responses exhibit a marked range of variation (23).

In the present study, the pattern of change of individual behavioral, neuroendocrine, and immune responses was examined in rats following acute and repeated noise exposure. We found that individual differences in suppression of general activity and ingestive behaviors during noise exposure significantly correlated with elevations in plasma corticosterone.

METHOD

Subjects

Male Sprague-Dawley rats (N = 60) (275–325 g) obtained from Simonson Laboratories were housed in groups of 3 with continuous access to food and water. Animals were maintained in constant temperature animal facilities under a reverse 12-hour red light 6:00 a.m. to 6:00 p.m., 12-hour white light cycle for two weeks prior to the experiments.

Behavioral Apparatus and Procedures

Behavioral characterization was carried out with the use of custom-designed residential activity chambers (RAC). Each of the RACs was located in a sound-attenuated, ventilated cabinet and consisted of two compartments: an activity/exploratory compartment (12″ × 12″ × 15″) and a smaller home compartment (5¾″ × 5¾″ × 4″).

The behavioral monitoring procedures have been previously

TABLE 1

BEHAVIORAL MEASURES FOLLOWING ACUTE AND REPEATED NOISE EXPOSURE

	Interval 10–60 Minutes*								
	Day of Treatment			Significance					
				Noise		Day		Noise × Day	
	1-Day	4-Day Mean (SD)	10-Day	F (df=1,54)	p	F (df=2,54)	p	F (df=4,54)	p
General Activity†									
Apparatus Control	78(20)	86(22)	79(30)						
				10.5	<0.002	4.9	<0.01	2.6	0.08
Noise Exposure	43(17)	75(48)	41(26)						
Ingestive Behavior (in seconds)									
Apparatus Control	176(175)	426(174)	515(338)						
				11.6	<0.001	7.2	<0.002	2.0	0.14
Noise Exposure	56(66)	156(109)	150(284)						

*Each experimental group contained 10 animals.

†Measured by composite number of cross-overs, rearings, home compartment entries/exits, and stimulus contacts.

described in detail (23). The behaviors assessed included movements of the animals within the activity/exploratory compartments (cross-overs), entries to and exits from the home compartment (intercompartment crossings), and duration of time in the home compartment (home duration). Rearing and contacts with either a hanging wire mesh object or a wire mesh window were monitored as indices of environmental interaction. Within the home compartment, contacts with a water sipper tube or with a recessed metal food container provided measures of drinking and eating, respectively. We have found high correlations between the amount of food consumed and duration of contact with the food trough, and between the volume of water consumed and duration of contact with water in the sipper tube.

Noise (108 dB) was delivered to the individual activity chambers via ⅜″ Realistic speakers suspended 3″ above each activity compartment within the sound-attenuated chamber. For experiments involving repeated daily exposure to noise, rats were transported from the animal housing facility to an experimental room, placed in individual containers, and exposed to a broad-banded noise (20–20,000 Hz) at 108 dB or quiet for one hour before being returned to their respective group cages. Apparatus control animals were treated identically with the exception of noise exposure. On the day of experimentation, beginning about 2–3 hours after the onset of their dark cycle, animals were placed for the first time in the behavioral apparatus or RAC. To insure relatively comparable exposure of all animals to both compartments, each rat was initially restricted to the activity compartment for 60 minutes followed by 30 minutes in the home compartment. After this 90-minute period of habituation, the barrier between the two compartments was removed and the rats were exposed to the noise (108 dB) or quiet for one hour. Immediately after noise offset, animals were removed and killed by decapitation.

Assay of Corticosterone

For the measurement of plasma corticosterone levels, approximately 5 ml of truncal blood was collected in plastic conical centrifuge tubes containing 200 μl of a solution of 50 mg/ml EDTA and 500 KIU of aprotoninin (Sigma Co., St. Louis, MO). Plasma was immediately prepared at 4°C and samples were frozen at −70°C until assay. Plasma corticosterone was measured using an antibody produced against corticosterone-21-hemisuccinate: bovine serum albumin (RSL, Carson, CA) as previously described (14). Plasma was heat denatured at 60°C prior to assay. The sensitivity of the assay is 12 pg and the interassay and intraassay coefficients of variation are about 17% and 11%, respectively.

Cytotoxicity Assay

NK cytotoxicity was assayed using splenic mononuclear cells isolated by sedimentation on Ficoll-Hypaque and treatment with carbonyl iron; a procedure that yields a 99% viable cell-suspension typically containing 95% lymphocytes and less than 5% monocytes (by Wright's stain) (17). Assay of NK cytotoxicity involved coincubation of effector cells with chromium-labelled YAC-1 murine lymphoma target cells at the effector:target (E:T) cell ratios of 100:1, 50:1, 25:1, and 12.5:1 in triplicate. After 4 hours of incubation, plates were centrifuged for 2 minutes at 500 × g and 100 μl of supernatant was harvested from each well. The amount of radioactivity was determined using a gamma-counter and for each E:T ratio the percent specific chromium release was determined. Chromium release data was expressed as lytic unit (LU) values using a least squares means computer program (SAS) (2).

Statistical Analysis

To evaluate potential systematic differences in the assay and measurement of NK cytotoxicity and of plasma corticosterone across the 1-, 4-, and 10-day treatment groups, home cage control animals (n=4) who were neither exposed to noise nor to the apparatus were run concurrently on each experiment day. In these home cage animals, mean (±SD) plasma levels of corticosterone [16.2±0.7, 17.3±0.9, and 12.2±0.3 μg/dl; $F(2,11)=0.21$, $p=0.81$] and mean values of splenic NK cytotoxicity [124.8±56.9, 110.4±24.3, 139.8±64.1 LU; $F(2,11)=0.33$, $p=0.73$] were similar across the three experiment days, respectively.

Behavioral measurement of general activity was expressed as a composite score of numbers of cross-overs, rearings, home compartment entries/exits, and stimulus contacts. Ingestive behavior was represented as total time (in sec) spent eating and drinking

TABLE 2

PLASMA CORTICOSTERONE AND SPLENIC NATURAL KILLER CYTOTOXICITY FOLLOWING ACUTE AND REPEATED NOISE EXPOSURE

	Day of Treatment			Significance					
				Noise		Day		Noise × Day	
	1-Day	4-Day Mean (SD)	10-Day	F	p	F	p	F	p
				(df = 1,54)		(df = 2,54)		(df = 4,54)	
Corticosterone (μg/dl)									
Apparatus Control	18.1(9.8)	12.8(6.5)	10.7(5.3)						
				23.8	<0.001	21.7	<0.001	7.2	<0.002
Noise Exposure	39.3(12.7)	15.7(5.4)	17.2(6.1)						
NK Activity (in LU)									
Apparatus Control	115.4(32.1)	173.9(51.5)	99.1(63.2)						
				4.2	<0.04	2.1	<0.13	4.3	<0.04
Noise Exposure	127.8(51.1)	175.4(53.6)	224.4(73.9)						

during the treatment interval.

To evaluate the effect of acute and repeated exposure to the noise stressor on the dependent variables including general activity, ingestive behaviors, plasma corticosterone and NK cytotoxicity, 2 (group:noise exposure, apparatus) × 3 (treatment days: 1-, 4-, 10-day) analyses of variance (ANOVAs) were used. Post hoc comparisons at the $p<0.05$ level were evaluated using Newman-Keuls tests. The relationship between the behavioral indices and changes in either plasma corticosterone levels or splenic NK activity was assessed using nonparametric Spearman correlation procedures.

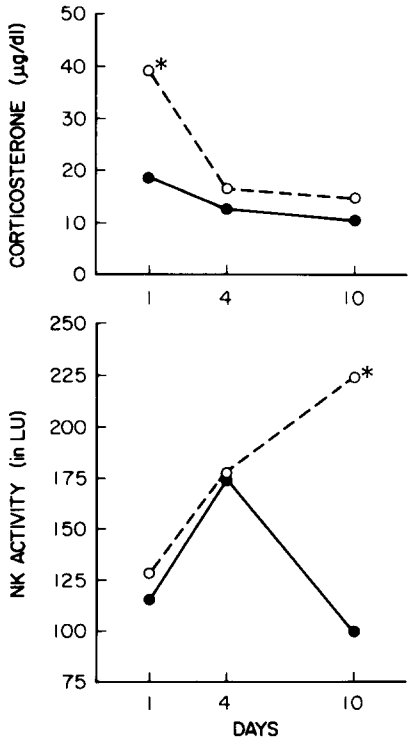

FIG. 1. Plasma corticosterone levels and splenic NK activity following 1, 4, and 10 days of exposure to noise (108 dB). *Indicates that noise-treated animals are significantly ($p<0.05$) different from their respective treatment day apparatus controls. Each experimental group contains 10 animals. ○: Noise exposure, ●: apparatus controls.

RESULTS

As described previously (23), characterization of the audiogenic stress response during noise exposure reveals a multiphasic response pattern involving an initial hyperactivity phase lasting up to 10 minutes followed by a period of behavioral suppression which persists for the remainder of 60-minute noise exposure. For the interval lasting from 10 to 60 minutes, noise exposure produced a significant suppression of both general activity, $F(1,54) = 10.5$, $p<0.002$, and ingestive behaviors, $F(1,54) = 11.6$, $p<0.001$ (Table 1). Day of treatment also yielded significant main effects for general activity, $F(2,54) = 4.9$, $p<0.01$, and for ingestive behaviors, $F(2,54) = 7.2$, $p<0.002$, with both behavioral measures increasing with repeated treatment. No significant interaction between noise exposure and day of treatment was demonstrated for either behavioral measure.

For plasma levels of corticosterone, significant main effects were found for noise exposure, $F(1,54) = 23.8$, $p<0.001$, day of treatment, $F(2,54) = 21.7$, $p<0.0001$, and the interaction between noise exposure and day of treatment, $F(4,54) = 7.2$, $p<0.002$ (Fig. 1 and Table 2). Post hoc comparisons demonstrated that the animals exposed to noise for one day had significantly ($p<0.05$)

TABLE 3

RELATIONSHIP BETWEEN PLASMA CORTICOSTERONE AND BEHAVIORAL MEASURES IN ALL SUBJECTS

	General Activity		Ingestive Behaviors	
Corticosterone	r	p	r	p
Day of Treatment				
1-Day group (n = 20)	−.60	<0.001	−.44	<0.03
4-Day group (n = 20)	−.36	0.06	−.34	.07
10-Day group (n = 20)	−.46	<0.02	−.46	<0.02

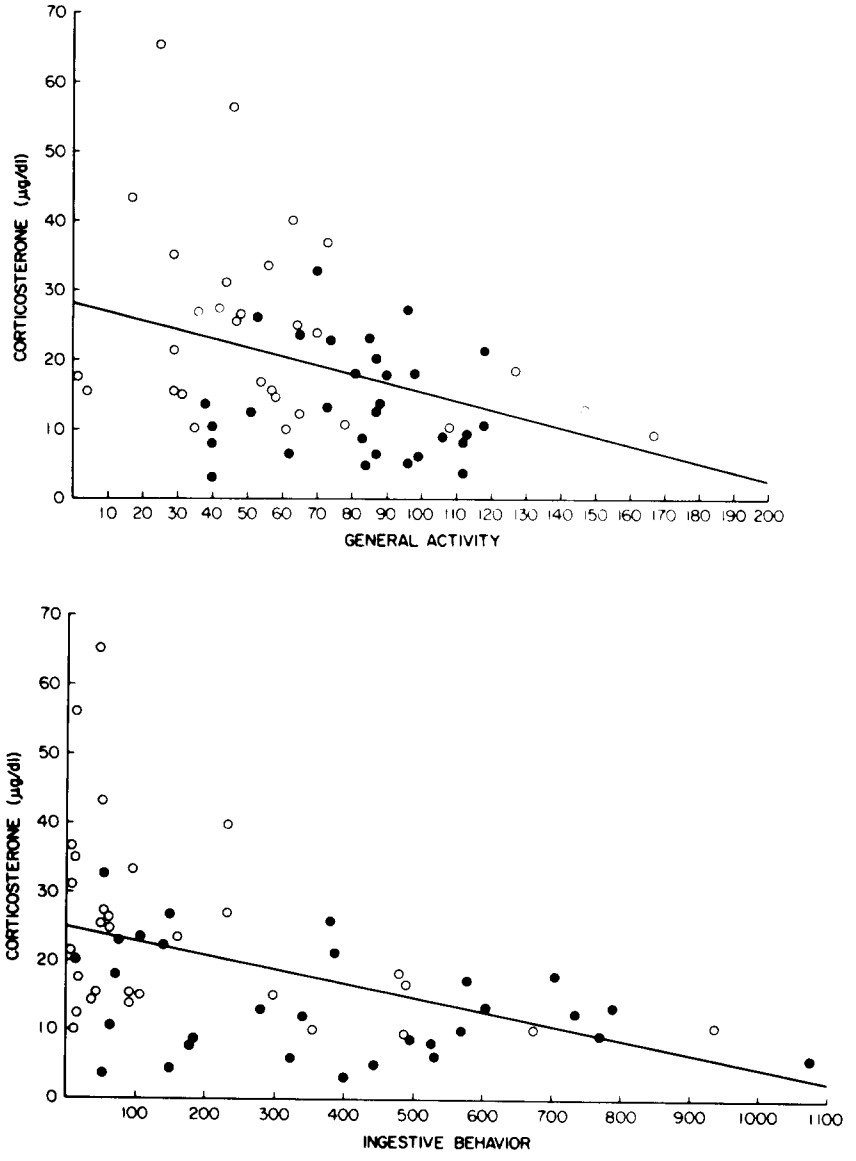

FIG. 2. Relationship between plasma levels of corticosterone and the behavioral measures of general activity and ingestive behaviors. The regression curve is based on values from the combined groups including noise- (○) and apparatus- (●) treated subjects across the 1, 4, and 10 days of treatment.

higher levels of corticosterone than the animals who had received either 4 or 10 days of repeated noise or exposure to the apparatus. For splenic NK cytotoxicity there was significant noise effect, $F(1,54)=4.2$, $p<0.04$, and a significant noise by day of treatment interaction, $F(2,54)=4.3$, $p<0.04$, in which post hoc comparisons demonstrated that animals exposed to 10 days of noise treatment had *greater* lytic unit values than their respective ($p<0.05$) controls (Fig. 1 and Table 2). Although the control rats demonstrated an increase in mean NK activity on day 4, this result was not statistically significant as compared to mean lytic activity on day 1 and no significant effect for day of treatment was found.

The relationship between individual differences in noise-induced suppression of the behavioral measures and elevated plasma levels of corticosterone is illustrated in Fig. 2. Noise-induced suppression of general activity was significantly correlated with elevated plasma levels of corticosterone for the combined groups ($r=-.37$, $p<0.003$, $N=60$; $y=-1.36x+28.4$) and for the noise-treated animals alone ($r=-.34$, $p=0.06$, $N=30$; $y=-1.22x+31.1$), but not for the apparatus controls ($r=-.06$, $p=0.75$, $N=30$; $y=-0.20x+15.5$) (Fig. 2). Suppression of ingestive behaviors was also correlated with elevated corticosterone levels for the combined groups ($r=-.45$, $p<0.001$, $N=60$; $y=-0.20x+24.8$) and for the noise-treated animals ($r=-.40$, $p<0.03$, $N=30$; $y=-0.24x+28.3$), but not for the control subjects ($r=-.33$, $p=0.08$, $N=30$; $y=-0.09x+17.4$). When the sample was examined with regard to day of treatment, negative correlations between the behavioral measures and values of plasma corticosterone were found within each day of treatment including the 1-, 4-, and 10-day groups (Table 3). Although the correlation between general activity and plasma corticosterone on day 4 explained only half the variance of the day 1 correlation, the magnitude of correlation for each day of treatment was not

statistically different ($z = 0.92$). Changes in splenic NK activity were not correlated with either of the behavioral measures.

DISCUSSION

Audiogenic stress produces a significant suppression of general activity and ingestive behaviors, acute activation of adrenal steroid secretion, and with 10 days of repeated treatment, a significant increase in splenic NK activity. Consistent with previous results (23), there is a marked range of individual differences in noise-induced suppression of general activity and ingestive behavior. The findings demonstrate that behavioral responsiveness correlated significantly with elevated plasma levels of corticosterone but not with changes in NK activity. Thus, the behavioral response appears to reflect the perceived aversiveness of the stimulus, corresponds to the neuroendocrine changes with stress, and is likely to be of potential importance in elucidating *individual* processes such as neurochemical differences that underlie behavioral and neuroendocrine responses to acute and chronic stress.

Exposure to noise produces alterations in both adrenal steroid secretion and splenic NK activity. Responses of plasma corticosterone levels acutely increase, and then return to baseline levels by 10 days of treatment. In contrast, the temporal pattern of change in NK activity is characterized by a progressive enhancement, with the average lytic activity increased by about 2-fold after 10 days of noise exposure. These observations that corticosteroid responses habituate following repeated stress exposure, whereas NK cell activity shows a progressive increase, confirm and extend the work of Monjan and Collector (20) and are consistent with our findings using immobilization stress (14).

The temporal dissociation between adrenal activation and NK responses following audiogenic stress is consistent with both our findings in rats using immobilization stress (14) and our clinical work in bereaved women (11). Alterations in NK cytotoxicity do not appear to be solely mediated by adrenocortical activity, and future studies are needed to investigate whether changes in other neuromodulatory systems such as the sympathetic nervous system might mediate stress-induced reductions in NK activity (3, 4, 15, 17). For example, the administration of stressful stimuli is associated with an activation of the sympathetic nervous system (19) and the release of norepinephrine which can bind to receptors on lymphocytes (1) and inhibit NK cells (5). Furthermore, chronic doses of norepinephrine (0.5 to 5 μg/kg) administered subcutaneously over 7 days have been shown to enhance NK cell responses in mice (4).

In summary, audiogenic stress produces behavioral suppression, activation of adrenal steroid secretion, and with repeated treatment an enhancement of NK activity. Importantly, the results of this study demonstrate that individual differences in behavioral responsiveness were consistently correlated with elevations in plasma corticosterone. Characterization of the severity of psychological distress in animal models of depression is likely to be important in the study of mechanisms that underlie stress-induced changes in neuroendocrine responses.

ACKNOWLEDGEMENTS

This work was partly supported by grants from VA Merit Review (M.R.I., B.L.H.), NIMH #MH 442701 (M.R.I.) and #MH 30914 (R.L.H.), NIDA #DA-01568 and DA-01994 (D.S.S.), the Pfizer Scholars Program (R.L.H.), and NIMH Research Scientist #MH-70183 (D.S.S.).

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