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Impaired Neuroendocrine and Immune Response to Acute Stress in Medication-Naive Patients With a First Episode of Psychosis

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Little is known about how the biological stress response systems—the autonomic nervous system (ANS), the hypothalamic-pituitary-adrenal (HPA) axis, and the immune system—function during psychosis. Results of studies on the effect of stress on the immune and autonomic system in patients with schizophrenia are inconsistent. The present study investigates whether the stress response is impaired in medication-naive patients with a first episode of psychosis. Ten male patients with a first episode of psychosis and 15 controls were exposed to the stress of public speaking. Parameters of the ANS (heart rate and catecholamines), the HPA axis (plasma adrenocorticotropic hormone [ACTH] and cortisol), and the immune system (number and activity of natural killer [NK] cells) were measured. Peak responses were calculated to examine the relationship between stress-induced activation of the different systems. Subjective stress and anxiety before and during the task were assessed. Patients and controls displayed similar autonomic responses to acute stress. However, there was an impaired HPA axis response, slow onset and return of ACTH, and flattened cortisol response and a reduced increase in number NK cells and NK cell activity in patients with a first episode of psychosis. Furthermore, in patients, the relationship between the different stress response systems was weaker or absent compared with controls. These findings indicate that impairments in stress processing are associated with the endophenotype of psychosis and are not a result of illness progression or antipsychotic medication.

Key words: schizophrenia/stress/autonomic nervous system/neuroendocrine/immune/first-episode psychosis

Introduction

Stress plays an important role in the onset and course of psychosis.[1,2] Stress factors such as (cumulative) life events,[3,4] daily hassles,[5] exposure to urban life,[6] and high levels of expressed emotions[3,7] can trigger or worsen psychosis in vulnerable individuals.[8] Such findings imply an altered sensitivity to stress in patients with psychosis.[9] However, the exact biological underpinnings of this phenomenon remain unclear.

Important biological systems involved in stress processing are the autonomic nervous system (ANS) and the hypothalamic-pituitary-adrenal (HPA) axis. Both the ANS and the HPA axis modulate the reactivity of the immune system, thereby implicating a close relationship between the different stress response systems.[10–16]

Several studies have examined the basal level of functioning of stress response systems in patients with psychosis, mostly with schizophrenia. With regard to the ANS, increased basal heart rate as well as reduced heart rate variability have been consistently resported.[17–22] Basal HPA axis functioning is undisturbed according to most studies[18,23] although hypercortisolemia has been described in patients with schizophrenia as well.[24,25]

The most robust immunological alteration that has been reported in patients with schizophrenia is a shift in the T helper (Th) 1/Th2 cytokine balance toward Th2.[26,27] In addition, abnormal numbers and activity of natural killer (NK) cells have been reported.[28,29] Recently, circumstantial evidence of the involvement of autoimmunity in the pathophysiology of schizophrenia has been reported.[26,30–32] However, a consistent pattern of immunological abnormalities has not been demonstrated so far.[31–33]

Studies in which patients and controls are challenged by a stressor have been performed to examine the reactivity of the stress response systems in patients with psychosis more directly. Reduced or normal autonomic responses (electrodermal and heart rate reactivity) to a laboratory stress challenge have been reported,[18,21,34–36] as well as diminished adrenaline and noradrenaline responses to surgical or psychological stress.[37–39]

Challenge studies also reported a blunted cortisol and/or adrenocorticotropic hormone (ACTH) response in anticipation of different stressful events, such as surgery,[38–40] lumbar puncture,[41] metabolic stress,[42] psychological tasks (mental arithmetics, cold pressor, and noise),[37] or public speaking.[18,35] In contrast, Brenner et al.[36] described only a trend toward a blunted cortisol response, whereas normal cortisol and normal or even elevated ACTH responses to metabolic stress have also been reported.[43–45]

Only one study examining the response of the immune system to a laboratory stress challenge has been published so far. This study showed that the proinflammatory cytokine response to abdominal surgery is inhibited in patients with schizophrenia.[40]

Despite reported evidence, the notion that the stress response in psychotic patients is disturbed is still controversial because the results in the literature are inconsistent and most studies have several limitations. First of all, most studies examined medicated patients, while several other studies reported effects of antipsychotic medication on the stress response systems.[46–50] Only three studies included medication-free patients, but the length of the medication-free period was limited to 3 weeks,[41] 4 weeks,[37] or 8–77 days.[24] Second, most studies focused on patients with a chronic course of schizophrenia (mean duration of illness between 7 and 16 years), the study by Albus et al.[37] being the only exception. The results of these studies do not allow to draw conclusions on the question whether a disturbed stress response is related to the progression of disease or already present at first presentation of symptoms. With regard to the type of stressor, different stress tests have been used. Because specific stressors can have different effects on the stress response systems,[18] differences in the type of stressor used can contribute to variation in the results. Finally, previous studies examined the stress response systems independently of each other, while interaction between the stress response systems could provide more useful information about stress processing in psychosis.

In summary, there is some evidence that the stress response in psychotic patients is disturbed, but the results of studies published so far are inconsistent and difficult to interpret because they differ with regard to medication status, duration of illness, and type of stressor. In the present study, we investigated whether altered stress responsiveness is already present during the first episode of psychosis in medication-naive patients using public speaking as a stressor. Specific endocrine and immunological parameters, as well as heart rate were measured before, during, and after stress exposure in patients and healthy control subjects.

Methods

Subjects

This study was performed at the Department of Psychiatry of the University Medical Center Utrecht (UMCU). Male patients with symptoms of a psychosis were eligible for this study. Patients with clear features of a manic or depressive episode and patients who had taken any antipsychotic medication in the past or any antidepressive or mood stabilizing medication in the previous 2 weeks were excluded.

Eleven patients (median age 23 y; interquartile range 19–29) were included after the diagnosis of first psychosis was made by the attending psychiatrist, and their diagnosis based on the Comprehensive Assessment of Symptoms and History (CASH) interview[51] was a psychotic disorder in the schizophrenic spectrum (eight patients with schizophrenia and three patients with a schizophreniform psychosis). The CASH interview was performed by an independent psychiatrist during follow-up. One of the eleven patients (diagnosis: schizophrenia) was excluded because of a positive urine screen for cocaine use. The median interval between first appearance of psychotic symptoms and the day of the test was 3 months (interquartile range 1–5 months). Severity of psychosis was assessed by the Positive and Negative Syndrome Scale (PANSS).[52] The median total PANSS score (scale range 30–210) at entry of the study was 77.5 (interquartile range 63–82) (see also table 1). Five patients were nonsmoking, one smoked less than 10 cigarettes a day, and the remaining four smoked between 10 and 25 cigarettes a day.

Healthy nonsmoking control males (median age 22 y; interquartile range 20–25) between 18 and 40 years were recruited by advertisements posted at the university and in regional newspapers. Subjects who responded were screened for psychiatric or somatic morbidity using the CASH interview, the Schedule for Affective Disorders and Schizophrenia—Lifetime version interview,[53] and a somatic screening list. Healthy subjects were excluded in case of a psychiatric disorder or when any of their first-degree relatives had a psychiatric diagnosis.

Both patients and healthy subjects were excluded in case of medical or neurological illness, use of immunosuppressive medication, use of anti-inflammatory agents (like

Table 1. Baseline Characteristics of Patients and Controls, Median (Quartiles)

Variable	Patients (n = 11)	Controls (n = 15)
Age (y)	23 (19–29)	22 (20–25)
Smoking (cigarettes/d)	2.5 (0–20)	0 (0–0)
0	5	15
1–10	1	0
11–20	4	0
Duration of psychotic symptoms (mo)	3 (1–5)	
PANSS score total	78 (63–82)	
Positive scale	23 (16–28)	
Negative scale	16 (11–19)	
General scale	38 (31–41)	

Note: PANSS, Positive and Negative Syndrome Scale.

nonsteroidal anti-inflammatory drugs) in the previous 2 weeks, and use of drugs or abuse of alcohol in the 3 months prior to entry. Individuals suffering from an infectious illness within 2 weeks before the experiment were rescheduled. All patients and controls gave written informed consent. The protocol was approved by the Medical Ethics committee of the UMCU.

Psychosocial Stress Test

The psychosocial stress test consisted of speaking in public while being recorded on video. This test has been shown to be effective in inducing a cortisol stress response in healthy subjects[54] and patients with schizophrenia.[18,35] Subjects were asked to take part in a study on psychological stress, without explicit information about the forthcoming challenge test. Subjects were requested to avoid tyramine-containing food during 24 h before the test session and to refrain from severe mental or physical exertion, eating, drinking, and smoking for at least 1 h before the experiment. The experimental session was conducted from 10 AM until 1 PM to minimize circadian variation. For details of the procedure, refer to Jansen et al.[55]

Data Collection

All subjects filled out the Perceived Stress Scale (PSS) to measure the degree to which situations in one's life are appraised as stressful during the last month.[56] At the beginning of the test session, the Spielberger state trait anxiety inventory (STAI)[57] was filled out to determine anticipation fear for the test session. Ten-point visual analog scales (VASs) for the assessment of subjective stress (nervousness and experiencing control) were filled out during the preparation period, the actual public speaking period, and the overall subjective stress during the total stress test.

Heart rate was recorded via three electrodes on the chest (Psylab data acquisition, Contact precision instruments). Eight blood samples were collected at regular intervals (refer to Jansen et al.[55]) for the measurement of cortisol, ACTH, adrenaline, noradrenaline, and NK cell number and activity.

Biochemical Analysis

Plasma samples were obtained within 30 min after collection of blood in plastic tubes containing EDTA as an anticoagulant and stored until analysis at −30°C for ACTH and cortisol and at −80°C for adrenaline and noradrenaline determination. ACTH and cortisol were measured using a standard immunometric technique on an Advantage Chemiluminescense System (Nichols Institute Diagnostics, San Juan Capistrano, California). Adrenaline and noradrenaline were measured using high-performance liquid chromatography with electrochemical detection after extraction over aluminium oxide (ClinRep).

NK cell activity was determined in heparinized blood samples using a standard ^{51}Cr release assay using K562 target cells as described.[58] Whole blood diluted 1:2 in RPMI-1640 was incubated for 4 h in 96-well round-bottom microtiter plates with 10^4 K562 cells labeled with ^{51}Cr. Maximal (MR) and spontaneous (SR) ^{51}Cr release were determined by incubating with 1% Triton X-100 and medium with 5% fetal calf serum, respectively. Specific killing was calculated using the formula [(cpm experimental sample − cpm SR)/(cpm MR − cpm SR)] × 100% (in which cpm stands for counts per minute). All measurements were performed in triplicate. The number of CD16/CD56+CD3− NK cells was determined in whole blood using dual-color fluorescence analysis using a FACS Calibur (Becton and Dickinson, Mountain View, California) using simultest antibodies against CD16/CD56 and CD3.

Statistical Analyses

Differences between the groups with regard to demographic variables and the results of the psychological questionnaires were examined with nonparametric tests (Mann-Whitney *U* test). Stress-induced changes on the biological response systems were analyzed using repeated measures MANOVA with time as within factor and group (patient or control) as the between factor. To examine the relationship between the stress-induced activation of the different systems, the peak response of each variable was calculated as the difference between the maximum and minimum of each time series. Subsequently, Spearman rank correlations were calculated between the different peak responses of each variable.

Results

Characteristics of the Study Population

Characteristics of patients and controls are shown in table 1. Patients and controls did not differ significantly on age.

Psychological Measures Before and During Public Speaking

Table 2 summarizes the results for the stress-related psychological questionnaires. PSS scores did not differ between patients and controls indicating that patients and controls experienced the same level of stress during the previous month. During the public speaking task, Spielberger anxiety scores were significantly higher in patients than in controls ($P = .002$). With regard to the VASs, no differences between patients and controls were observed in nervousness before and during the public speaking task or in the extent of having control during the preparation phase. However, patients were significantly more nervous after the public speaking task ($t = 70$ min; $P = .001$) and experienced significantly less control during speaking in public ($P = .035$).

Table 2. Results of Stress Questionnaires, Median (Quartiles)

Questionnaire	Variable	Patients	Controls
PSS	Total score	23 (14–34)	15 (11–20)
STAI	Total score	45 (37–55)	29 (24–34)[a]
Subjective well-being	Nervousness before test	5 (3–6)	7 (3–8)
	Nervousness during test	5 (4–7)	5 (3–7)
	Nervousness after test	4 (3–5)	2 (2–3)[a]
	Nervousness total	4 (3–5)	6 (2–7)
	Extent of control before test	6 (3–7)	4 (2–4)
	Extent of control during test	5 (4–7)	4 (2–4)[a]

Note: PSS, Perceived Stress Scale; STAI, state trait anxiety inventory.
[a]Significant group differences.

Biological Response Systems

At baseline, patients showed a significantly higher heart rate than controls ($P = .002$). There were no statistically significant differences at baseline in any of the other parameters tested.

The results of statistical analysis of the changes in biological parameters during the stress test are listed in table 3. Six of the seven measurements of the ANS, the HPA axis, and the immune system showed a significant increase during the psychosocial stress test (time effect: $P < .001$). These findings indicate that the laboratory stressor was sufficiently robust to induce discernible neurobiological responses. Adrenaline did not alter in response to the stressor (time effect not significant).

Heart rate was significantly higher in first-episode patients compared with controls irrespective of time (group effect $P = .004$; time × group interaction: not significant [ns]). Baseline plasma noradrenaline and the increase in noradrenaline in response to the psychological stressor did not differ between patients and controls (group: ns; time: $P < .001$; time × group interaction: ns).

With regard to the HPA axis measures, patients showed significantly lower cortisol and ACTH responses to the psychological stressor than controls (time × group effect $P = .042$ for cortisol, $P = .047$ for ACTH). Both the onset and recovery of the ACTH response were slower in patients than in controls, whereas the cortisol response to the stressor was flattened in patients (figure 1).

The response of the immune system to the stressor differed significantly between patients and controls. In controls, the stressor induced an immediate increase in NK cell activity and NK cell number. In contrast, there was no significant change in NK cell activity and NK cell numbers in patients (figure 1) (time × group effect $P = .035$ and $P = .020$, respectively). The difference in response resulted on average in a lower NK cell activity in patients compared with controls (group effect $P = .018$).

Correlations Between the Peak Responses of the Biological Stress Parameters

The peak response in patients and controls was reached at $t = 15$ min for NK cells, $t = 20$ for heart rate, $t = 25$ for ACTH and noradrenaline, and $t = 35$ for cortisol. Using a Spearman rank correlation analyses of values obtained at these time points, significant correlations were observed between ACTH and cortisol in both patients and controls (0.64 and 0.79, respectively). In the control group, we also observed significant positive associations between heart rate and cortisol (0.69), heart rate and ACTH (0.62), heart rate and NK cell activity (0.60), NK cell activity and cortisol (0.60) as well as NK cell activity and ACTH (0.68) (no corrections for multiplicity were applied). In contrast, in patients, the associations between these parameters did not significantly differ from 0, indicating an impaired interaction between the systems in the patient group.

Discussion

Our results showed a diminished response of specific neuroendocrine and immune factors to a psychosocial stressor in male medication-naive patients with a first episode of psychosis as compared with matched controls, while the autonomic response was intact. Furthermore, we observed no association between the different parameters of the stress response system in patients, while these were clearly present in controls. These findings indicate that a different biological response to stress is already present in medication-naive patients with a first episode of psychosis.

Our finding of an altered neuroendocrine response to psychological stress in medication-naive patients with first-episode psychosis is consistent with previous findings in—mainly chronic and medicated—patients with schizophrenia.[18,35–37,41] It therefore supports the notion that the alteration in the neuroendocrine response observed in patients with schizophrenia does not represent a medication effect. In addition, these findings suggest that altered responsiveness of the stress system is not the result of the disease progression.

In our study, no differences between NK cell activity at baseline in patients and controls were observed. These data are in line with some earlier studies,[59–61] although others reported increased[29] or decreased NK cell activity[62,63] in patients with schizophrenia. NK cell activity is considered to be an important parameter of innate cellular immune function. After acute psychological stress, sympathetic arousal and peripheral catecholamine increases are mainly responsible for alterations in NK cell function. β2-Adrenergic receptors on NK cells, in particular, are involved.[64,65] We showed here that the response of NK cells to stress was decreased in patients with a first episode of psychosis although the noradrenaline response to the stressor was similar in both groups. These

Table 3. Autonomic, Neuroendocrine, and Immune Responses to the Public Speaking Task in First-Episode Patients and Healthy Controls

Variable	Time (min)	First episodes, n = 11, mean ± SEM	Healthy controls, n = 15, mean ± SEM	MANOVA (P-value)		
				Within-groups		Between-groups
				Time	Time × group	
Heart rate (bpm)	-10	79.73 ± 2.99	66.94 ± 2.25	.000[a]	.245	.004[a]
	7.5	82.44 ± 2.99	74.62 ± 2.43			
	20	87.79 ± 3.73	77.32 ± 2.69			
	27.5	82.48 ± 2.92	69.84 ± 2.27			
	32.5	80.83 ± 3.07	68.41 ± 1.91			
	42.5	81.64 ± 3.44	68.39 ± 2.30			
	60	79.36 ± 3.19	67.11 ± 2.17			
	95	76.91 ± 3.32	64.78 ± 2.19			
Adrenalin (pmol/ml)	0	0.32 ± 0.09	0.49 ± 0.19	.544	.290	.655
	15	0.33 ± 0.05	0.49 ± 0.10			
	25	0.36 ± 0.07	0.3 ± 0.08			
	35	0.38 ± 0.09	0.29 ± 0.07			
Noradrenalin (pmol/ml)	0	1.98 ± 0.29	1.85 ± 0.26	.000[a]	.336	.580
	15	2.15 ± 0.38	2.52 ± 0.32			
	25	2.57 ± 0.61	2.9 ± 0.37			
	35	2.25 ± 0.38	2.61 ± 0.30			
Cortisol blood (nmol/ml)	0	0.30 ± 0.02	0.32 ± 0.01	.000[a]	.042[a]	.402
	15	0.34 ± 0.03	0.38 ± 0.02			
	25	0.37 ± 0.03	0.44 ± 0.03			
	35	0.35 ± 0.04	0.44 ± 0.03			
	50	0.36 ± 0.05	0.4 ± 0.03			
	70	0.34 ± 0.04	0.32 ± 0.02			
	120	0.28 ± 0.03	0.25 ± 0.01			
ACTH (ng/l)	0	23.4 ± 2.97	21.53 ± 2.47	.001[a]	.047[a]	.899
	25	27.5 ± 2.63	37.77 ± 5.93			
	35	33.5 ± 6.05	33.15 ± 5.03			
	50	32.6 ± 4.94	27.47 ± 3.48			
NKCA (%)	0	24.99 ± 7.72	27.43 ± 2.67	.007[a]	.035[a]	.018[a]
	15	24.20 ± 4.88	48.36 ± 4.45			
	25	21.93 ± 5.87	39.21 ± 5.58			
	35	23.30 ± 5.37	37.85 ± 3.55			
	120	18.02 ± 3.70	28.57 ± 2.33			
NK cells, n (×1000)	0	226.82 ± 72.2	156.48 ± 22.72	.000[a]	.020[a]	.294
	15	277.60 ± 84.76	465.23 ± 72.34			
	25	236.47 ± 54.69	390.83 ± 66.59			
	35	196.05 ± 53.04	300.17 ± 46.48			
	120	157.88 ± 36.82	169.67 ± 22.14			

Note: ACTH, Adrenocorticotropic hormone; bpm, beats per minute; NK, natural killer; NKCA, Natural Killer Cell Activity; SEM, standard error of the mean.
[a]Significant results.

results could imply that there is a dysregulation of the interaction between the sympathetic nervous system and the immune system in the stress regulation of first-episode psychotic patients.

The finding that a different biological stress response is already present at first appearance of psychotic symptoms could indicate that this phenomenon belongs to the endophenotype of the schizophrenic disorder. However, because we have included only patients with florid psychotic symptoms, we cannot conclude from our results whether the impaired stress response is a state or trait characteristic. To address this issue, one should investigate stress sensitivity when the psychosis is fully in remission. Furthermore, because several earlier studies[66–68] reported similar (although less pronounced) alterations in various stress responses in patients with psychosis or schizophrenia and first-degree relatives, one should examine the biological stress response in first-degree relatives of patients with first-episode psychosis in order to study a possible genetic origin of the inadequate stress processing.

With regard to the regulation of the stress response, this study is the first to indicate a disturbed interaction between the different stress response systems in patients

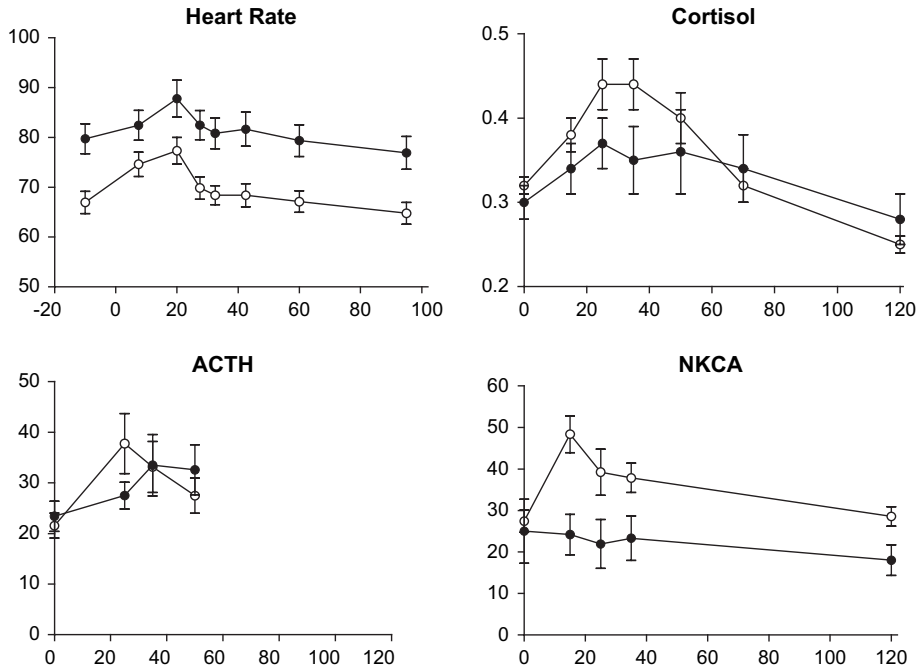

Fig. 1. Mean and Standard Error of the Mean of Different Biological Stress Parameters. Closed dots show the results of the patients. Open dots are the results of the control group.

experiencing a first episode of psychosis, although the data are predominantly descriptive. To further unravel the underlying neurobiological mechanisms, a possible approach could be to assess the sensitivity of the immune system to agents associated with the HPA axis (eg, glucocorticoid agonist dexamethasone) or the ANS (eg, β2-adrenergic agonist terbutaline).[69]

Theoretically, it may be possible that the blunted response to a psychosocial stressor reflects misinterpretation in patients of the situation or the stress stimulus itself. In that case, diminished stress perception may contribute to the impaired stress response. However, the stress questionnaires clearly showed that patients experienced stress. In addition, the heart rate response and the noradrenaline response to the stressor were similar in patients and controls. Thus, it is unlikely that the observed blunted response of the HPA axis and of NK cells in patients is the result of inappropriate stress perception. Therefore, we propose that the neurobiological regulation of the stress response is impaired in patients with a first episode of psychosis.

Our finding of normal baseline cortisol levels in patients with schizophrenia is in line with our previous work[18,35] but in contrast with other studies.[49] The reason may be that in our studies, patients are always tested when they are accommodated to their hospital admission, which is not usually the case in other studies. In addition, our repeated measurement design may add to the validity of this finding. It is therefore considered that methodological differences between studies account for the differences found in literature.

The results of our study should be interpreted with caution because of its limitations such as small group size and the inclusion of smokers in the patient group. Smoking has been reported to negatively influence plasma cortisol levels and could influence NK cell function.[70–72] To limit the effect of smoking, subjects refrained from smoking 1 h before the test session, as has been done in other challenge studies.[18,35,36,42] To examine the possible confounding effect of smoking in our data, we compared the peak response of smokers and nonsmokers of the different biological parameters with a *t* test. We observed no effect of smoking on cortisol or ACTH, but there was a significant effect of smoking on NK cell number and activity ($P = .05$). Restricting the repeated measurement analysis to nonsmokers was not possible due to the limited number of nonsmoking patients.

In conclusion, this study showed that a blunted biological stress response was already present in medication-naive patients with a first episode of psychosis, indicating that impairments in stress processing are either related to the psychosis or already present before the onset of the first psychotic episode and maybe considered as an endophenotype or vulnerability factor. Future studies are warranted to examine the possible genetic origin of the impaired stress response as well as the mechanisms underlying the disturbed interactions between the stress response systems.

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