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Effect of Ethanol on Hypothalamic–Pituitary–Adrenal System Response to Psychosocial Stress in Sons of Alcohol-Dependent Fathers

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Familial risk and environmental stress promote the development of alcohol dependence. This study tested two hypotheses: that a family history for alcoholism is associated with (i) a greater stress response and (ii) more effective stress response dampening by alcohol. We studied 29 high-risk subjects with a paternal history of alcoholism (PHA) and 23 family history negative (FHN) controls all aged 18–26 years, who were recruited using a representative sample of the local area population. Psychosocial stress was induced by having subjects deliver a speech and perform mental arithmetics in front of an audience on two separate days, after drinking either placebo or alcohol (0.6 g/kg) in a randomized double-blind crossover design. Plasma cortisol and adrenocorticotropin (ACTH) were measured up to 90 min after the test. The stress task induced a phasic increase of both hormones in PHA and FHN subjects during all experimental conditions except in tests where FHN subjects received alcohol during the second day. ACTH secretion was higher in PHA subjects during placebo experiments, but equal to controls after alcohol administration. The alcohol-induced attenuation of ACTH response was statistically significant in PHA, but not FHN, subjects. Cortisol response was higher in PHA than FHN probands if placebo was administered during the first test, but equal if subjects received alcohol first. The increased stress response and its stronger dampening by alcohol in sons of alcoholic fathers suggest a mechanism by which predisposition to develop alcohol use disorders might be expressed, implying that a transient favorable alcohol effect might occur in PHA, but not FHN, subjects.
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INTRODUCTION

Alcohol dependence has long been recognized as a familial disorder (Cotton, 1979), and genetic factors account for most of the increased risk in male (Goodwin *et al*, 1974; Prescott and Kendler, 1999) and female (Heath *et al*, 1997) children of alcohol-dependent parents. In search for underlying mechanisms, a number of studies investigated neurobiological alterations, most often in response to alcohol challenges, in offspring of alcoholics who have not yet developed alcohol-related disorders themselves (see for review Newlin and Thomson, 1990). Concerning the activity of the hypothalamic–pituitary–adrenal (HPA) system, lower levels of adrenocorticotropin (ACTH; Schuckit *et al*, 1988) and cortisol (Schuckit *et al*, 1987) were found after ingestion of the rather high dosage of 0.88 g/kg ethanol in sons of alcoholics compared to control males, while this difference was not apparent after 0.6 g/kg in the same studies. Another study found no cortisol changes after ingestion of 0.25, 0.5, and 0.75 g/kg ethanol, while a rise in β-endorphin after the medium and high dose was observed in offspring of alcoholics but not in control subjects (Gianoulakis *et al*, 1996). Stimulation of the HPA system by i.v. administration of the opiate antagonist naloxone resulted in an increased cortisol response in high-risk subjects, while response to adrenal stimulation with ACTH (1–24) was similar to low-risk controls (Wand *et al*, 1999a, b).

Another factor long known to increase the risk for development and maintenance of alcohol use disorders is environmental stress. According to the tension-reduction

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hypothesis proposed by Conger (1956), the reinforcement by reduction of negative feelings due to alcohol's anxiolytic effect is an important underlying mechanism (see for review Sinha, 2001). One possible mechanism by which a positive family history increases the risk for alcoholism might consist in an interaction between genetic factors and the effect of stress. Preliminary evidence for this notion comes from an epidemiological study conducted in a Honduran community, which found a modulating effect of occupational/economic stress on the association between the dopamine receptor D2 *Taq*I polymorphism and alcoholism (Madrid *et al*, 2001). Interactions between genetic factors and stress were also tested experimentally by subjecting individuals with and without a family history of alcoholism to aversive electric shocks or public speaking tasks while measuring heart rate response, skin conductance level, or pulse transit time as indicators of the stress response. Several authors found a more pronounced stress response (Finn *et al*, 1990) and a stronger stress-dampening effect of alcohol (Levenson *et al*, 1987; Finn *et al*, 1990; Conrod *et al*, 1998) in high-risk subjects, while Sinha *et al* (1998) found this difference only in daughters of alcoholics.

These studies, however, did not appropriately consider the core parameter that defines a biological stress response, that is, activation of the HPA system. Recently, a standardized laboratory procedure of inducing psychosocial stress that reliably produces autonomic activation and HPA system stimulation was described (Trier Social Stress Test, TSST; Kirschbaum *et al*, 1993). We therefore used the TSST to investigate the HPA system reactivity to psychosocial stress, and its alteration by moderate alcohol intoxication, in healthy young subjects with a paternal history of alcohol dependence (PHA) compared to male control subjects with a negative family history of alcohol use disorders (FHN).

Our two main hypotheses were that (i) the stress response would be more pronounced in PHA than FHN subjects being sober and (ii) alcohol would attenuate the stress response in both groups, albeit with a stronger effect in PHA subjects. To test these hypotheses, we investigated all subjects twice, with alcohol and placebo administration on alternate days. Doing so, we had to respect that repetitive exposure to the TSST in the same subjects can result in lower stress response during the second test (Gerra *et al*, 2001; Kirschbaum *et al*, 1995). This issue was accounted for in two ways: as a first step, we analyzed only data from the respective first test days, that is, when subjects were test-naive, thereby excluding a test repetition effect. Second, when performing the repeated-measures analysis, which compared data of both experimental days, the administration sequence (subjects receiving either alcohol first and placebo on the second day or vice versa) was considered as an additional factor. Therefore, risk group, treatment, and administration sequence defined a $2 \times 2 \times 2$ design.

In an exploratory approach, we also investigated whether stress response and the alcohol effect were influenced by the amount of prior or current alcohol use, use of substances other than alcohol, individual or parental psychiatric comorbidity, or the personality trait variable of sensation seeking. The latter was considered meaningful since high scores of the closely related trait of novelty seeking are associated with early manifestation of alcohol use disorders in sons of alcoholics (Cloninger, 1987; Mc Court *et al*, 1993), raising the possibility of an interaction with stress response to modulate risk of alcoholism.

MATERIALS AND METHODS

Subjects

Recruitment of subjects was based on a prospective longitudinal epidemiologic and family genetic study of a representative general population sample of a total of 3021 adolescents of the Munich area (Lieb *et al*, 2000). Twice before entering the present study, participants of this survey were assessed for early developmental stages of psychopathology by means of the Munich Composite International Diagnostic Interview (M-CIDI; Lachner *et al*, 1998). In the family history section of the baseline investigation, 115 of 1533 male adolescents reported that their father, but not their mother, had an alcohol problem. These subjects were provisionally supposed to have a positive paternal history of alcoholism (PHA). For the control group, 62 male study participants who did not report parental alcohol problems were matched for age, history of depressive or anxiety disorders, personal alcohol and substance use disorders, and subjects' reports on parental depressive and anxiety disorders. They were provisionally supposed to be FHN for alcoholism. Subjects were recruited as part of the final (third wave) follow-up interview. Subjects agreeing to participate gave written consent to contact their biological fathers and mothers for direct or telephone diagnostic interviews. The parental interviews consisted of the alcohol use disorders section of the M-CIDI. Additional questions were asked concerning whether they had ever suffered from symptoms of psychiatric disorders, namely generalized anxiety disorder, panic attacks, phobias, depression, mania, psychotic disorders, or eating disorders, and whether alcohol had ever caused health or social problems in their parents, brothers, or sisters (ie the four grandparents and blood-related uncles and aunts of the study participants).

Based on these parental interviews, the inclusion criteria for PHA subjects were (i) confirmation of a DSM-IV diagnosis of alcohol dependence in the father and (ii) the absence of an alcohol-related disorder in the mother to exclude possible effects of fetal alcohol syndrome. FHN subjects were included if M-CIDI interviews ascertained the absence of alcohol dependence or abuse in both biological parents, and both parents denied alcohol-related problems in their first-grade relatives. General inclusion criteria applying for both groups were social alcohol drinking at least once monthly and consenting to abstain from any illegal drug use during the experimental period.

In a high percentage of subjects selected from the epidemiological survey, the inclusion criteria were not fulfilled (see Table 1). Therefore, to increase the sample size available for experimental investigation, subjects were encouraged to tell their friends about the possibility of participation in the study. Each study participant could suggest only one supplementary person. Thereby, 12 additional FHN subjects were recruited. These individuals and their parents underwent the same interviewing procedure as described above to scrutinize inclusion, exclusion, and matching criteria. All subjects were of Caucasian ethnicity.

Table 1 Recruitment of Subjects and Reasons for Dropout

	Paternal history of alcoholism	Family history negative
Assessed for eligibility	115	84
Excluded for parental reasons		
Parents refused being interviewed	7	3
Paternal alcohol-related disorder not matching inclusion criteria	34	9
Maternal alcohol dependence	4	1
Alcohol-related disorders in second-degree relatives not matching inclusion criteria	Not applying	13
Subjects declined to participate	9	5
Subjects meeting exclusion criteria (see text)	10	7
Randomized to receive alcohol/placebo during first test	51 (26/25)	46 (23/23)
Withdrawal of consent before first experiment (no more time $n=10$, refusal of blood drawing $n=2$, relocated $n=3$, no reasons given $n=18$)	15 (9/6)	18 (8/10)
Withdrawal of consent during experiments (phobic reaction during stress test $n=1$, no more time $n=1$, no reasons given $n=5$)	5 (3/2)	2 (1/1)
Excluded from analysis (tested positive for cannabinoids $n=2$, outlier of basal hormone values $n=3$)	2 (0/2)	3 (1/2)
Analyzed	29 (14/15)	23 (13/10)

Eligible subjects were invited for a screening visit performed by a psychiatrist. A medical history was taken, and a physical examination, routine laboratory tests, and a psychiatric interview were performed including questions for adverse life events and the Beck depression inventory (BDI). Current active alcohol or substance abuse and dependence were excluded by asking the respective CIDI questions and by confirming that liver enzymes and mean erythrocyte corpuscular volume were within the normal range. Participants also completed the sensation seeking scale according to Zuckerman *et al* (1980). Exclusion criteria and numbers of excluded subjects were as follows: subjects not wanting to drink alcohol (one PHA, four FHN), not wanting to abstain from cannabinoid use (three PHA, one FHN), active alcohol abuse together with increased liver enzymes (two PHA), current depressive disorder (two PHA, two FHN), a history of pancreatitis (one PHA), and a parental history of a psychotic disorder (one PHA). The study procedures were explained including announcement of an unspecified 'stress test', and subjects were informed that they would receive alcohol on both test days with 'differing dosages'. They were instructed to abstain from any illegal substance use throughout the study period and from alcohol use for 3 days before each experimental day, and to have lunch at least 1 h before arrival to the laboratory. Written informed consent was obtained from all subjects. The study protocol was approved by the local ethical committee and performed in accordance with the Declaration of Helsinki. Subjects were paid for participation.

Experimental Procedures

Subjects were tested twice using the TSST (Kirschbaum *et al*, 1993), which required them to deliver a self-disclosing 5-min free speech and 5 min of mental arithmetics in front of three observers unknown to them while being video- and audiotaped. Before the stress test, alcohol or placebo was administered orally in a double-blind, placebo-controlled crossover design with a minimum of 1 week between study days. The experimenter was blind against the risk status of the subjects.

Subjects reported at the laboratory at 1300 h, were asked for recent alcohol use, life events, and number of cigarettes smoked today, and they completed the State Anxiety Inventory (Laux *et al*, 1981). Recent illegal substance use was excluded by urine screening for amphetamines, cannabinoids, benzodiazepines, opiates, and cocaine (Roche Diagnostics, Indianapolis, IN). Cigarette smoking and food intake were not allowed during the experiment. At 1315 h, an i.v. line was established and 40 ml/h of 0.9% saline was infused to keep the line open for blood drawings. At 1350 h, a basal blood sample was drawn. At 1400 and 1420 h, laboratory-grade ethanol was administered orally, diluted in ice-cold grapefruit juice to give a concentration of 15% (v/v). The volume was divided into two equal portions each consumed during 5 min. The same volume of plain grapefruit juice was given on placebo days. During drinking, subjects wore a nose-peg to disguise the smell of alcohol. When asked for the alcohol content of their beverage, 86% of the subjects correctly stated the placebo beverage as 'low' in alcohol content. The alcohol beverage was judged 'high' in alcohol content by 54% of PHA and 46% of FHN subjects, 'low' by 23% of PHA and 37% of FHN subjects, while 23% of PHA and 17% of FHN subjects answered they could not guess the alcohol content.

At 1420 and 1430 h, basal blood samples were drawn. At 1435 h, the subjects were instructed about the procedures of the test and were given 10 min to prepare for their speech. At 1450 h, three health-care professionals walked into the room to act as an audience, and the stress test was performed as described by Kirschbaum *et al* (1993). Blood samples were taken at the beginning of the preparation time, immediately before, and 10, 20, 30, 45, 60, and 75 min after beginning of the speech. Immediately after the stress test, subjects completed four 100 mm visual analogue scales

(VAS) to rate their agreement with the statements 'I could cope with the situation', 'I felt the stress situation was very demanding', 'I felt the situation was stressful', and 'I felt the situation was unpleasant'. Seven breath alcohol concentration (BrAC) measurements were taken throughout the test session using an Alcotest 7410 breathanalyzer (Draeger Sicherheitstechnik, Lübeck, Germany).

Sample Treatment and Hormone Measurements

Blood samples were drawn into devices pretreated with EDTA and aprotinin (2000 kallikrein-inhibiting units per 7 ml of blood, Bayer Leverkusen, Germany). The blood was chilled on ice immediately, spun at 1500*g* within 60 min, and the plasma was frozen at −80°C. ACTH was measured without extraction by an ^{125}I-immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA) with a detection limit of 4 pg/ml. The monoclonal antibody did not crossreact with α-MSH, β-MSH, β-LPH, or β-endorphin. Inter- and intra-assay coefficients of variation at 20 pg/ml were below 8%. Cortisol measurement was by radioimmunoassay with ^{125}I (ICN Biochemicals, Costa Mesa, CA) at a detection limit of 1.6 ng/ml. Crossreactivities were 37% with prednisolone, 33% with 11-deoxycortisol, and 3% with 11-hydroxyprogesterone. Inter- and intra-assay coefficients of variation at 20 and 40 ng/ml were below 7%.

Statistical Methods

The main outcome variables to test the hypotheses concerning stress response were ACTH and cortisol levels after onset of the stress (ie instruction of the subjects about the test procedure). In order to reduce the number of tests, we calculated the gross area under the time curve (AUC) defined by the measurements 3–11 of ACTH and cortisol according to the trapezoid rule, instead of evaluating the hormone level at single time points. Secondary variables were baseline hormone secretion, the subjectively perceived stress level (expressed as the mean ratings of the four VAS scales), and the individuals' characteristics given in Table 2. To test the effects of treatment and risk group on hormone secretion in subjects who were naive to the test situation, a two-factorial multivariate analysis of covariance (MANCOVA) was performed with ACTH and cortisol AUC during the respective first stress test as dependent variables. Thereby, 'treatment' (alcohol *vs* placebo) and 'group' (PHA *vs* FHN) were between-subjects factors with two levels, respectively. The basal ACTH and cortisol levels immediately preceding stimulus onset (ie third measurement) were included as covariates.

The intraindividual comparison between first and second tests was performed by a three-factorial repeated-measures MANCOVA with ACTH and cortisol AUC as dependent variables. In this analysis, 'treatment' was a within-subjects factor, and 'group' and 'administration sequence' (placebo first *vs* alcohol first) were between-subjects factors. The baseline ACTH and cortisol levels were included as covariates.

The secondary variables were examined for factor effects partly exploratively and partly inferentially. All subjects' characteristics with a continuous data structure given in Table 2 were tested for effects of risk group and administration sequence in two-factorial univariate analyses of variance. Group differences in the nominal variables were examined by Fisher's exact test. Further analysis of simple effects in cases of significant interactions in the MANCOVA

Table 2 Subject's Characteristics (mean and SD) by Experimental Group and Sequence of Alcohol Administration

Alcohol administration sequence	**First placebo/second alcohol**		**First alcohol/second placebo**	
Family history for alcohol dependence	**Negative ($n=10$)**	**Paternal alcoholism ($n=15$)**	**Negative ($n=13$)**	**Paternal alcoholism ($n=14$)**
Age (years)	20.3±2.75	20.3±3.0	20.3±2.0	20.0±2.9
Drinks per week[a]	3.3±3.2	10.7±8.7	6.1±4.3	7.0±6.0
Beck depression inventory	3.1±6.6	4.2±5.6	4.0±6.1	3.7±2.4
Cigarettes smoked on the placebo day	1.1±2.6	2.3±3.1	2.0±4.3	2.2±2.7
Cigarettes smoked on the alcohol day	0.8±1.9	1.8±2.7	1.6±3.2	2.6±2.8
STAI score before placebo test[b]	31.3±7.8	35.3±6.1	37.9±7.0	36.1±4.7
STAI score before alcohol test[b]	31.3±9.6	36.7±6.0	38.7±5.5	36.9±5.6
Sensation seeking scale[a]	19.2±6.7	24.1±5.7	21.8±6.8	24.1±5.2
Regular smokers (%)[c]	2 (20)	10 (66)	4 (31)	9 (64)
Illegal drug use during past 6 months (%)	3 (30)	7 (47)	3 (23)	7 (50)
Prior history of alcohol use disorder (%)	2 (20)	6 (40)	7 (54)	6 (43)
Individual history of affective, anxiety, or somatoform disorder (%)	1 (10)	3 (20)	1 (10)	0 (0)
Parental history of psychiatric symptoms (%)[c]	3 (30)	13 (87)	3 (23)	6 (43)
Alcohol-related problems in grandparents (%)	0	5 (33)	0	7 (50)

STAI, state anxiety inventory (Laux *et al*, 1981).
[a]Significant effect for group (ANOVA).
[b]Significant effect for administration sequence (ANOVA).
[c]Significant difference between experimental groups (Fisher's exact test).

was performed by tests with contrasts. As a nominal level of significance, $\alpha = 0.05$ was accepted. For all *post hoc* tests (univariate F-tests and tests with contrasts), the significance level was corrected according to the Bonferroni procedure in order to keep the type I error less than or equal to 0.05.

RESULTS

Breath Alcohol Concentration

BrAC during the time course of the experiments involving alcohol administration is depicted in Figure 1. ANOVA revealed no significant effects of risk group or administration sequence on the area under the time curve of all BrAC measurements. BrAC AUC had no significant effect when included as a covariate into the MANCOVAs testing the alcohol influence on stress response.

Main Effects on Stress Response in Test-Naive Subjects

Hormone secretion in response to the respective first stress test was significantly influenced by a group × treatment interaction (Wilks' multivariate test of significance, $F[2,45] = 4.1$, $p = 0.023$). The interaction effect was remarkable for both ACTH and cortisol AUC (univariate F-tests, $p < 0.05$, respectively). The influence of group and treatment, *per se*, was not statistically significant. The covariates (baseline hormone level) had a significant effect on AUC ($F[2,45] = 35.8$ for ACTH and 70.0 for cortisol, $p < 0.001$, respectively), which justified their inclusion into the model.

For specific evaluation of our hypotheses, the source of the factor interaction was analyzed by tests with contrasts. Comparison of risk groups within treatment modalities revealed significantly higher endocrine response in PHA than FHN subjects during placebo sessions ($p < 0.05$ for ACTH and cortisol AUC in tests with contrasts, respectively), but no significant group difference during alcohol sessions (Figure 4). When the treatment effect was evaluated within groups, alcohol was found to decrease hormone secretion significantly compared to placebo in PHA ($p < 0.05$ for ACTH and cortisol AUC in tests with contrasts, respectively), but not in FHN subjects (Figure 4).

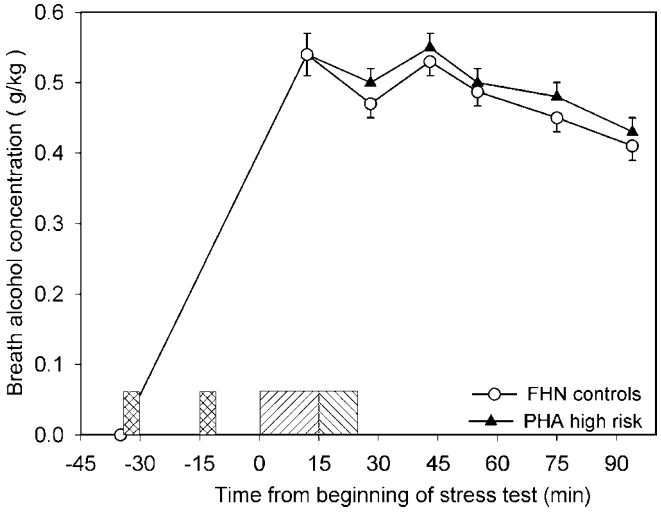

Figure 1 Mean and SEM of breath alcohol concentration. Cross-hatched bars: drinking periods; upward hatched bars: preparation time (beginning at clock time 1440 h); downward hatched bars: speech and mental arithmetics.

Main Effects on Stress Response During Repeated Testing

When the results of both experimental days were compared in a repeated-measures MANCOVA, a significant effect of treatment was noted (Wilks' multivariate test, $F[2,45] = 11.5$, $p < 0.001$), caused by a significant influence on both ACTH and cortisol AUC (univariate F-tests, $p < 0.05$, respectively, Figures 2 and 3). The interaction between treatment and risk group also influenced hormone secretion (Wilks' multivariate test, $F[2,45] = 8.6$, $p = 0.001$), and this effect was significant for ACTH (univariate F-tests, $p < 0.05$), but not cortisol AUC.

Administration sequence (ie alcohol/placebo *vs* placebo/alcohol) also interacted with treatment ($F[2,45] = 8.8$, $p = 0.001$), affecting both ACTH and cortisol ($p < 0.05$, respectively). Both covariates associated significantly with the dependent variables ($p < 0.001$ for basal ACTH and basal cortisol, respectively), which justified their inclusion into the model. Risk group, administration sequence, and their interaction did not significantly influence hormone secretion, and there was no significant three-factorial interaction between treatment, risk group, and administration sequence.

Again, to test our specific hypotheses, the risk group × treatment interaction affecting ACTH was further analyzed for simple effects. During placebo experiments, ACTH AUC was higher in PHA than FHN subjects (mean and SD: 2336 ± 893 *vs* 1902 ± 753 pg min/ml, $p < 0.05$, tests with contrasts), whereas after alcohol administration there was no significant group difference (1699 ± 755 *vs* 1720 ± 694 pg min/ml for PHA and FHN, NS in tests with contrasts, respectively). Analysis of the alcohol effect within PHA subjects revealed marginally less ACTH secretion during alcohol compared to placebo sessions ($p = 0.07$ in tests with contrasts). In the FHN subjects, no such treatment effect could be found.

Tests with contrasts to elucidate the source of the interaction between treatment and administration sequence revealed a significant treatment effect on ACTH and cortisol for subjects receiving placebo first and alcohol during the second test ($p < 0.05$ in tests with contrasts, respectively), but not for subjects receiving alcohol first and placebo second. Evaluation of the influence of administration sequence within treatment modalities revealed no significant effects.

Additional tests with contrasts were performed to test for differences between risk groups during all four experimental conditions (Figure 4). Hormone AUC was significantly higher in PHA than FHN subjects in the first test if placebo was given, caused by a group effect on both ACTH and cortisol ($p < 0.05$, respectively). Risk groups did not differ significantly in the first test upon alcohol administration, or during the respective second tests.

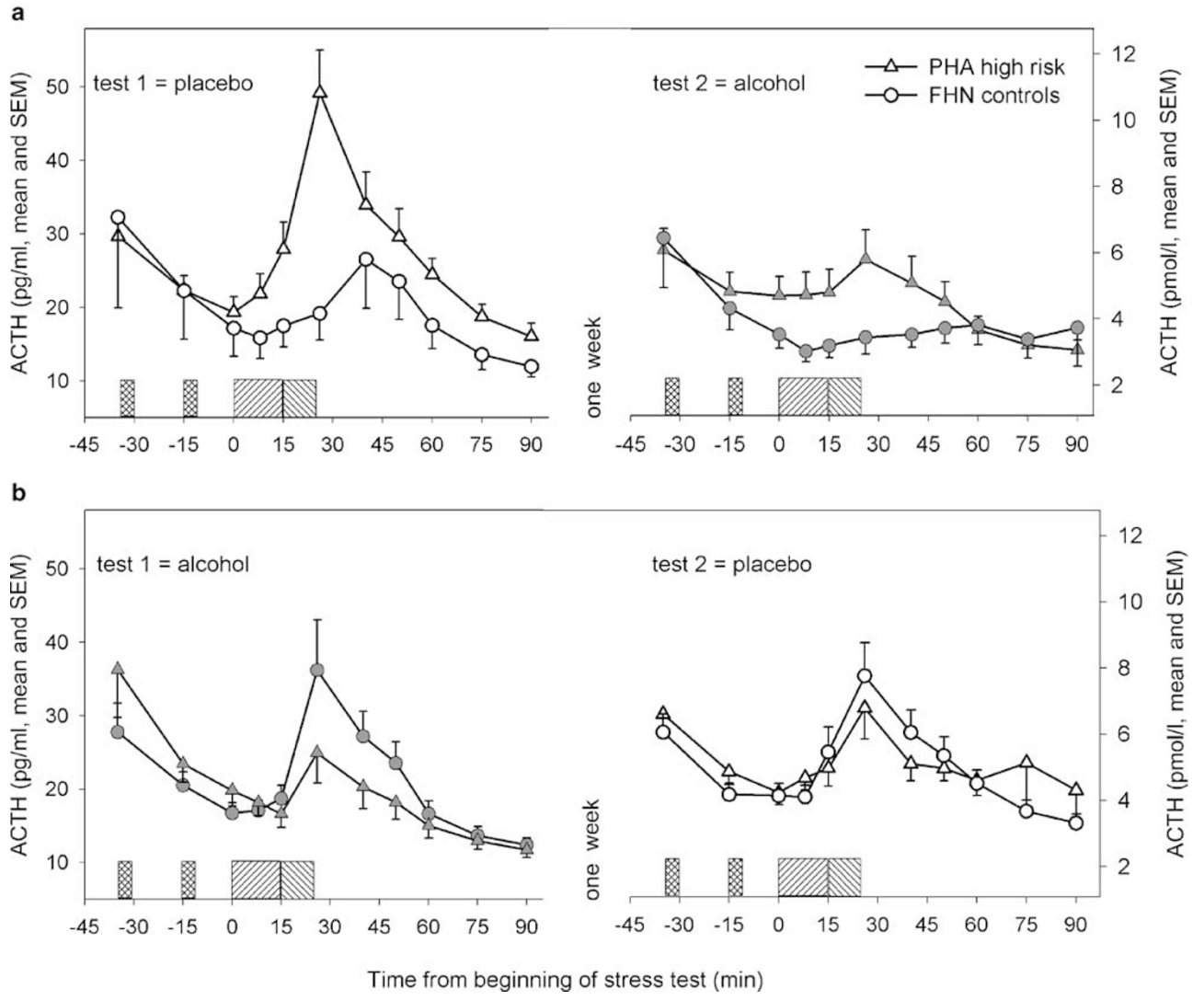

Figure 2 ACTH secretion during two consecutive stress experiments. (a) First placebo (open symbols), second alcohol (filled symbols). (b) First alcohol, second placebo. Cross-hatched bars: drinking periods; upward hatched bars: explanation of and preparation for test (beginning at clock time 1440 h); downward hatched bars: speech and mental arithmetics.

Baseline Hormone Secretion

Main effects on the three hormone measurements performed during the baseline period, prior to the stress test, were evaluated by a MANOVA with repeated measures with ACTH and cortisol as dependent variables. Thereby, 'time' and 'treatment' were within-subjects factors with three and two levels, respectively, and 'risk group' and 'administration sequence' were between-subjects factors. A significant effect for time was noted ($F[4,45] = 20.6$, $p < 0.001$, Figures 2 and 3), which significantly influenced both ACTH and cortisol (univariate F-tests, $p < 0.05$, respectively). Effects of all other factors and their interactions were not statistically significant.

Subjective Perception of the Stress Test

The mean rating of the four VAS items was analyzed in a MANOVA with 'experimental session' (first *vs* second test day), 'administration sequence', and 'risk group' as influential factors. The perceived stress level was significantly influenced by experimental session (Wilks: $F[1,48] = 18.1$, $p < 0.001$) and by risk group ($F[1,48] = 8.1$, $p = 0.007$), but not by any factor interaction. Mean and SD stress ratings of FHN *vs* PHA subjects were 47.2 ± 23.1 *vs* 64.1 ± 21.7 during the first and 38.8 ± 21.4 *vs* 50.9 ± 21.1 during the second test day. The absence of a significant interaction between experimental session and administration sequence indicates that alcohol had no effect on perceived stress.

Inter-relations between the levels of subjectively perceived stress and the endocrine stress response were analyzed by calculating Pearson's correlation coefficients. In the 23 FHN subjects, correlation of the mean stress ratings with ACTH AUC showed a trend during placebo ($r = 0.40$, $p = 0.056$) and a significant association during alcohol experiments ($r = 0.45$, $p < 0.05$). Significant correlations with cortisol were found during both placebo and alcohol sessions ($r = 0.44$ and 0.43, $p < 0.05$, respectively). In the 29 PHA subjects, no correlation

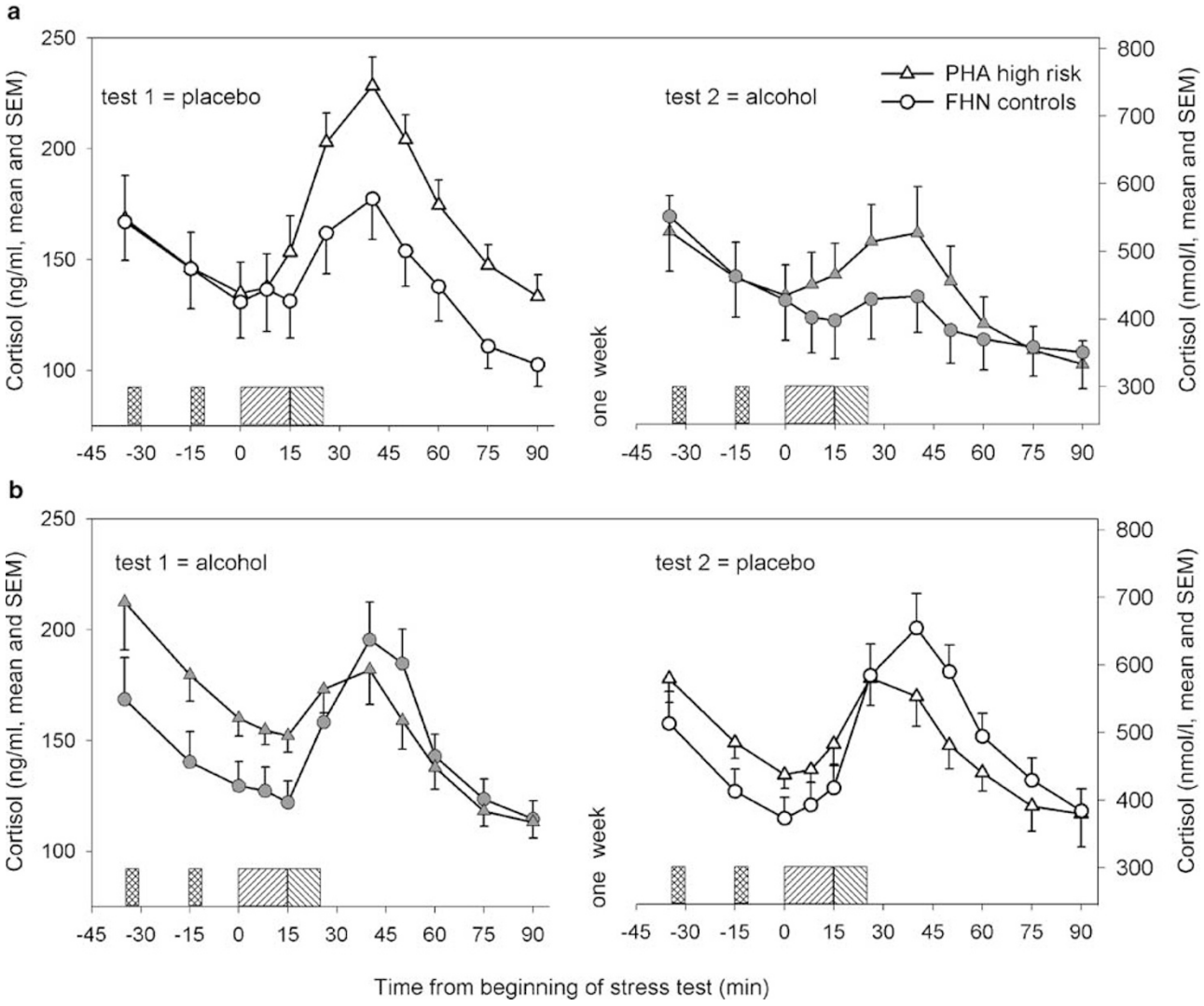

Figure 3 Cortisol secretion during two consecutive stress experiments. (a) First placebo (open symbols), second alcohol (filled symbols). (b) First alcohol, second placebo. Cross-hatched bars: drinking periods; upward hatched bars: explanation of and preparation for test (beginning at clock time 1440 h); downward hatched bars: speech and mental arithmetics.

between subjective stress perception and hormone AUC could be detected ($r = 0.03$ and 0.01 for ACTH, and $r = 0.13$ and 0.04 for cortisol, NS, respectively). Stress ratings, obtained during placebo and alcohol experiments, were significantly correlated for both groups ($r = 0.68$, $p < 0.001$ for FHN, and $r = 0.43$, $p < 0.05$ for PHA).

Effect of Confounding Variables

Measures that were considered as potentially confounding factors are described in Table 2. Two-factorial ANOVAs revealed a significant effect of risk group on drinks per week ($F[1,58] = 5.6$, $p = 0.022$), sensation seeking scale ($F[1,58] = 4.5$, $p = 0.04$), and an effect of administration sequence on the state anxiety (STAI) score ($F[1,48] = 4.3$, $p = 0.044$ and 4.2, $p = 0.047$ for placebo and alcohol days, respectively). Of the confounding variables with nominal data structure, Fisher's exact test indicated significant group differences in the distribution frequency for regular smoking and parental history of affective symptoms. These variables and the VAS ratings of perceived stress were simultaneously included as covariates into the three-factorial repeated-measures MANCOVA testing the main effects on ACTH and cortisol AUC. Significant effects were noted for the STAI score ($F[2,39] = 4.9$, $p = 0.012$) and for the interaction between sensation seeking scale and treatment ($F[2,39] = 4.6$, $p = 0.016$), while the other covariates had no effect on hormone AUC. The effects of group, treatment, their interaction, and administration sequence remained statistically significant as in the model described above.

To analyze a possible confounding effect of the method of subjects' recruitment, the 11 FHN study participants drawn from the epidemiologic survey were compared with the 12 FHN subjects recruited from the friends of the initial study participants. A MANCOVA with 'method of recruitment' (two levels), 'treatment', and 'administration sequence' as influential factors revealed no significant effects on ACTH and cortisol secretion.

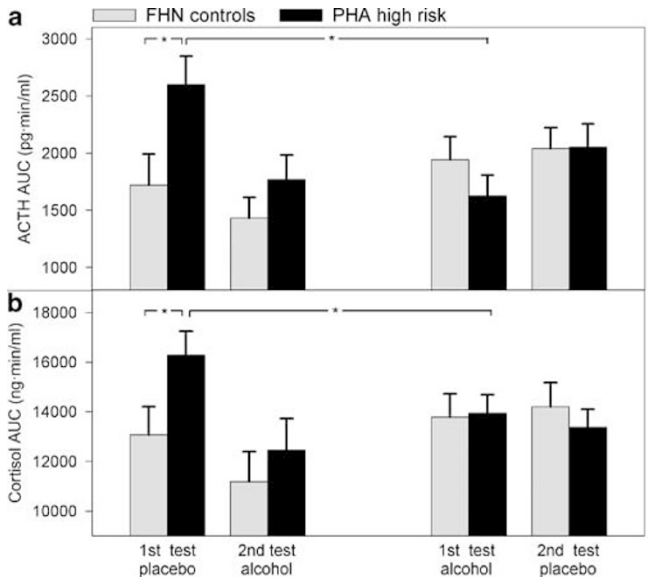

Figure 4 Mean and SEM AUC of hormone secretion during two consecutive stress tests in subjects receiving either placebo first and alcohol second, or vice versa. (a) ACTH, (b) cortisol secretion. $^{*}p<0.05$ in tests with contrasts.

DISCUSSION

The experimental paradigm reliably elicited an endocrine stress response, which was significantly attenuated, but not completely abolished by alcohol. Therefore, interpretation of our results is not complicated by a floor effect. A significant interaction between alcohol effect and administration sequence influenced both ACTH and cortisol secretion. This is most probably due to a repetition effect resulting in habituation, since in prior studies HPA response was diminished during the second test in the majority of sober subjects when the TSST was repeated after 1 week (Kirschbaum *et al*, 1995; Gerra *et al*, 2001). Therefore, alcohol administered during the second test acted synergistically with the habituation effect, resulting in more effective dampening of the HPA response than if alcohol was administered during the first test.

To avoid this interaction, a separate evaluation was performed including only results of the respective first experiments, during which subjects were naive to the test situation. This analysis supported our hypotheses, since the stress-induced ACTH and cortisol response was more pronounced in sober PHA than FHN subjects, while the absence of risk group difference in participants who were moderately intoxicated suggests a stronger stress response dampening alcohol effect in PHA subjects.

Evaluation of both experimental days in a repeated-measures MANCOVA led to similar results for ACTH. Regarding cortisol, univariate tests revealed no influence of risk group. This might be due to a marginally significant interaction between risk group $\times$ alcohol effect $\times$ administration sequence ($p<0.065$ in univariate F-test), which was caused by the fact that risk group differences in the alcohol effect on cortisol secretion were apparent in the subjects receiving placebo first, but not if alcohol was given first (Figure 4). This finding might be explained by effects of alcohol on memory encoding during the first test. Recall of these memories affects the cortisol response upon repetition of the experiment in some subjects (Kirschbaum *et al*, 1995). This process might have been modulated by alcohol in a way that masks risk group differences. Unfortunately, the design of our study does not allow for testing of this hypothesis.

The family history effect on stress response cannot be explained by the risk group differences in current alcohol drinking, cigarette smoking, recent illegal drug use, sensation seeking traits, state anxiety, or parental psychiatric symptoms, since these covariates *per se* had no significant effect in the repeated-measures MANCOVA and did not alter the above-described group effects.

The observed group differences in the endocrine stress response are consistent with prior reports on autonomic parameters of response to aversive electric shocks and public speaking, where an increased stress-dampening effect of alcohol was found in high-risk subjects (Levenson *et al*, 1987; Finn *et al*, 1990; Conrod *et al*, 1998). In a recent study, Dai *et al* (2002) used a similar design as reported here to investigate HPA response to stress and alcohol in high-risk subjects. In contrast to our results, they found higher baseline and stress-induced ACTH secretion in FHN compared to PHA subjects. Several methodological differences might explain this difference: first, the stressor that employed consisted of a mathematical problem solving task with time pressure and monetary award, while our paradigm included a strong social component in addition to cognitive demands. This different quality of stress might be the reason why we observed substantially more stimulation of ACTH and cortisol secretion in both PHA and FHN subjects, and why risk groups responded differently compared to Dai *et al*'s study. Second, Dai *et al* recruited subjects by newspaper advertisements and bulletin boards in colleges/universities, which might have introduced a response bias. In contrast, we contacted all PHA and most FHN study participants directly via a sample representing the general population, thereby avoiding preselection other than by definition of risk status. Third, alcoholism in the paternal grandfather was mandatory for the definition of a high-risk status in Dai *et al*'s study, while it was optional in ours. However, the presence or absence of alcoholism in grandparents of our study participants did not affect hormone secretion when included as an influential factor into a MANOVA analyzing ACTH and cortisol secretion in PHA subjects. Fourth, the group differences in stress-induced ACTH secretion observed by Dai *et al* were partly caused by higher baseline levels in the low-risk subjects. In our study, baseline hormone levels prior to the TSST were rather high, decreased significantly over time, but were not influenced by risk group or treatment (first three measurements in Figures 2 and 3). This indicates a short-lived endocrine response caused by introducing subjects to the laboratory environment. Two other studies also found similar baseline ACTH (Schuckit *et al*, 1988, Wand *et al*, 1999a) and cortisol levels (Schuckit *et al*, 1987) between risk groups, while one found lower ACTH in family history positive subjects (Waltman *et al*, 1994). In our study, only the response to the TSST and the alcohol effect thereupon differed between risk groups, while baseline hormone secretion did not. Together with the

findings of Dai *et al*, this suggests that the genetic risk factors for alcoholism might be specifically associated with an exaggerated response to psychosocial stress, but not to all other forms of stress.

Contrary to our expectations, the dampening of stress hormone response by alcohol was not paralleled by any effect on how subjects perceived the stress situation. Two issues might offer an explanation: first, our results are analogous to those in prior studies. Levenson *et al* (1987) and Sinha *et al* (1998) found no alcohol effect on anxiety, *per se*, in a study of family history positive and negative subjects that employed a public speaking task, but did find that autonomic responses were dampened by alcohol. Other authors found that alcohol reduced anxiety, but during anticipation of electric shocks (Finn *et al*, 1990; Conrod *et al*, 1998). Thus, our timing to administer the subjective perceptions questionnaire after the TSST might have been inappropriate. Second, our VAS scales could have assessed emotions more directly than they did. Our questions were designed to appraise cognitive aspects of the recent stress situation, whereas the tension-reducing effect of alcohol may have occurred in emotional responses that were not assessed directly.

Another issue important for the interpretation of stress perception is that its subjective scores did not correlate with the endocrine stress response in PHA subjects during placebo or alcohol experiments, while there was a significant positive correlation in FHN controls. This suggests that the physiological response to a stressor and the subjective perception thereof might be dissociated in sons of alcoholics, regardless of whether the stress situation was encountered while being sober or intoxicated.

The increased stress response in healthy PHA subjects contrasts with findings in alcohol-dependent patients after 3–4 weeks of abstinence, in whom saliva cortisol levels were unaffected by a public speaking task, indicating an absent endocrine stress response (Lovallo *et al*, 2000). This difference might be due to long-lasting effects of chronic alcohol intoxication on HPA functioning and suggests that endocrine abnormalities observed in alcoholics during early abstinence cannot be interpreted as pre-existing risk factors for the development of alcoholism. In untreated patients with major depression, basal cortisol was increased, but cortisol response to the TSST was equal to healthy controls matched for sex and age (Young *et al*, 2000). In another study investigating a nonclinical sample of adult women, HPA response to the TSST correlated with childhood abuse, adulthood traumas, and severity of depression, but not with severe negative life events (Heim *et al*, 2002). In our study, all participants denied childhood abuse, and signs of psychopathology that might be secondary to adverse experiences in early life did not differ between risk groups. Therefore, our finding of an increased stress response might reflect some specific pathology affecting sons of alcoholics, rather than to be generally associated with psychiatric morbidity.

Several limitations restrict interpretation of our results. First, this study included only male subjects due to the problem of controlling for menstrual cycle effects in a repeated-measures design extending over a minimum of 1 week. Second, our efforts to match groups for confounding factors were only partly successful. Due to the limited validity of the subjects' initial accounts and diagnoses, alcohol drinking and cigarette smoking during the experimental period as well as sensation seeking scores and parental psychiatric symptoms differed between risk groups. However, statistical compensation for these factors did not alter the main effects. Third, although one of the strengths of our study is that it was based on a randomly selected representative population sample, we had to compensate for a high dropout rate by including 12 friends of the initial study participants. Although only one acquaintance was accepted from every initial participant and the recruitment method did not affect the endocrine stress response, the final sample may not be entirely representative of the male population. Other negative aspects of our recruitment method were that there was no possibility to increase the rather small sample size of PHA subjects available for evaluation, and that we could not recruit enough subjects with alcoholic grandparents to study the effect of a multigenerational family history.

In conclusion, our data on HPA system activation suggest that environmental stress and paternal history of alcoholism can interact to produce an exaggerated endocrine stress response, which is normalized by alcohol. This combination is of the kind considered by Koob and Le Moal (2001) to be detrimental to internal homoeostasis and promote the development of alcohol use disorders. We assume that alcohol might have a transient favorable effect in paternal history positive subjects experiencing psychosocial stress. If so, our results may reflect a mechanism by which predisposition to develop alcohol use disorders is expressed. We expected this finding to be accompanied by a more rewarding subjective alcohol effect, but our methodologically weak rating scales failed to detect an overall alcohol effect on stress perception when recalled after completion of the stress test. However, our results do raise the possibility that subjective stress experience might be dissociated from physiological stress response in sons of alcoholics, an unexpected finding that needs replication by further experiments before it can be interpreted.

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