Available online at www.sciencedirect.com
SCIENCE DIRECT
Pharmacology, Biochemistry and Behavior 83 (2006) 239–248
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Gender specific effects of a mild stressor on alcohol cue reactivity in heavy social drinkers

J. Nesic [1], T. Duka *

Department of Psychology, School of Life Sciences, University of Sussex, Falmer, Brighton BN1 9QG, UK

Received 7 June 2005; received in revised form 23 January 2006; accepted 1 February 2006
Available online 10 March 2006

Abstract

Rationale: Stress plays an important role in the development and maintenance of alcohol-abuse. Some of the effects of stress on alcohol-related behaviours, however, appear to be gender-dependent.
Aim: The present study set out to examine the effects of stress on feelings of desire for alcohol, skin conductance response and alcohol consumption in the presence of alcohol-related cues in relation to gender. Participants were heavy non-dependent alcohol drinkers.
Methods: Thirty-two (16 males) participants drinking more than 21 units of alcohol per week were randomly allocated to undergo the experimental stress (based on the 'Trier Social Stress' Test) or the non-stress procedure before the alcohol cue exposure procedure, during which participants handled and smelled their preferred drink. Mood and saliva cortisol level changes were used as indices of the stress effects, while alcohol craving, skin conductance and alcohol consumption were the cue reactivity measures.
Results: Self ratings of anxiety and tension increased and cortisol levels remained high in the stress compared to the non-stress condition; no gender differences were found. Stress induced gender-specific effects with regard to skin conductance response and alcohol consumption measurements. Stressed females did not show an increase from baseline in the skin conductance response during the alcohol cue-exposure session, which was observed in the non-stressed females; they also consumed less alcohol than males under stress.
Conclusion: Female participants respond less to alcohol-related cues when in a negative mood state. Such a finding suggests that females when in a negative mood may be less sensitive to positive incentive processes mediating cue reactivity compared to males.

Keywords: Craving; Skin conductance; Negative mood; Cortisol

1. Introduction

Exposure to psychosocial stressors has been found to increase alcohol consumption in social drinkers and alcoholics (Miller et al., 1974). In addition, the hypothalamo-pituitary-adrenocortical (HPA) axis has been suggested to play a crucial role in stress-induced drug-related behaviours and disruption in the HPA reactivity to stress appears to constitute vulnerability to drug-related behaviours (Sinha et al., 2003). More generally, stress is considered to be an important factor in the initiation, maintenance and relapse to alcohol abuse (Brady and Sonne, 1999; Brown et al., 1990; Marlatt, 1979; Wolffgramm and Heyne, 1991; Pohorecky, 1991).

In view of the higher prevalence of alcoholism in men compared to women, (Dawson and Archer, 1992) it is interesting to note that the relationship between stressful events and alcohol drinking was found to be stronger in male than in female drinkers (Cooper et al., 1992; Pohorecky, 1991). Could these observations be the result of a lower response to stress in females compared to males? The aim of the present study was to examine gender differences in the effects of stress on mood, HPA activation as measured by salivary cortisol secretion and alcohol-related behaviours in a cue reactivity paradigm.

It has been suggested that stress activates the brain reward circuits thus increasing their sensitivity to the reinforcing properties of drugs and, consequently, increasing motivation to

* Corresponding author. Tel.: +44 1273 678 879; fax: +44 1273 678058.
E-mail address: t.duka@sussex.ac.uk (T. Duka).
[1] Present address: Department of Pharmacology and Therapeutics, University of Brighton, UK.

0091-3057/$ - see front matter © 2006 Published by Elsevier Inc.
doi:10.1016/j.pbb.2006.02.006

use drugs (Piazza and Le Moal, 1997, 1998). Animal studies have indeed shown that stress activates drug-related behaviours via stimulation of the mesolimbic dopamine (incentive) system by glucocorticoids (Piazza and Le Moal, 1997, 1998). For instance, administration of corticosterone was found to facilitate, while reduction in corticosterone levels decreased, alcohol consumption in rats (relative to water consumption; Fahlke et al., 1994a, 1995, 1994b). Consistent with this suggestion, craving for alcohol has been shown to increase following stress (Sinha and O'Malley, 1999). Similarly, alcohol-dependent patients with post-traumatic stress disorder (PTSD) reported increased craving for alcohol following exposure to personalized trauma imagery (Coffey et al., 2002).

Drug-related cues have also been suggested to activate the incentive system (Stewart et al., 1984). The present study set out to examine the effects of stress on alcohol-cue reactivity in order to evaluate further the hypothesis that stress activates the incentive system. A cue reactivity paradigm was included that involves exposure to a cue or a set of cues (e.g. sight and smell of a favourite drink) and measurements of subjective, physiological and behavioural responses were taken (Drummond, 2000).

Alcohol cue-exposure has fairly consistently been found to increase craving in non-dependent (Walitzer and Sher, 1990; Schulze and Jones, 1999, 2000) as well as in dependent drinkers (Saladin et al., 2002; Cooney et al., 1997; Davidson et al., 2003; Jansma et al., 2000; Rubonis et al., 1994; Coffey et al., 2002). Physiological cue reactivity has also been well documented. Exposure to alcohol olfactory stimulus has been found to increase salivation (Rubonis et al., 1994; Saladin et al., 2002) and skin conductance (Kaplan et al., 1985; Stormark et al., 1995) in alcoholics compared to non-dependent drinkers. Social drinkers exposed to experimentally conditioned alcohol cues also showed increased skin conductance (Field and Duka, 2002).

Facilitatory effects of stress on cue reactivity as measured by cue-induced reinstatement of alcohol seeking have been demonstrated in rats (Liu and Weiss, 2002). However, very few studies have examined the effects of stress on cue-reactivity in humans and the data are equivocal, probably due to using different type of stressors or different populations. In one study, employing alcoholics undergoing treatment, negative mood induction was found to augment the effects of alcohol cue exposure on subjective craving (Cooney et al., 1997). In non-dependent social drinkers, negative mood induction prior to exposure to gustatory alcohol stimulus (consumption of half a pint of low-alcohol beer) was also found to increase craving for alcohol (Willner et al., 1998). This effect of stress, however, was observed only in male participants while in females stress reduced liking of alcohol (Willner et al., 1998) suggesting that effects of stress on incentive value of alcohol are gender-dependent. In contrast, Coffey et al. (2002) did not find augmentation of alcohol and cocaine cue-induced craving by stressful imagery in alcohol- and cocaine-dependent PTSD patients, respectively, although both stress and drug cue alone were effective in inducing craving. Similarly, Jansma et al. (2000) did not find any effects of distressed mood on subjective and cardiovascular alcohol cue reactivity in alcoholic patients. Regarding alcohol consumption measurements, while negative mood induction in combination with alcohol gustatory cue exposure was found to enhance the incentive value of alcohol in heavy social drinkers (increased operant responding for alcohol reinforcement; Willner et al., 1998), de Wit et al. (2003) reported non-specific increase in alcohol as well as placebo consumption following stress and a priming dose of alcohol. However, the participants in the latter study were not heavy social drinkers. With the exception of Willner et al. (1998) gender effects were not reported in the above-mentioned studies. Thus the present study set out to examine the effects of stress and alcohol-related cues on all three aspects of cue reactivity (craving, physiological responses and consumption) in heavy social drinkers with respect to gender.

Whereas a decreased responsiveness of HPA axis to stress has been observed in abstinent alcoholics (Adinoff et al., 2005), a hyper-HPA responsiveness can increase vulnerability to drug-related behaviours (Sinha et al., 2003). There is evidence for a gender difference in HPA responsiveness to stress with females being less responsive (Kirschbaum et al., 1999, 1992). Thus in the present study we examined changes in cortisol levels in response to stress also with regard to gender.

Psychosocial stressors have been found to increase alcohol consumption in social drinkers (Higgins and Marlatt, 1975; Pelham et al., 1997; de Wit et al., 2003). Thus the present study employed stress-induction method based on the Trier Social Stress Test (TSST; Kirschbaum et al., 1993), a method that has been found to reliably produce a physiological response in volunteers (cortisol, heart rate, ACTH; Kirschbaum et al., 1993, 1995).

The effectiveness of cue-exposure to induce craving and physiological reactivity depends on the type of alcohol stimuli used, with greater reactivity observed in response to favourite alcohol beverages (Staiger and White, 1991). Thus participants in the present study were presented with their favourite type of drink (beer, white wine, red wine or vodka and orange juice) in order to improve the ecological validity of the experimental manipulation. The effects of stress on alcohol-related behaviours were not tested immediately but approximately 1 h after the stress manipulation. In a naturalistic situation, individuals often experience stress in situations where drinking cues are not present (e.g. work environment), and it is subsequently that they may become exposed to such cues (at home or in the bar). In order to be as closely as possible to the naturalistic situation participants were exposed to alcohol-related cues not immediately after stress but with some delay.

2. Materials and methods

2.1. Participants

Thirty-two heavy social drinkers (16 male, 16 female) aged 18–31 (mean=21.84, SE=0.56) consuming on average 27.5–90.3 alcohol units per week (mean=41.92, SE=2.27) took part in this experiment. Participants were recruited via Experimental Psychology participant pool and were mostly students at the

University of Sussex. Participants were only permitted to take part in this study if they were between 18 and 40 years of age and if they consumed 21 or more alcohol units per week (the maximum recommended weekly alcohol intake for men; Department of Health; Health, 1992), as reported in the Alcohol Use Questionnaire (Mehrabian and Russell, 1978), which was included in the general recruitment questionnaire.

Participants were generally in good health verified by a medical interview taken by a qualified person and their weights were within 15% of the normal weight limit for their heights. Participants were instructed to avoid high fat containing meals for 24 h prior to the testing session and to have a light dinner on the evening before the testing session and, if they attended the later testing slot (12:30 pm) to have a light breakfast but not later than 10 am. Participants were also asked to refrain from drinking alcohol for 12 h, taking sleeping pills and other sedatives for 48 h and taking illicit drugs for at least 5 days before the testing session.

All participants gave their informed consent before taking part in this study. The study was approved by the University of Sussex Ethics Review Committee for the use of human in compliance with the Declaration of Helsinki for human participants. Participants received payment for taking part in the experiment.

2.2. Experimental design

Participants were tested individually in a between-subjects design, fully balanced for gender and time of day (testing session starting at 10:00 am vs. 12:35 pm). Participants were randomly allocated to the experimental condition (stress vs. non-stress) whilst all participants underwent the cue-exposure procedure.

2.3. Stress-induction

Initially participants were informed via standardised written instructions about the requirements of the procedure (2 min). Participants were required to prepare (8 min) and deliver a 5-min speech as part of a fictional job interview. Participants were informed that their presentation would be recorded and that it would later be analysed by three independent observers for right or wrong (for the interview) non-verbal behaviour. The speech outline written during the speech preparation period was not available to participants during the speech delivery. The participants had to perform the speech standing up in front of the experimenter (who was taking notes as they were speaking) and were instructed to look at themselves on the TV screen in order to be able to assess their performance afterwards. If the participants finished their speech in less than 5 min the experimenter waited for 15 s before prompting them to continue and if the participants stopped again before the 5 min were up, there was a pause of 20 s after which the experimenter asked standardised questions until the end of the 5-min period. Participants were then asked to write down what they thought was the best and the worst aspect in their performance (2 min). After this, participants were instructed to perform a mental arithmetic task (serially subtracting number 7 from 1013 whilst keeping continuous eye contact with the experimenter) for 5 further minutes. On every mistake participants were asked to start counting again from 1013. Whenever necessary during the task performance, the participants were reminded to keep eye contact or to count faster.

In the control (non-stress) condition participants were initially given standardised written information about the procedure (2 min). Participants were first asked to spend some time (8 min) looking through an art history book (Art Through the Ages by de la Croix et al., 1991). Afterwards, they were allowed 6 min to assess 10 paintings from different art periods by marking their liking of each painting on a visual analogue scale ('not at all'–'very much'). Following this, the participants were given a booklet of 20 dot-to-dot pictures and asked to complete as many of them as they wanted during the 6-min period. Thus the total duration of the non-stress procedure was the same as the duration of the stress-induction.

2.4. Alcohol drinks

During the recruitment phase prior to the experiment, participants were able to choose which alcoholic drink they would like to consume in the experimental session (beer, red or white wine or vodka and orange). The drinks were matched as closely as possible for alcohol content (9% in beer, 12% in wine, 37.5% in vodka) and quantity, so that each participant received a total of 3 alcohol units divided into six 60-ml cups (252 ml white or red Chilean wine, ASDA; 330 ml beer Carlsberg Special Brew; or 300 ml vodka and orange juice mix containing 81 ml of 37.5% v/v vodka). During the cue-exposure phase, participants were asked to take one of the cups and hold it for 30 s and then to smell the drink for another 30 s. Following the cue exposure, participants were instructed to drink as much as they wanted.

2.5. Subjective ratings

2.5.1. Alcohol Use Questionnaire

Alcohol Use Questionnaire (AUQ; Mehrabian and Russell, 1978) is a self-report questionnaire, which establishes the average weekly alcohol intake over a 6-month period and with information about patterns of drinking provides the AUQ score.

2.5.2. Mood questionnaires

Changes in mood were measured using POMS (McNair et al., 1971) and KUSTA (Binz and Wendt, 1990; Wendt et al., 1985). POMS is a list of 72 mood-related adjectives, which are rated on a 5-point scale, ranging from "not at all" [0] to "extremely" [4] and are grouped into 8 factors (anxiety, depression, anger, vigour, fatigue, confusion, friendliness and elation). KUSTA consists of three 17-point bipolar scales that measure mood, activity and tension/relaxation and three 17-point scales ranging from 'not at all' [1] to 'extremely strong' [17] that measure happiness, anxiety and anger). POMS and KUSTA ratings were taken at baseline and immediately after stress. KUSTA ratings were in addition taken shortly before the cue exposure procedure.

2.5.3. Desire for Alcohol Questionnaire

Desire for Alcohol Questionnaire (DAQ; Love et al., 1998) is a 14-item questionnaire which measures four different aspects of craving for alcohol: mild desire, strong desire and intention to drink, negative reinforcement and loss of control over alcohol use. Participants are required to rate how much each statement applies to them at that particular moment on a Likert-type 7-point scale, ranging from 'strongly disagree' [1] to 'strongly agree' [7]. DAQ was administered immediately after the main stress/non-stress manipulation and immediately after the alcohol cue exposure phase.

2.6. Physiological measurements

2.6.1. Salivary cortisol

Saliva samples were collected using the salivettes (Sarstedt). Participants were instructed to place the cotton swab in their mouth and chew on it gently for 2 min. The participants then replaced the swab into the salivette, which was sealed and stored in a freezer at −20 °C until analysis. Saliva samples were taken shortly before and approximately 50 min after the end of the stress/non-stress procedure (some minutes before the cue reactivity procedure). From previous pilot studies it was established that the peak of changes in cortisol levels following the stress procedure was 30 min. Our measurements of stress effects on alcohol-related behaviours in the present study were made-for the reasons explained in the introduction—1 h and 15 min following the start of the stress procedure. Thus the cortisol levels measurements were taken also at the same time following the stress procedure. Saliva samples were analysed using DELFIA assays (Wood et al., 1997).

2.6.2. Blood Alcohol Levels (BAL)

Participants were breathalysed using Lion Alcolmeter. The measurements were taken at the beginning of experimental session and 5 min after the end of alcohol consumption phase. Additional measurements were taken, if necessary, at 20-min intervals after the end of the test session, until BAL were equal to or below 0.4%.

2.6.3. Skin conductance

Skin conductance measurements were taken over a period of approximately 190 s using an Electronic Development skin conductance instrument, which consisted of two electrodes interfaced to a PC for data processing. The circuit generates a constant current of 10 μA, which passes through the skin via 2 silver electrodes. The voltage between these 2 electrodes is measured and the resistance of the skin can then be determined by: Resistance = Voltage/Current. Electrodes were attached with surgical tape to distal phalanxes of second and fourth fingers of the non-dominant hand. Measurements were recorded at 1-s intervals. Measurements from the last 90 s were broken down into three 30-s time bins and an average value was calculated for each time bin. The three time bins corresponded to pre-cue reactivity period (baseline bin), period of handling the drink (take the drink bin), and period of smelling the drink (smell the drink bin).

2.7. Alcohol consumption measurements

Participants were video recorded during the alcohol consumption phase and the dependent measures were latency to the first sip, and cumulative alcohol intake in "drinking units" over the fifteen 1-min time bins. "Drinking units" (number of cups) per minute were calculated by dividing the total number of cups consumed during the whole session by the total number of sips per session to provide the part of the cup equivalent to one sip; subsequently, the number of sips that participants took in each 1-minute bin was multiplied by this average size of sip.

2.8. Procedure

Each participant reported to the human psychopharmacology laboratory either at 10:00 am or 12:30 pm. Upon the arrival participants' BAL were tested and their height and weight were measured. Participants were then given a light brunch and the experimental procedure started approximately 45 min after participants' arrival to the laboratory.

2.8.1. Stress procedure

Participants were taken to the experimental cubicle where they completed POMS and KUSTA questionnaires and provided saliva sample (baseline measurements). Participants then underwent either the stress or the non-stress procedure, after which they completed again the POMS, KUSTA and DAQ questionnaires (post-stress measurements). Participants were then occupied by performing simple cognitive tasks, which have not been evaluated. Subsequently participants provided a saliva sample in the salivette and completed the KUSTA questionnaire again (post-stress/pre-cue-exposure measurements). Participants were then fitted with electrodes for the measurement of skin conductance for the cue reactivity session.

2.8.2. Cue exposure

Participants were presented with a tray with alcoholic drinks and underwent the alcohol cue-exposure procedure during which skin conductance measurements were taken [time bin 1 = baseline, 2 = take the drink, 3 = smell the drink]. Participants were then asked to complete the DAQ (cue-exposure measurement). Subsequently (approximately 1 h and 15 min from the beginning of the stress-induction), participants were allowed to freely consume alcohol for 15 min. Five minutes later BAL were measured and participants were debriefed and paid for their participation. Participants were allowed to leave the laboratory only after their BAL have had dropped below 0.4%.

2.9. Statistical analyses

A series of independent t-tests were performed in order to explore potential differences in characteristics of participants between the two experimental conditions (stress vs. non-stress), and between gender.

All the analyses were done with gender and time of day (morning vs. afternoon testing slot) as between-subject factors in addition to the experimental condition. If no interaction

involving gender or time of day was found the data were reanalysed with the non-significant factor (gender and/or time of day) omitted.

Repeated measures ANOVAs were used for mood variables as measured by POMS and KUSTA with condition (stress vs. non-stress) being the between-subject factor and the time point [baseline vs. post-stress] being the within-subject factor. Additional repeated measures ANOVAs were performed on KUSTA factors with time point [baseline vs. post-stress/pre-cue-exposure] as within-subject factor and condition (stress vs. non-stress) as between-subject factor.

Univariate ANOVAs were performed for cortisol change from baseline (post-stress/pre-cue-exposure levels expressed as percentage of baseline) with condition (stress/non-stress) as the between-subject factor. Cortisol levels at baseline were also analysed using an independent sample *t*-test for any differences between condition or gender.

The four DAQ factors were analysed separately using repeated measures ANOVAs with time (post-stress vs. post-cue exposure measurement) as within- and condition as between-subject factors.

Repeated measures ANOVAs were also performed for the skin conductance measurement with time (baseline, take the drink and smell the drink time bin) as within- and condition as between-subject factors.

Variables related to alcohol consumption (latency, total number of cups consumed) were evaluated using univariate ANOVAs (condition as the between-subject factor), except for the cumulative alcohol consumption data (fifteen 1-min time bins), which were analysed using a MANOVA (condition, gender and time of day as the between-subject factors). Since there were no significant differences between conditions with respect to the type of drink participants chose to consume during the experiment (Pearson's chi-squared not significant), this variable was not included as a factor in subsequent analyses.

In addition, a microstructural analysis of alcohol consumption was performed as it is thought to provide more insight into basic processes that generate consumption (after Kissileff et al., 1982). A quadratic function $y=a+bx+cx^2$ was fitted to cumulative intake data, where 'y' represents intake, 'x' represents time, 'b' is a linear coefficient and represents the initial rate of consumption, 'c' is a quadratic coefficient which represents the rate of deceleration and 'a' represents intercept (constant) which reflects the size of initial sip (after Kissileff et al., 1982). The two coefficients (b and c) were only analysed if a main effect or an interaction was found with the MANOVA on the cumulative data.

3. Results

3.1. Population characteristics

General characteristics of the participants allocated to the stress and non-stress group are presented in Table 1. There were no significant differences on any of the measurements between the two experimental conditions or between gender, or between gender within each condition.

Table 1
Group characteristics (mean, SEM) of participants in the non-stress and stress groups, separately for males and females

	Non-stress		Stress	
	Male	Female	Male	Female
Age	22.38 (1.51)	20.88 (0.48)	21.88 (0.95)	22.25 (1.36)
Body Mass Index (BMI; kg/m^2)	22.50 (0.99)	21.72 (0.52)	22.97 (0.82)	22.57 (0.58)
Alcohol intake (units/week)	44.91 (3.59)	40.01 (3.56)	40.71 (3.57)	42.06 (7.14)
Alcohol intake/ weight (units consumed in a week/kg)	0.62 (0.07)	0.69 (0.07)	0.59 (0.05)	0.66 (0.11)
AUQ-score	52.91 (6.46)	63.81 (10.64)	53.64 (6.48)	69.11 (23.52)

3.2. Mood

Gender and time of day were not significant factors in the initial repeated measures ANOVAs of mood variables (F's $\leq$ 5.22, p's $\geq$ 0.03, $\alpha'=0.008$ for KUSTA, 0.006 for POMS), thus the analysis was repeated with these factors omitted.

Repeated measures ANOVAs of mood variables at baseline and immediately after stress procedure have revealed significant time x condition interactions for anxiety as measured by POMS ($F[1,30]=10.271$, $p<0.01$; Fig. 1a) and by KUSTA ($F[1,30]=17.375$, $p<0.001$; Fig. 1b) as well as for the tension/relaxation scale of the KUSTA ($F[1,30]=12.539$, $p<0.01$; Fig. 1c). The observed interactions remained significant after Holme's correction, which was applied to control for the inflated chance of type 1 error resulting from the number of analyses performed for the factors derived from the POMS questionnaire (8; $\alpha'=0.05/8=0.006$) and for the scales from the KUSTA questionnaire (6; $\alpha'=0.05/6=0.008$). Post-hoc t-tests confirmed that the interactions were due to an increase in anxiety (both in POMS and KUSTA) and tension (in KUSTA) in the stress but not in the non-stress condition (see comparisons on Fig. 1a–c).

ANOVA of KUSTA factors for the measurements post-stress/pre-cue-exposure versus baseline revealed that anxiety and tension were both still significantly elevated, in comparison to baseline, in the stress but not in the non-stress group ($F[1,30]=8.294$, $p<0.01$, and $F[1,30]=5.054$, $p<0.05$). Gender and time of day were not significant factors in these analyses.

3.3. Salivary cortisol

Gender and time of day did not significantly influence changes in cortisol levels (F's <1.90, p's >0.25). Cortisol baseline levels (nmol/L) did not differ between the two conditions (mean $\pm$ SEM, stress 9.9 $\pm$ 1.3, non-stress: 12.8 $\pm$ 2.0; $t[30]=-1.208$, $p>0.1$) or between males and females (mean $\pm$ SEM, females: 11.8 $\pm$ 2.2, males: 11.0 $\pm$ 1.0; $t[30]=-0.326$, $p>0.1$). Cortisol data were analysed in terms of cortisol levels post-stress expressed as percentage of baseline levels (see Fig. 2) to minimise the variability in individual differences in response to stress. Inspection of box plots of the cortisol distribution in the stress and non-stress condition

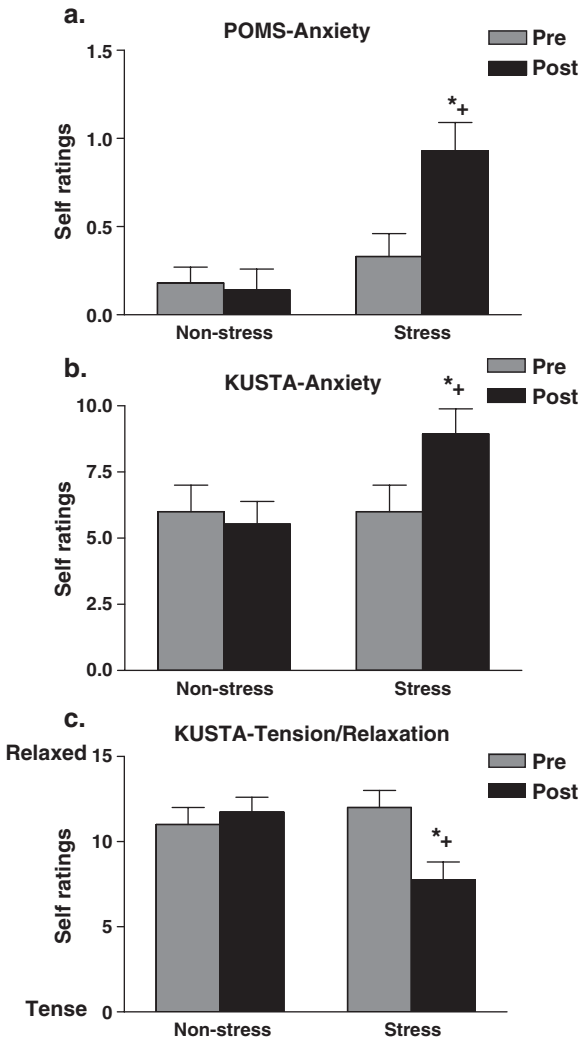

Fig. 1. Anxiety ratings (mean±SEM) in POMS (a) and KUSTA (b) as well as ratings in the bipolar scale tense/relaxed from KUSTA (c). Values at baseline (pre-) and post-stress are given for the non-stress and stress condition. $*p<0.05$ compared to the pre-stress measurement. $+p<0.05$ compared to the non-stress condition.

revealed greater variability of cortisol change in the stressful condition. There was only one outlier in each of the conditions and they were excluded from the subsequent analysis. Outliers were defined by SPSS software and had values more than 1.5 of the inter-quartile range (IQRs) computed from Tukey's hinges in the box-plots.

Univariate ANOVA with condition as the between-subject factor produced a significant main effect of experimental condition ($F[1,28]=4.777$, $p<0.05$), which reflects a significant reduction in cortisol levels in the non-stress condition (significantly different from 0: $t[14]=-6.785$, $p<0.01$) and no change in the stressful condition (see Fig. 2).

3.4. DAQ

Gender and time of day did not have an effect on DAQ ratings (F's<3.88, p's>0.05) thus the analysis was repeated with these two factors omitted. There was a main effect of time for two of the DAQ factors, both of which were significantly

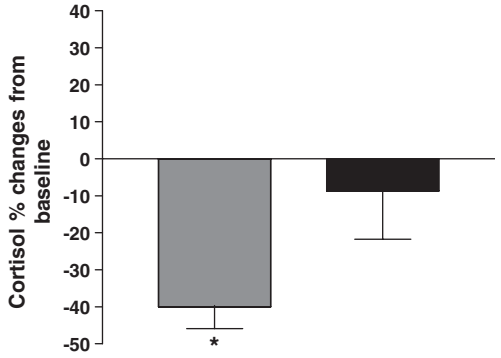

Fig. 2. Percent (%) changes (mean±SEM) from baseline in salivary cortisol levels (nmol/L) after the non-stress (light bars) and stress (dark bars) condition. $*p<0.05$ compared to stress condition.

lower than α' obtained through Holme's correction. Mild Desire and Strong Desire ratings increased following the alcohol cue-exposure ($F[1,30]=10.827$, $p<0.01$, $\alpha'=0.0167$ and $F[1,30]=13.115$, $p<0.01$, $\alpha'=0.0125$, respectively; see Table 2). However, no main effects of condition or interactions involving condition were observed in these analyses.

3.5. Skin conductance

ANOVA revealed a time of day by gender by condition by time bin interaction, which approached significance ($F[2,48]=3.182$; $p=0.050$). A subsequent ANOVA separately for males and females did not show any significant effects involving time of the day so the analysis was repeated excluding the factor time of the day. ANOVA with the factors gender, condition and time bin showed a time effect ($F[2,56]=6.39$, $p<0.01$) indicating an increase in skin conductance response over time, and a time×condition×gender interaction effect ($F[2,56]=4.45$, $p<0.05$; see Table 3). The source of interaction was further examined applying repeated measures ANOVA separately for males and females. A time×condition interaction was found in females ($F[2,28]=6.68$, $p<0.01$) but not in males ($F[2,28]=$

Table 2
DAQ ratings for the 4 factors positive/negative reinforcement, mild desire, strong desire and loss of control over drinking at the end of the stress procedure (before the cue exposure) and immediately after the cue exposure

	Non-stress		Stress	
	Male	Female	Male	Female
Measurements (post-stress/pre-cue exposure)				
Pos/Neg reinforcement	11.4 (1.1)	8.6 (1.6)	14.2 (1.7)	10.9 (1.1)
Mild desire	20.1 (1.4)	17.8 (3.0)	24.9 (1.7)	17.8 (1.8)
Strong desire	7.1 (1.2)	7.4 (1.6)	9.6 (1.6)	5.5 (1.1)
Control	4.4 (0.8)	2.9 (0.5)	3.8 (0.8)	4.1 (1.0)
Measurements (post-cue exposure)				
Pos/Neg reinforcement	11.5 (1.6)	8.3 (1.1)	13.8 (1.4)	10.1 (1.3)
Mild desire*	20.8 (1.7)	22.5 (2.4)	25.6 (0.9)	20.0 (2.8)
Strong desire*	9.6 (1.9)	11.1 (2.3)	10.2 (1.5)	10.0 (2.2)
Control	4.6 (0.9)	3.5 (0.8)	4.4 (1.0)	5.4 (0.8)

Values represent mean (SEM) of participants in the non-stress and stress groups and for males and females separately. $*p<0.05$, main effect of time.

Table 3
Skin conductance levels during pre-cue reactivity period (baseline time bin), period of handling the drink (take drink time bin), and period of smelling the drink (smell drink time bin)

Measurements during cue exposure procedure	Male		Female	
	Non-stress	Stress	Non-stress	Stress
Baseline time bin	16.64 (1.2)	18.25 (3.9)	14.90 (1.0)	10.12 (1.0)
Take drink time bin	17.17 (1.2)	20.06 (5.5)	17.96 (1.6)*	9.47 (1.1)
Smell drink time bin [a]	19.54 (1.7)	19.62 (4.9)	16.88 (1.2)*	10.81 (0.9)

Values are in iS [mean (SEM)] of male and female participants in the non-stress and stress groups; $*p<0.05$ (paired *t*-tests compared to baseline).
[a] Main effect of time bin. Each time bin represents values recorded at 1 μs intervals and averaged over a period of 30 s.

Table 4
Linear ('*b*') and quadratic ('*c*') coefficient values of cumulative alcohol consumption curves, following the stress procedure and exposure to alcohol cues

Mode of alcohol consumption	Male		Female	
	Non-stress	Stress	Non-stress	Stress
Linear coefficient ('*b*')	0.401 (0.094)	0.510 (0.086)*	0.388 (0.084)	0.255 (0.058)
Quadratic coefficient ('*c*')	−0.012 (3.04)	−0.015 (0.004)	−0.008 (0.003)	−0.005 (0.002

Values represent mean (SEM) of male and female participants in the non-stress and stress groups; $*p<0.05$ compared to females in the same condition.

1.39, $p>0.1$). Tests of within-subject contrasts with the factor time repeated showed an interaction at baseline vs. take drink time bin ($F[1,14]=10.77$, $p<0.01$). Post-hoc *t*-tests revealed that while females in the non-stress condition showed a significant increase in skin conductance during take the drink time bin compared to baseline ($t[8]=-3.702$, $p<0.01$), those in the stress condition did not. There was also an interaction at the take the drink vs. smell the drink time ($F[1,14]=5.55$, $p<0.05$) due to a small (non-significant) decrease and increase of skin conductance levels in the non-stress and stress condition, respectively. Post hoc paired *t*-tests, however, showed that the skin conductance levels at the smell the drink time were still significantly higher than baseline in females in the non-stress condition ($t[8]=-2.845$, $p<0.05$).

In female participants, main effects of time ($F[2,28]=4.10$, $p<0.05$) and condition ($F[1,14]=17.82$, $p<0.01$) were also found, while the main effect of time only approached significance in males ($F[2,28]=3.19$, $p=0.058$). A separate analysis on baseline data showed an effect of gender with males having higher skin conductance ratings at baseline than females ($F[1,28]=5.23$, $p<0.05$).

3.6. Alcohol consumption

Time of day did not have an effect in the MANOVA ($F[15,10]=3.477$, $p<0.05$) thus the analysis was repeated with the factor time of the day omitted. A significant condition × gender interaction was revealed with males consuming larger amounts of alcohol than females under stress ($F[15,14]=2.55$, $p<0.05$; see Fig. 3). Subsequent between-subject *t*-tests of the coefficients in each of the two experimental groups revealed a significant gender difference in the linear coefficient ('b') only in the stressful condition, with male participants showing greater initial rate of consumption than their female counterparts ($t[14]=2.453$, $p<0.05$; see Table 4).

No differences between the experimental groups were observed with respect to other alcohol consumption variables.

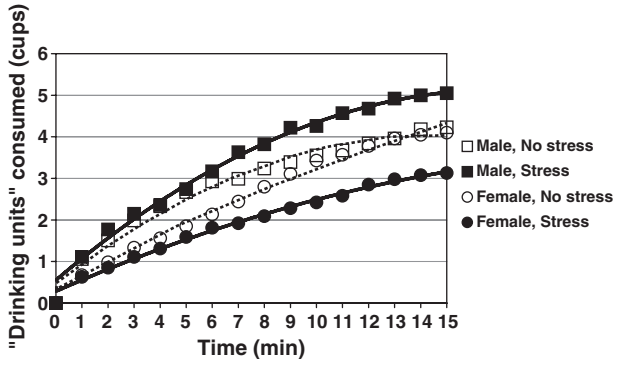

Fig. 3. Cumulative number of "drinking units" (cups; mean) that participants consumed in 1-min over the period of 15 min, during which time participants could consume alcohol freely. Values are given for males and females in the stress and non-stress condition.

4. Discussion

The stress induction procedure was successful in increasing anxiety and tension in the sample of heavy non-dependent alcohol drinkers employed in the present study. The effects of the stressor appeared to be long lasting since more than 1 h after the initiation of the stressful procedure anxiety and tension were still significantly higher than baseline in the stressed group. In the stressful condition cortisol levels also did not show the expected diurnal decrease, which was observed in the non-stressful condition. Contrary to the findings from Kirschbaum et al. (1992) that men show greater cortisol response to psychosocial stressors than women, no gender differences in cortisol responsiveness were observed in this study. However, since heavy alcohol use has been found to impair cortisol responsiveness to stress (Errico et al., 2002; Lovallo et al., 2000; both studies looked at abstinent alcoholics), it is possible that heavy alcohol use in the sample of participants in the present study may have overshadowed any underlying gender differences in the HPA responsiveness to stress.

Craving for alcohol reflected in the factors mild as well as strong desire for alcohol increased over time in the presence of alcohol-related cues, but it was not differentially affected by stress. Lack of baseline measurements of craving in the present study does not allow us to be conclusive with regard to a stress effect on craving. However, no change in the craving ratings between the two groups following the alcohol cue presentation allows us to be conclusive with regard to a lack of interaction between cue reactivity and stress. Lack of a finding of stress in the present study contradicts the findings of Cooney and colleagues (Cooney et al., 1997) who reported in alcoholic

patients an increase in self-reported urge to drink following negative mood induction as well as following cue-exposure. Therefore lack of the effectiveness of stress in modulating craving for alcohol in the present study may be due to that participants were heavy social drinkers and not alcoholic patients. Such conclusion is in agreement with reports from the pre-clinical literature that stress exposure in the absence of cues reinstates alcohol seeking only in alcohol-dependent rats (Liu and Weiss, 2002). Furthermore, Koob and Le Moal's negative emotion alliesthesia model of drug dependence (Koob and Le Moal, 2001) proposes that with time alcoholics experience more negative mood that leads them to drink excessively.

Regarding the physiological skin conductance response, participants overall showed a significant increase in skin conductance with time of cue exposure, i.e. as they handled the cups containing their favourite type of drink and then as they smelled the drink. Such linear increase in skin conductance extends the findings from the cue-exposure study by Greeley et al. (1993) who observed a similar linear increase in subjective craving with increased duration of cue exposure in heavy social drinkers. In the absence of measurements in the presence of a control cue it is difficult to speculate that the increase in skin conductance observed during the alcohol cue trial or the gender-influenced increases in alcohol consumption following stress represent a conditioned response to alcohol related cue.

Nevertheless, skin conductance response to alcohol related cues was differentially affected by stress in male and female participants. Stressed female participants did not show an increase from baseline in their skin conductance response in the presence of alcohol-related cues as their non-stressed counterparts did, suggesting that stress had an inhibitory effect on the response to alcohol-related cues. Stressed females had lower skin conductance response than non-stressed females already at baseline before the cue exposure; this might reflect a differential response to stress. However, since there was no measurement of skin conductance before the stress procedure we cannot be certain. Interestingly alcohol consumption data further confirmed this conclusion, as stressed female participants showed also a lower initial rate of alcohol consumption compared to stressed male participants. Since this difference in consumption was reflected in the linear coefficient of the cumulative intake curve, which, according to Kissileff's interpretation of human feeding pattern (Kissileff et al., 1982) reflects motivation to consume as well as hedonic value of the food, it could be suggested that stress reduces motivation to consume and/or the hedonic value of the drinks in females compared to males. The gender differences observed in the present study appear to be in agreement with the report by Willner et al. (1998) who, using a negative mood induction procedure in social drinkers, observed increased motivation to obtain the alcohol reinforcer in males but not in females and reduced liking of the alcohol reinforcer in female but not in male social drinkers. It may be that female alcohol drinkers have more positive mental representations of the reinforcer than males, and thus experience of negative mood due to stress does not induce reinforcer approach behaviour since it does not match the positive appetitive state (Stewart et al., 1984). The suggestion that appetitive processes may have stronger influence on alcohol cue reactivity in female compared to male social drinkers is in line with the findings from the smoking literature that female but not male smokers respond to the presentation of smoking cues with an increase in cigarette craving (Field and Duka, 2004) and that olfactory and taste cues are an important determinant of satisfaction from smoking in female, but not male smokers (Perkins et al., 2001). The results of the present study therefore suggest that stress-induced negative mood may be acting by blocking the activation of appetitive processes in the presence of alcohol-related cues in female drinkers. On the other hand, subjective craving measure did not show an interaction between gender and stress manipulation, therefore this interpretation of the physiological and behavioral data needs to be taken with caution. Further studies on gender and stress effects on alcohol cue reactivity are required in order to understand fully the gender differences found in the present study. It should be pointed out however that gender effects with regard to cue reactivity might be different in alcoholics and social drinkers. For instance, the studies of Willner et al. (1998) and Cooper et al. (1992), as well as the present study, all examining social drinkers, point out to a stronger relationship between drinking and stress in men. Yet female alcoholics are more likely to use alcohol for the purpose of altering mood, and they show greater cue reactivity after negative mood induction compared to their male counterparts (Rubonis et al., 1994).

There are some limitations to the present study with regard to alcohol consumption measurements. Firstly, as it was not placebo-controlled, it is not known if the observed effects of stress on behavioural cue reactivity are selective for alcohol. de Wit et al. (2003) did not report any selective enhancement of alcohol consumption compared to placebo following a mild stressor in moderate social drinkers. Secondly, participants in the present study knew that they would consume alcohol later in the testing session and this may have influenced subjective ratings of craving. Furthermore, the observed effects on alcohol consumption may have been influenced by participants' awareness that they were being observed and evaluated, since this factor has been suggested to alter consumption (Clements et al., 1996). Also, the time of day when participants took part in the experiment is not the usual time people go drinking (evening) so they may have been restrained from rapid consumption by extra-experimental factors, such as plans for the afternoon (Clements et al., 1996). Another possible limitation of the present findings is that there was a wide range of habitual alcohol consumption in the study sample, which might have confounded some of the findings. However, there were no gender differences between the groups with regard to habitual consumption of alcohol.

In summary, alcohol cue reactivity was observed in a sample of heavy social drinkers, yet neither craving (in the absence or in the presence of cues) nor physiological cue-reactivity and alcohol consumption appeared to be influenced by stressful manipulation in the experimental sample as a whole. However, under stress female participants did not show physiological cue reactivity in the presence of alcohol-related cues and showed lower alcohol consumption compared to males under stress.

These findings suggest that female participants under stress show reduced sensitivity to the incentive value of alcohol-related cues.

References

Adinoff B, Junghanns K, Kiefer F, Krishnan-Sarin S. Suppression of the HPA axis stress-response: implications for relapse. Alcohol Clin Exp Res 2005; 29:1351–5.

Binz U, Wendt G. KUSTA: brief rating scale for mood and activation. Beltz Test, Beltz Test; 1990.

Brady KT, Sonne SC. The role of stress in alcohol use, alcoholism treatment, and relapse. Alcohol Res Health 1999;23:263–71.

Brown SA, Vik PW, McQuaid JR, Patterson TL, Irwin MR, Grant I. Severity of psychosocial stress and outcome of alcoholism treatment. J Abnorm Psychol 1990;99:344–8.

Clements K, Glautier S, Stolerman IP, White J-AW, Taylor C. Classical condition in humans: nicotine as CS and alcohol as US. Human Psychopharmacology 1996;11:85–95.

Coffey SF, Saladin ME, Drobes DJ, Brady KT, Dansky BS, Kilpatrick DG. Trauma and substance cue reactivity in individuals with comorbid posttraumatic stress disorder and cocaine or alcohol dependence. Drug Alcohol Depend 2002;65:115–27.

Cooney NL, Litt MD, Morse PA, Bauer LO, Gaupp L. Alcohol cue reactivity, negative-mood reactivity, and relapse in treated alcoholic men. J Abnorm Psychol 1997;106:243–50.

Cooper ML, Russell M, Skinner JB, Frone MR, Mudar P. Stress and alcohol use: moderating effects of gender, coping, and alcohol expectancies. J Abnorm Psychol 1992;101:139–52.

Davidson D, Tiffany ST, Johnston W, Flury L, Li TK. Using the cue-availability paradigm to assess cue reactivity. Alcohol Clin Exp Res 2003;27:1251–6.

Dawson DA, Archer L. Gender differences in alcohol consumption: effects of measurement. Br J Addict 1992;87:119–23.

de Wit H, Soderpalm AH, Nikolayev L, Young E. Effects of acute social stress on alcohol consumption in healthy subjects. Alcohol Clin Exp Res 2003; 27:1270–7.

Drummond DC. What does cue-reactivity have to offer clinical research? Addiction 2000;95(Suppl 2):S129–44.

Errico AL, King AC, Lovallo WR, Parsons OA. Cortisol dysregulation and cognitive impairment in abstinent male alcoholics. Alcohol Clin Exp Res 2002;26:1198–204.

Fahlke C, Engel JA, Eriksson CJ, Hard E, Soderpalm B. Involvement of corticosterone in the modulation of ethanol consumption in the rat. Alcohol 1994a;11:195–202.

Fahlke C, Hard E, Thomasson R, Engel JA, Hansen S. Metyrapone-induced suppression of corticosterone synthesis reduces ethanol consumption in high-preferring rats. Pharmacol Biochem Behav 1994b;48:977–81.

Fahlke C, Hard E, Eriksson CJ, Engel JA, Hansen S. Consequence of long-term exposure to corticosterone or dexamethasone on ethanol consumption in the adrenalectomized rat, and the effect of type I and type II corticosteroid receptor antagonists. Psychopharmacology (Berl) 1995;117:216–24.

Field M, Duka T. Cues paired with a low dose of alcohol acquire conditioned incentive properties in social drinkers. Psychopharmacology (Berl) 2002; 159:325–34.

Field M, Duka T. Cue reactivity in smokers: the effects of perceived cigarette availability and gender. Pharmacol Biochem Behav 2004;78:647–52.

Greeley JD, Swift W, Prescott J, Heather N. Reactivity to alcohol-related cues in heavy and light drinkers. J Stud Alcohol 1993;54:359–68.

Health Do. The health of the nation—a strategy for health in England. London: HMSO; 1992.

Higgins RL, Marlatt GA. Fear of interpersonal evaluation as a determinant of alcohol consumption in male social drinkers. J Abnorm Psychol 1975; 84:644–51.

Jansma A, Breteler MH, Schippers GM, de Jong CA, Van Der Staak CF. No effect of negative mood on the alcohol cue reactivity of in-patient alcoholics. Addict Behav 2000;25:619–24.

Kaplan RF, Cooney NL, Baker LH, Gillespie RA, Meyer RE, Pomerleau OF. Reactivity to alcohol-related cues: physiological and subjective responses in alcoholics and nonproblem drinkers. J Stud Alcohol 1985;46:267–72.

Kirschbaum C, Wust S, Hellhammer D. Consistent sex differences in cortisol responses to psychological stress. Psychosom Med 1992;54:648–57.

Kirschbaum C, Pirke KM, Hellhammer DH. The 'Trier Social Stress Test'—a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 1993;28:76–81.

Kirschbaum C, Prussner JC, Stone AA, Federenko I, Gaab J, Lintz D, et al. Persistent high cortisol responses to repeated psychological stress in a subpopulation of healthy men. Psychosom Med 1995;57:468–74.

Kirschbaum C, Kudielka BM, Gaab J, Schommer NC, Hellhammer DH. Impact of gender, menstrual cycle phase, and oral contraceptives on the activity of the hypothalamus–pituitary–adrenal axis. Psychosom Med 1999; 61:154–62.

Kissileff HR, Thornton J, Becker E. A quadratic equation adequately describes the cumulative food intake curve in man. Appetite 1982;3:255–72.

Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 2001;24:97-129.

Liu X, Weiss F. Additive effect of stress and drug cues on reinstatement of ethanol seeking: exacerbation by history of dependence and role of concurrent activation of corticotropin-releasing factor and opioid mechanisms. J Neurosci 2002;22:7856–61.

Lovallo WR, Dickensheets SL, Myers DA, Thomas TL, Nixon SJ. Blunted stress cortisol response in abstinent alcoholic and polysubstance-abusing men. Alcohol Clin Exp Res 2000;24:651–8.

Love A, James D, Willner P. A comparison of two alcohol craving questionnaires. Addiction 1998;93:1091–102.

Marlatt GA. A cognitive-behavioural model of the relapse process; 1979.

McNair D, Lorr M, Droppleman L. Profile of Mood States, manual, San Diego; 1971.

Mehrabian A, Russell JA. A questionnaire measure of habitual alcohol use. Psychol Rep 1978;43:803–6.

Miller PM, Hersen M, Eisler RM, Hilsman G. Effects of social stress on operant drinking of alcoholics and social drinkers. Behav Res Ther 1974;12:67–72.

Pelham WE, Lang AR, Atkeson B, Murphy DA, Gnagy EM, Greiner AR, et al. Effects of deviant child behavior on parental distress and alcohol consumption in laboratory interactions. J Abnorm Child Psychol 1997; 25:413–24.

Perkins KA, Gerlach D, Vender J, Grobe J, Meeker J, Hutchison S. Sex differences in the subjective and reinforcing effects of visual and olfactory cigarette smoke stimuli. Nicotine Tob Res 2001;3:141–50.

Piazza PV, Le Moal M. Glucocorticoids as a biological substrate of reward: physiological and pathophysiological implications. Brain Res Brain Res Rev 1997;25:359–72.

Piazza PV, Le Moal M. The role of stress in drug self-administration. Trends Pharmacol Sci 1998;19:67–74.

Pohorecky LA. Stress and alcohol interaction: an update of human research. Alcohol Clin Exp Res 1991;15:438–59.

Rubonis AV, Colby SM, Monti PM, Rohsenow DJ, Gulliver SB, Sirota AD. Alcohol cue reactivity and mood induction in male and female alcoholics. J Stud Alcohol 1994;55:487–94.

Saladin ME, Drobes DJ, Coffey SF, Libet JM. The human startle reflex and alcohol cue reactivity: effects of early versus late abstinence. Psychol Addict Behav 2002;16:98-105.

Schulze D, Jones BT. The effects of alcohol cues and an alcohol priming dose on a multi-factorial measure of subjective cue reactivity in social drinkers. Psychopharmacology (Berl) 1999;145:452–4.

Schulze D, Jones BT. Desire for alcohol and outcome expectancies as measures of alcohol cue-reactivity in social drinkers. Addiction 2000;95:1015–20.

Sinha R, O'Malley SS. Craving for alcohol: findings from the clinic and the laboratory. Alcohol Alcohol 1999;34:223–30.

Sinha R, Talih M, Malison R, Cooney N, Anderson GM, Kreek MJ. Hypothalamic-pituitary-adrenal axis and sympatho-adreno-medullary responses during stress-induced and drug cue-induced cocaine craving states. Psychopharmacology (Berl) 2003;170:62–72.

Staiger PK, White JM. Cue reactivity in alcohol abusers: stimulus specificity and extinction of the responses. Addict Behav 1991;16:211–21.

Stewart J, de Wit H, Eikelboom R. Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. Psychol Rev 1984;91:251–68.

Stormark KM, Laberg JC, Bjerland T, Nordby H, Hugdahl K. Autonomic cued reactivity in alcoholics: the effect of olfactory stimuli. Addict Behav 1995; 20:571–84.

Walitzer KS, Sher KJ. Alcohol cue reactivity and ad lib drinking in young men at risk for alcoholism. Addict Behav 1990;15:29–46.

Wendt G, Binz U, Muller AA. KUSTA (Kurz-Skala Stimmung/Aktivierung): a daily self-rating scale for depressive patients. Pharmacopsychiatry 1985; 18:118–22.

Willner P, Field M, Pitts K, Reeve G. Mood, cue and gender influences on motivation, craving and liking for alcohol in recreational drinkers. Behav Pharmacol 1998;9:631–42.

Wolffgramm J, Heyne A. Social behavior, dominance, and social deprivation of rats determine drug choice. Pharmacol Biochem Behav 1991;38:389–99.

Wood PJ, Kilpatrick K, Barnard G. New direct salivary cortisol and cortisone assays using the DELFIA system. ACB National Meeting Focus; 1997. p. 86.