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Serum NPY and BDNF response to a behavioral stressor in alcohol-dependent and healthy control participants

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Abstract

Rationale—Neuropeptide Y (NPY) and brain-derived neurotrophic factor (BDNF) have been implicated in both the stress response and alcohol addiction. However, few studies have assessed the NPY and BDNF response to stress in alcohol-dependent participants and the concurrent measure of NPY and BDNF has not been reported in human participants.

Objective—The purpose of this study was to concurrently assess serum NPY and BDNF, as well as adrenocorticotropin (ACTH) and cortisol, in control and race- and aged-matched abstinent alcohol-dependent participants in response to a stress-inducing public-speaking task.

Methods—Basal and post-stress serum values of NPY and BDNF, as well as ACTH and cortisol, were assessed in 14 abstinent alcohol-dependent and ten healthy control male participants.

Results—Basal measures were stable over short periods of time and stress induced a significant increase in both NPY ($p=0.002$) and BDNF ($p=0.006$) as well as ACTH ($p<0.001$) and cortisol ($p<0.007$). Alcohol-dependent and control groups did not significantly differ on any basal or stress-induced measure. Basal and delta responses of NPY and BDNF were not significantly correlated, and delta peak responses of NPY and BDNF did not correlate with one another or with their respective ACTH and cortisol responses.

Conclusions—These findings reveal that both serum NPY and BDNF are responsive to behavioral stressors, although their regulatory mechanisms appear to differ from one another and those of the hypothalamic–pituitary–adrenal axis. Differences in basal and stress-induced responses of NPY and BDNF were not supported between control and abstinent alcohol-dependent subjects.

Keywords

Brain-derived neurotrophic factor; Neuropeptide Y; Adrenocorticotrophic hormone; Cortisol; Stress; Psychological; Alcoholism

Introduction

Neuropeptide Y (NPY) and brain-derived neurotrophic factor (BDNF) provide protective and neuroadaptive functions within the central nervous system (CNS). Both neuropeptides have been implicated in the modulation of stress response. NPY (or NPY mRNA) is co-released with norepinephrine (Perkins et al. 2009), is anxiolytic (Heilig et al. 1993; Yang and Gaydos 2010), and is altered following both acute (Thorsell et al. 1998) and chronic stress (Thorsell et al. 1999) in rodents. Acute stressors also elicit variable loci-dependent changes in the central expression of BDNF, although the direction and region of these changes varies across paradigms (Acuna et al. 2010; Adlard et al. 2004; Requena et al. 2010; Shi et al. 2010). Hippocampal BDNF, for example, is higher in rats exhibiting higher rates of voluntary exercise (Johnson et al. 2003) and are increased following 1 week of exercise (Adlard et al. 2004).

The role of NPY and BDNF in humans, however, has been complicated by difficulties in assessing neuropeptide functioning centrally, the variable permeability of the blood–brain barrier to neuropeptides, and the peripheral production of NPY and BDNF (Fujimura et al.

2002). Studies assessing basal or provoked measures of NPY or BDNF in humans generally support the relationship between NPY/BDNF and stress, although the data are limited. Serial cerebrospinal fluid concentrations of NPY are decreased in participants with post-traumatic stress disorder (Sah et al. 2009) and cortisol and NPY positively correlate with stress in participants immediately and 24 h following a physical and psychological stressor (Morgan et al. 2002). Intense (Rojas Vega et al. 2006) and moderate (Gold et al. 2003) exercise also increase serum BDNF in healthy humans. These studies support the relationship between NPY/BDNF and stress, although the data are limited.

Given the established connection between stress and NPY/BDNF, coupled with the role of stress in both the development and persistence of alcohol dependence, the relationship between these neuropeptides and alcohol intake has been an area of significant interest. In rodents, acute (Kinoshita et al. 2000) and chronic (Goldstone et al. 2009; Roy and Pandey 2002) alcohol intake decreases central NPY expression. Following the cessation of chronic alcohol intake, NPY levels are altered for at least 72 h (Goldstone et al. 2009). The administration of NPY decreases alcohol reinstatement in rodents both with (Cippitelli et al. 2010) and without (Gilpin et al. 2003) exposure to a stressor. Furthermore, intrathecal NPY administration suppressed anxiety-like and arousal behaviors in rats bred for high alcohol preference (Gilpin et al. 2011). Infusion of NPY into the central amygdala produced the same effect and reduced alcohol intake (Zhang et al. 2010). Genetic variation in the NPY promoter regions also appears to affect the interplay between stress and alcohol intake. Rhesus macaques with the -1002T>G variant of NPY promoter demonstrated higher levels of arousal and increased alcohol consumption in response to a stressor (Lindell et al. 2010). Despite this provocative literature, to our knowledge serum NPY has not been assessed in alcohol-dependent subjects.

Variable loci-dependent, including striatal (McGough et al. 2004) and hippocampal (Tapia-Arancibia et al. 2001), changes in central BDNF expression also occur following chronic alcohol intake. In mice, BDNF administered into the dorsolateral striatum attenuated responding for ethanol (Jeanblanc et al. 2009) whereas the inhibition of BDNF expression increases ethanol self-administration (Jeanblanc et al. 2009). In alcohol-dependent participants, measures of serum or plasma BDNF concentrations have been mixed. Huang et al. (2008a) reported serum BDNF levels higher at 1 day relative to 7 days abstinence (although neither time point was significantly different than controls), Lee et al. (2009) found elevated plasma BDNF levels at 1 day abstinence relative to controls, and Joe et al. (2007) reported lower plasma BDNF concentrations at 30 days abstinence relative to controls. No difference in serum BDNF was found by Heberlein et al. (2010) between 1, 7, and 14 days of abstinence or between alcohol-dependent subjects and healthy controls and Umene-Nakano et al. (2009) reported similar null findings in depressed subjects with and without alcohol dependence. Both positive (Huang et al. 2008b) and negative (Heberlein et al. 2010) correlations between BDNF concentrations and alcohol withdrawal severity have been observed. Although no association between the BDNF Val66Met (G196A) polymorphism and alcohol dependence has been observed (Matsushita et al. 2004; Muschler et al. 2011; Tsai et al. 2005), this polymorphism has been related to alcohol relapse (Wojnar et al. 2009).

Thus, there exists a complex interplay between NPY/ BDNF, stress, and alcohol intake. Despite the preponderance of preclinical studies supporting a role for NPY and BDNF in the stress response, the peripheral response of these measures have received limited attention in clinical investigations. The goal in the present study was to (1) assess the response of NPY and BDNF to a psychological stressor and (2) determine if there were differences in basal and stress-induced changes in serum NPY/BDNF in abstinent alcohol-dependent participants relative to controls. We utilized the Trier Social Stress Task (Kirschbaum et al. 1993), a standardized stress protocol shown to induce subjective and physiological stress responses. Participants were a subset of a larger sample participating in a study assessing alterations in stress reactivity in alcohol-dependent participants.

Methods

Alcohol-dependent participants

Fourteen male alcohol-dependent participants were recruited from patients in residential treatment for alcohol dependence at the Dallas VA Medical Center and Homeward Bound, Inc. Patients met Diagnostic and Statistical Manual of Mental Disorders– Version IV (DSM-IV) criteria for alcohol dependence, reported alcohol as their primary drug of choice and heavy drinking for at least 90 days prior to admission, and were between 4 and 6 weeks abstinent at the time of the study. Exclusion criteria included other active mood, anxiety (except PTSD), psychotic, eating, and cognitive DSM-IV Axis I disorders not associated with alcohol use, major medical disorders or medical disorders affecting the hypothalamic–pituitary–adrenal (HPA) axis or autonomic nervous system, body mass index of ≥ 30 , major head trauma, use of any medications that may interfere with HPA axis or autonomic nervous system functioning [e.g., psychotropics (including benzodiazepines, antidepressants, antipsychotics, mood stabilizers, anxiolytics, or beta blockers), anti-hypertensives (except hydrochlorothiazide), or hypoglycemic agents] within 2 weeks of the study.

Healthy controls

Ten male controls reported no lifetime history of a DSM-IV Axis I substance dependence disorder (except nicotine) or an active non-substance use disorder. Other inclusion/exclusion criteria were similar to that of alcohol-dependent participants. Controls with a single first-degree or two second-degree relatives with substance dependence disorder were excluded.

Clinical assessment

After a complete description of the study was provided to the participants, consent was obtained. Participants were financially compensated for their participation. Study approval was obtained from the Institutional Review Board of the University of Texas Southwestern Medical Center at Dallas and the VA North Texas Health Care System. Participants obtained a medical exam, routine laboratory testing, electrocardiogram, and urine drug screens. Axis I disorders were assessed using the Structured Clinical Interview for DSM-IV Lifetime (First et al. 1996). Depressive symptoms were assessed with the Beck Depression Inventory (BDI) (Beck et al. 1979) and anxiety symptoms with the State Trait Anxiety Inventory (STAI) (Spielberger 1971). The Drinker Inventory of Consequences–Lifetime Consequences (Miller et al. 1995) assessed lifetime severity of alcohol-related problems. A

Time-Line Follow Back (Sobell and Sobell 1978) assessed 90-day and lifetime drinking history. Alcohol-dependent participants were housed on a residential treatment unit until studies were initiated.

Trier social stressor task

Participants arrived at 1700 h and were provided dinner. An intravenous catheter was inserted at 1800 h, and the Brief Symptom Inventory (BSI) (Derogatis and Melisaratos 1983) was administered 45 min later. The BSI uses 53 questions to assess cognitive, sensory, and affective changes. Nine symptoms clusters are measured as well as three global measures (Positive Symptom Distress Index (*severity* of response), positive symptom total (*frequency* of response; PST), and Global Severity Index (*severity frequency* of response; GSI). The task was begun at 1900 h. The Trier Social Stress Test (TSST), performed in front of a male and female confederate, involved a 5-min preparation period for a 5-min public-speaking task, followed by an arithmetic task for an additional 5 min. Participants were then asked “How stressful did you experience the experiment?” (scored 1 to 5). Basal blood samples were taken at 1845 and 1855 h followed by post-TSST samples at 1920, 1930, 1940, 1950, 2000, and 2010 h. Preliminary analyses of all time points in four individuals (two controls and two patients) revealed peak NPY and BDNF responses at 1920 and 1930 h. Due to the expense of the NPY and BDNF assays, subsequent assays were only performed for the 1845, 1855, 1920, and 1930 h samples in order to assess basal and peak concentrations. Results below include the relevant samples from the initial four subjects.

Bioassays

Serum BDNF and NPY were collected in redtopped vacutainer tubes and kept at room temperature for 30 min following collection (allowing coagulation). Plasma adrenocorticotropin (ACTH) and cortisol were collected in purple-topped vacutainer tubes with EDTA and were processed immediately following collection. Samples were then centrifuged (2,500, 4°C, 10 min). Samples were placed in cryovials and frozen at –80°C until assayed. NPY was measured by radioimmunoassay (ALPCO Diagnostics, Salem, NH). Calibrators were 9.4 to 300 pmol/L, the lowest detectable concentration was 3 pmol/L, and intra- and inter-assay variability was 4.5–9.2%. BDNF was measured using a sandwich ELISA kit (ChemiKine, Millipore Corp.). Calibrators were 7.8 to 500 pg/mL, the lowest detectable concentration was 7.8 pg/mL, and intra- and inter-assay variability was between 3.7% and 8.5%. ACTH was measured using an ELISA kit (Biomerica, Newport Beach, CA). Calibrators were 7 to 535 pg/mL, the lowest detectable concentration was 0.46 pg/mL, and intra- and inter-assay variability was 3.1–6.2%. Cortisol was measured using an EIA kit (Diagnostic Systems Labs, Webster, TX). Calibrators were 0.5–60 µg/dL, the lowest detectable concentration was 0.1 µg/dL, and intra- and inter-assay variability was 2.4–12%. Samples were diluted 1:50 to 1:100.

Statistical analysis

Comparisons between basal measures were assessed with paired *t* tests. Repeated measures analysis of variance assessed change over time (pre- to peak post-stress response) and group (control vs. alcohol dependent) interactions, followed by post hoc *t* tests to determine group

differences in basal, peak, and delta (peak– basal) concentrations. Analyses of covariance were used to assess the potential influence of BSI scores on the BDNF and NPY comparisons. The relationship between basal and delta () peak response was assessed with Pearson's r ($p<0.01$). There were missing BDNF values for one control and one patient and NPY values for one patient, so these participants were not included in the respective analysis. Raw BSI scores were utilized.

Results

Demographics and clinical characteristics (Table 1)

Control and alcohol-dependent patients did not differ in race, employment status, and age, although alcohol-dependent participants had fewer years of education and were more likely to be smokers. The patient group also had higher BDI and STAI (Spielberger 1971) scores compared with controls. Liver enzymes (aspartate aminotransferase and alanine aminotransferase) were not significantly different between groups. Three patients had active co-morbid substance dependence; one with cocaine dependence, one with marijuana dependence, and one with cocaine, amphetamine, and cannabis dependence. Two patients had PTSD.

TSST-induced stress

Both groups found the public-speaking task equally stressful, based upon both subjective and biological measures. Both alcohol-dependent (4.0 ± 1.2) and control (4.0 ± 0.5) groups endorsed marked subjective stress ($t=0.00$, $df=17.7$, $p=1.00$). A significant time effect (mean basal vs. peak) was observed for both ACTH ($F=14.34$, $p=0.001$) and cortisol ($F=9.02$, $p=0.007$), with no significant group $\times$ time effect for either hormone (ACTH: $F=0.06$, $p=0.81$; cortisol: $F=0.32$, $p=0.58$) (see Fig. 1).

Basal and post-stress response of NPY and BDNF (Table 2; Fig. 1)

No significant differences were evident between the two basal measures of NPY ($t=0.33$, $df=23$, $p=0.97$) or BDNF ($t=0.43$, $df=18$, $p=0.66$), revealing short-term stability of the neuropeptides at rest. Thus, the average of both basal measures was used for subsequent analyses. Slightly more subjects showed higher net peak concentrations for NPY and BDNF at the second (1930 h), relative to the first (1920 h), post-TSST time point (NPY, 12 of 23 subjects (52%); BDNF, 14 of 22 subjects (64%).

A significant time effect (pre- vs. post-stress) was observed for both NPY ($F=13.22$, $p=0.002$) and BDNF ($F=9.50$, $p=0.006$), with no significant group (NPY: $F=26$, $p=0.61$; BDNF: $F=0.18$, $p=0.67$) or group $\times$ time (NPY: $F=0.87$, $p=0.36$; BDNF: $F=0.00$, $p=0.99$) effect for either hormone. Post hoc comparisons revealed no significant basal, peak, or group differences of serum NPY or BDNF concentrations.

Mood and anxiety symptoms (Table 3)

Although median BSI scores of both groups tended to be low at baseline, alcohol-dependent subjects scored significantly higher on somatization, obsessive–compulsive, depression, anxiety, and psychoticism as well as the GSI and PST global scores. As BDNF and NPY

have been related to depressive and/or anxiety symptoms (Duncan et al. 2009; Hashimoto 2007; Shimizu et al. 2003), the global score PST was considered as a covariate in the BDNF- and NPY-repeated measures and post hoc *t* test comparisons. (PST was chosen as it demonstrated the greatest difference between groups. Only one covariate was assessed due to the relatively small sample size). The time effect for NPY no longer reached significance ($F=3.41$, $p=0.08$); the time effect for BDNF persisted ($F=4.63$, $p=0.046$). No significant group differences over time were revealed. No significant group differences became evident in the post hoc comparisons (baseline, peak, and peak) when PST was considered as a covariate. In addition, correlations between baseline, peak, and peak with PST did not yield significant relationships in the combined sample.

Relationship between NPY, BDNF, ACTH, and cortisol

There were no significant relationships between basal and delta peak concentrations of NPY, BDNF, ACTH, or cortisol in either group. Within-group peak responses were significantly correlated for ACTH and cortisol (control: $r=0.92$, $n=10$, $p<0.001$; alcohol dependent: $r=0.98$, $n=13$, $p<0.001$), but no other neuropeptide/hormone peak response relationships were evident. Since almost all (83%) participants endorsed high subjective stress (four or five), an association between subjective stress and neuropeptides increase was not assessed.

Discussion

To our knowledge, this is the first published study to assess (1) the concurrent NPY and BDNF (as well as ACTH and cortisol) response to stress, (2) two consecutive basal measures of NPY and BDNF, (3) NPY in alcohol-dependent participants, and (4) the NPY and BDNF responses to stress in alcohol-dependent participants. The findings demonstrate that both serum NPY and BDNF concentrations significantly increase in response to an acute psychosocial stressor, although these changes were somewhat mitigated by baseline mood and anxiety symptoms. Similar basal measures suggest relative stability of NPY and BDNF over short periods of time. Neither basal nor peak measures of NPY or BDNF differed between control and 4–6-week abstinent alcohol-dependent participants, suggesting either a resistance to alcohol-induced modulation or a normalization by 4 weeks abstinence.

The NPY and BDNF responses to stress did not significantly correlate with each other or with the ACTH or cortisol response. These findings may suggest different sources, secretory dynamics, and/or regulatory mechanisms for NPY, BDNF, and pituitary–adrenal responses. NPY is co-released with norepinephrine from peripheral sympathetic neurons (Perkins et al. 2009), so the increase following stress is not unexpected. Other peripheral sources of NPY include platelets (Myers et al. 1993) and adipocytes (Yang et al. 2008), although their relevance to stress-induced NPY is unknown. Increases in plasma NPY following US Navy survival school (Morgan et al. 2002) and public speaking also suggest NPY responsivity to variable levels of stress. Peripheral sources of BDNF include platelets (Fujimura et al. 2002), peripheral blood mononuclear cells (Lee and Kim 2010), and vascular cells (Scarisbrick et al. 1993), although the mechanism of BDNF release during stress has not been identified. Our present findings, coupled with those observed following exercise (Gold et al. 2003; Rojas Vega et al. 2006), suggest that BDNF increases in response to both

physical and psychological stressors and can occur within 20-min post-stress. Although the time required to release BDNF during a stressor is unknown, our findings suggest that 20 min post-stress initiation is sufficient for BDNF to be released into the vasculature and taken up into the platelets. Presumably, BDNF is subsequently released into the serum from platelets during the coagulation process (Karege et al. 2005).

Our null group basal BDNF findings are similar to those of others (Heberlein et al. 2010; Huang et al. 2008b; Umene-Nakano et al. 2009). Although the higher BDNF concentrations reported by Lee et al. (2009) in alcohol-dependent subjects may have been due to the presence of withdrawal, Joe et al. (2007) found lower BDNF levels in a 30-day abstinent, healthy population of alcohol-dependent subjects similar to ours. Possible explanations for these differences include the use of fasting, morning plasma measures by Joe et al. (2007) compared with the present sample of post-prandial, evening serum levels, particularly as BDNF has been shown to exhibit a diurnal cycle similar to cortisol (Begliomini et al. 2008). In addition, reports of basal BDNF concentrations have fluctuated markedly between laboratories (Heberlein et al. 2010; Huang et al. 2008b; Joe et al. 2007; Umene-Nakano et al. 2009), suggesting differences in assay techniques. The serum BDNF concentrations reported in this manuscript are within the range usually observed in previous studies (Karege et al. 2005).

Unexpectedly, our alcohol-dependent population did not demonstrate a muted ACTH or glucocorticoid response to the behavioral stressor relative to controls, as has been reported following public-speaking stressors (Junghanns et al. 2003; King et al. 2006; Lovallo et al. 2000), exercise (Coiro et al. 2007), cold pressor and mental arithmetic (Errico et al. 1993), and isometric handgrip and public speaking (Bernardy et al. 2003). Other studies have also revealed a blunted ACTH and/or cortisol response in alcohol-dependent subjects administered various pharmacological stimuli (Adinoff et al. 2005; Coiro and Vescovi 1999; Wand and Dobs 1991). There is no straightforward explanation for the present finding. It is possible that the NPY and BDNF responses would have shown group differences if measured in a patient group demonstrating subdued ACTH and/or cortisol stress responses.

Strengths of the present study included concurrent measures of BDNF and NPY during a psychosocial stressor, documented by both subjective stress and ACTH/ cortisol measures. Controls were similar in age and race to the alcohol-dependent participants. Alcohol-dependent participants were post-withdrawal and not taking medications that interfered with CNS, HPA axis, or autonomic nervous system functioning. However, serum measures may have missed group differences that occurred following peak effects and the period of abstinence may have missed changes present earlier in the withdrawal process. Although the sample size was modest, the findings were relatively unequivocal. Although several studies have noted a relationship between nicotine use/administration and BDNF and NPY concentrations (Bhang et al. 2010; Chen et al. 2007; Frankish et al. 1995; Kim et al. 2007; Li et al. 2000), the unequal distribution of nicotine dependence between the control and alcohol-dependent groups would have tended to result in a Type I, rather than Type II, error. The alcohol-dependent subjects also endorsed higher mood and anxiety symptoms relative to controls, which appeared to contribute to the NPY and BDNF response. Thus, subjects

with greater mood and anxiety symptoms may show a heightened NPY and BDNF stress response. As only men were assessed, these findings may not be applicable to women.

These findings support the release of both peripheral NPY and BDNF during psychological stress yet suggest that distinct mechanisms underlie their release. Both peripheral NPY and BDNF are widely expressed, although their presence in certain tissues serve unknown functions and act via unclear mechanisms. Future research should be directed toward examining the physiological role of both neuropeptides in mediating their unique contributions to the acute stress response.

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References

- Acuna MJ, Martin JC, Graciani M, Cruces A, Gotor F. A comparative study of the sexual function of institutionalized patients with schizophrenia. *J Sex Med.* 2010; 7(10):3414–3423. [PubMed: 20456629]
- Adinoff B, Krebaum SR, Chandler PA, Ye W, Brown MB, Williams MJ. Dissection of hypothalamic-pituitary-adrenal axis pathology in 1-month-abstinent alcohol-dependent men, part 2: response to ovine corticotropin-releasing factor and naloxone. *Alcohol Clin Exp Res.* 2005; 29:528–537. [PubMed: 15834217]
- Adlard PA, Perreau VM, Engesser-Cesar C, Cotman CW. The timecourse of induction of brain-derived neurotrophic factor mRNA and protein in the rat hippocampus following voluntary exercise. *Neurosci Lett.* 2004; 363:43–48. [PubMed: 15157993]
- Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. *Arch Gen Psychiatry.* 1979; 4:561–571. [PubMed: 13688369]
- Begliuomini S, Lenzi E, Ninni F, Casarosa E, Merlini S, Pluchino N, Valentino V, Luisi S, Luisi M, Genazzani AR. Plasma brain-derived neurotrophic factor daily variations in men: correlation with cortisol circadian rhythm. *J Endocrinol.* 2008; 197:429–435. [PubMed: 18434373]
- Bernardy NC, King AC, Lavallo WR. Cardiovascular responses to physical and psychological stress in female alcoholics with transitory hypertension after early abstinence. *Alcohol Clin Exp Res.* 2003; 27:1489–1498. [PubMed: 14506411]
- Bhang SY, Choi SW, Ahn JH. Changes in plasma brain-derived neurotrophic factor levels in smokers after smoking cessation. *Neurosci Lett.* 2010; 468:7–11. [PubMed: 19850105]
- Chen H, Hansen MJ, Jones JE, Vlahos R, Bozinovski S, Anderson GP, Morris MJ. Regulation of hypothalamic NPY by diet and smoking. *Peptides.* 2007; 28:384–389. [PubMed: 17207894]
- Cippitelli A, Damadzic R, Hansson AC, Singley E, Sommer WH, Eskay R, Thorsell A, Heilig M. Neuropeptide Y (NPY) suppresses yohimbine-induced reinstatement of alcohol seeking. *Psychopharmacology (Berl).* 2010; 208:417–426. [PubMed: 20012021]
- Coiro V, Vescovi PP. Effect of cigarette smoking on ACTH/ cortisol secretion in alcoholic after short- and medium-term abstinence. *Alcohol Clin Exp Res.* 1999; 23:1515–1518. [PubMed: 10512318]
- Coiro V, Casti A, Jotti GS, Rubino P, Manfredi G, Maffei ML, Melani A, Volta E, Chiodera P. Adrenocorticotrophic hormone/cortisol response to physical exercise in abstinent alcoholic patients. *Alcohol Clin Exp Res.* 2007; 31:901–906. [PubMed: 17386066]

- Derogatis LR, Melisaratos N. The brief symptom inventory: an introductory report. *Psychol Med*. 1983; 13:595–605. [PubMed: 6622612]
- Duncan LE, Hutchison KE, Carey G, Craighead WE. Variation in brain-derived neurotrophic factor (BDNF) gene is associated with symptoms of depression. *J Affect Disord*. 2009; 115:215–219. [PubMed: 18842305]
- Errico AL, Parsons OA, King AC, Lavallo WR. Attenuated cortisol response to biobehavioral stressors in sober alcoholics. *J Stud Alcohol*. 1993; 54:393–398. [PubMed: 8341041]
- First, MH.; Spitzer, RL.; Gibbon, M.; Williams, JBW. Structured clinical interview for DSM-IV axis I disorders—patient edition (SCID-I/P, version 2.0). Biometrics Research Department, New York State Psychiatric Institute; New York: 1996.
- Frankish HM, Dryden S, Wang Q, Bing C, MacFarlane IA, Williams G. Nicotine administration reduces neuropeptide Y and neuropeptide Y mRNA concentrations in the rat hypothalamus: NPY may mediate nicotine's effects on energy balance. *Brain Res*. 1995; 694:139–146. [PubMed: 8974638]
- Fujimura H, Altar CA, Chen R, Nakamura T, Nakahashi T, Kambayashi J, Sun B, Tandon NN. Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. *Thromb Haemost*. 2002; 87:728–734. [PubMed: 12008958]
- Gilpin NW, Stewart RB, Murphy JM, Li TK, Badia-Elder NE. Neuropeptide Y reduces oral ethanol intake in alcohol-preferring (P) rats following a period of imposed ethanol abstinence. *Alcohol Clin Exp Res*. 2003; 27:787–794. [PubMed: 12766623]
- Gilpin NW, Henderson AN, Badia-Elder NE, Stewart RB. Effects of neuropeptide Y and ethanol on arousal and anxiety-like behavior in alcohol-preferring rats. *Alcohol*. 2011; 45:137–145. [PubMed: 20880660]
- Gold SM, Schulz KH, Hartmann S, Mladek M, Lang UE, Hellweg R, Reer R, Braumann KM, Heesen C. Basal serum levels and reactivity of nerve growth factor and brain-derived neurotrophic factor to standardized acute exercise in multiple sclerosis and controls. *J Neuroimmunol*. 2003; 138:99–105. [PubMed: 12742659]
- Goldstone AP, de Hernandez CG, Beaver JD, Muhammed K, Croese C, Bell G, Durighel G, Hughes E, Waldman AD, Frost G, Bell JD. Fasting biases brain reward systems towards high-calorie foods. *Eur J Neurosci*. 2009; 30:1625–1635. [PubMed: 19811532]
- Hashimoto K. BDNF variant linked to anxiety-related behaviors. *Bioessays*. 2007; 29:116–119. [PubMed: 17226799]
- Heberlein A, Muschler M, Wilhelm J, Frieling H, Lenz B, Groschl M, Kornhuber J, Bleich S, Hillemeier T. BDNF and GDNF serum levels in alcohol-dependent patients during withdrawal. *Prog Neuropsychopharmacol Biol Psychiatry*. 2010; 34:1060–1064. [PubMed: 20553781]
- Heilig M, McLeod S, Brot M, Heinrichs SC, Menzaghi F, Koob GF, Britton KT. Anxiolytic-like action of neuropeptide Y: mediation by Y1 receptors in amygdala, and dissociation from food intake effects. *Neuropsychopharmacology*. 1993; 8:357–363. [PubMed: 8099792]
- Huang MC, Chen CH, Liu SC, Ho CJ, Shen WW, Leu SJ. Alterations of serum brain-derived neurotrophic factor levels in early alcohol withdrawal. *Alcohol Alcohol*. 2008; 43:241–245. [PubMed: 18326550]
- Jeanblanc J, He DY, Carnicella S, Kharazia V, Janak PH, Ron D. Endogenous BDNF in the dorsolateral striatum gates alcohol drinking. *J Neurosci*. 2009; 29:13494–13502. [PubMed: 19864562]
- Joe KH, Kim YK, Kim TS, Roh SW, Choi SW, Kim YB, Lee HJ, Kim DJ. Decreased plasma brain-derived neurotrophic factor levels in patients with alcohol dependence. *Alcohol Clin Exp Res*. 2007; 31:1833–1838. [PubMed: 17850220]
- Johnson RA, Rhodes JS, Jeffrey SL, Garland T Jr, Mitchell GS. Hippocampal brain-derived neurotrophic factor but not neurotrophin-3 increases more in mice selected for increased voluntary wheel running. *Neuroscience*. 2003; 121:1–7. [PubMed: 12946694]
- Jungmanns K, Backhaus J, Tietz U, Lange W, Bernzen J, Wetterling T, Rink L, Driessen M. Impaired serum cortisol stress response is a predictor of early relapse. *Alcohol Alcohol*. 2003; 38:189–193. [PubMed: 12634269]

- Karege F, Bondolfi G, Gervasoni N, Schwald M, Aubry JM, Bertschy G. Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. *Biol Psychiatry*. 2005; 57:1068–1072. [PubMed: 15860348]
- Kim TS, Kim DJ, Lee H, Kim YK. Increased plasma brain-derived neurotrophic factor levels in chronic smokers following unaided smoking cessation. *Neurosci Lett*. 2007; 423:53–57. [PubMed: 17662528]
- King A, Munisamy G, de Wit H, Lin S. Attenuated cortisol response to alcohol in heavy social drinkers. *Int J Psychophysiol*. 2006; 59:203–209. [PubMed: 16359745]
- Kinoshita H, Jessop DS, Finn DP, Coventry TL, Roberts DJ, Ameno K, Ijiri I, Harbuz MS. Acute ethanol decreases NPY mRNA but not POMC mRNA in the arcuate nucleus. *Neuro-report*. 2000; 11:3517–3519.
- 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. [PubMed: 8255414]
- Lee BH, Kim YK. BDNF mRNA expression of peripheral blood mononuclear cells was decreased in depressive patients who had or had not recently attempted suicide. *J Affect Disord*. 2010; 125:369–373. [PubMed: 20207421]
- Lee BC, Choi IG, Kim YK, Ham BJ, Yang BH, Roh S, Choi J, Lee JS, Oh DY, Chai YG. Relation between plasma brain-derived neurotrophic factor and nerve growth factor in the male patients with alcohol dependence. *Alcohol*. 2009; 43:265–269. [PubMed: 19560628]
- Li MD, Kane JK, Parker SL, McAllen K, Matta SG, Sharp BM. Nicotine administration enhances NPY expression in the rat hypothalamus. *Brain Res*. 2000; 867:157–164. [PubMed: 10837809]
- Lindell SG, Schwandt ML, Sun H, Sparenborg JD, Bjork K, Kasckow JW, Sommer WH, Goldman D, Higley JD, Suomi SJ, Heilig M, Barr CS. Functional NPY variation as a factor in stress resilience and alcohol consumption in rhesus macaques. *Arch Gen Psychiatry*. 2010; 67:423–431. [PubMed: 20368518]
- 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; 24:651–658. [PubMed: 10832906]
- Matsushita S, Kimura M, Miyakawa T, Yoshino A, Murayama M, Masaki T, Higuchi S. Association study of brain-derived neurotrophic factor gene polymorphism and alcoholism. *Alcohol Clin Exp Res*. 2004; 28:1609–1612. [PubMed: 15547445]
- McGough NN, He DY, Logrip ML, Jeanblanc J, Phamluong K, Luong K, Kharazia V, Janak PH, Ron D. RACK1 and brain-derived neurotrophic factor: a homeostatic pathway that regulates alcohol addiction. *J Neurosci*. 2004; 24:10542–10552. [PubMed: 15548669]
- Miller, WR.; Tonigan, JS.; Longabaugh, R. The drinker inventory of consequences (DrInC) An instrument for assessing adverse consequences of alcohol abuse. National Institutes of Health; Rockville: 1995.
- Morgan CA 3rd, Rasmusson AM, Wang S, Hoyt G, Hauger RL, Hazlett G. Neuropeptide-Y, cortisol, and subjective distress in humans exposed to acute stress: replication and extension of previous report. *Biol Psychiatry*. 2002; 52:136–142. [PubMed: 12114005]
- Muschler MA, Heberlein A, Frieling H, Vogel N, Becker CM, Kornhuber J, Bleich S, Hillemacher T. Brain-derived neurotrophic factor, Val66Met single nucleotide polymorphism is not associated with alcohol dependence. *Psychiatr Genet*. 2011; 21:53–54. [PubMed: 20978454]
- Myers AK, Torres Duarte AP, Zukowska-Grojec Z. Immunore-active neuropeptide Y (NPY) in plasma and platelets of rat and mouse strains and human volunteers. *Regul Pept*. 1993; 47:239–245. [PubMed: 8234908]
- Perkins KA, Lerman C, Mercincavage M, Fonte CA, Briski JL. Nicotinic acetylcholine receptor beta2 subunit (CHRNA2) gene and short-term ability to quit smoking in response to nicotine patch. *Cancer Epidemiol Biomarkers Prev*. 2009; 18:2608–2612. [PubMed: 19755656]
- Requena J, Aranaz JM, Gea MT, Limon R, Miralles JJ, Vitaller J. [Evolution of the adverse effects prevalence related to healthcare in hospitals of the comunidad valenciana]. *Rev Calid Asist*. 2010; 25(5):244–249. [PubMed: 20488742]

- Rojas Vega S, Struder HK, Vera Wahrmann B, Schmidt A, Bloch W, Hollmann W. Acute BDNF and cortisol response to low intensity exercise and following ramp incremental exercise to exhaustion in humans. *Brain Res.* 2006; 1121:59–65. [PubMed: 17010953]
- Roy A, Pandey SC. The decreased cellular expression of neuropeptide Y protein in rat brain structures during ethanol withdrawal after chronic ethanol exposure. *Alcoholism, Clinical and Experimental Research.* 2002; 26:796–803.
- Sah R, Ekhtor NN, Strawn JR, Sallee FR, Baker DG, Horn PS, Geraciotti TD Jr. Low cerebrospinal fluid neuropeptide Y concentrations in posttraumatic stress disorder. *Biol Psychiatry.* 2009; 66:705–707. [PubMed: 19576571]
- Scarlsbrick IA, Jones EG, Isackson PJ. Coexpression of mRNAs for NGF, BDNF, and NT-3 in the cardiovascular system of the pre- and postnatal rat. *J Neurosci.* 1993; 13:875–893. [PubMed: 8441013]
- Shi SS, Shao SH, Yuan BP, Pan F, Li ZL. Acute stress and chronic stress change brain-derived neurotrophic factor (BDNF) and tyrosine kinase-coupled receptor (TrkB) expression in both young and aged rat hippocampus. *Yonsei Med J.* 2010; 51:661–71. [PubMed: 20635439]
- Shimizu E, Hashimoto K, Okamura N, Koike K, Komatsu N, Kumakiri C, Nakazato M, Watanabe H, Shinoda N, Okada S, Iyo M. Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biol Psychiatry.* 2003; 54:70–75. [PubMed: 12842310]
- Sobell MB, Sobell LC. Behavioral treatment of alcohol problems. Plenum Press, New York Speilberger CD (1971) Trait-state anxiety and motor behavior. *J Mot Behavior.* 1978; 3:265–279.
- Tapia-Arancibia L, Rage F, Givalois L, Digneon P, Arancibia S, Beauge F. Effects of alcohol on brain-derived neurotrophic factor mRNA expression in discrete regions of the rat hippocampus and hypothalamus. *J Neurosci Res.* 2001; 63:200–208. [PubMed: 11169630]
- Thorsell A, Svensson P, Wiklund L, Sommer W, Ekman R, Heilig M. Suppressed neuropeptide Y (NPY) mRNA in rat amygdala following restraint stress. *Regul Pept.* 1998; 75–76:247–254.
- Thorsell A, Carlsson K, Ekman R, Heilig M. Behavioral and endocrine adaptation, and up-regulation of NPY expression in rat amygdala following repeated restraint stress. *Neuroreport.* 1999; 10:3003–3007. [PubMed: 10549813]
- Tsai SJ, Liao DL, Yu YW, Chen TJ, Wu HC, Lin CH, Cheng CY, Hong CJ. A study of the association of (Val66Met) polymorphism in the brain-derived neurotrophic factor gene with alcohol dependence and extreme violence in Chinese males. *Neurosci Lett.* 2005; 381:340–343. [PubMed: 15896496]
- Umene-Nakano W, Yoshimura R, Ikenouchi-Sugita A, Hori H, Hayashi K, Ueda N, Nakamura J. Serum levels of brain-derived neurotrophic factor in comorbidity of depression and alcohol dependence. *Hum Psychopharmacol.* 2009; 24:409–413. [PubMed: 19548207]
- Wand GS, Dobs AS. Alterations in the hypothalamic-pituitary-adrenal axis in actively drinking alcoholics. *J Clin Endocrinol Metab.* 1991; 72:1290–1295. [PubMed: 2026749]
- Wojnar M, Brower KJ, Strobbe S, Ilgen M, Matsumoto H, Nowosad I, Sliwerska E, Burmeister M. Association between Val66-Met brain-derived neurotrophic factor (BDNF) gene polymorphism and post-treatment relapse in alcohol dependence. *Alcohol Clin Exp Res.* 2009; 33:693–702. [PubMed: 19170664]
- Yang Z, Gaydos LM. Reasons for and challenges of recent increases in teen birth rates: a study of family planning service policies and demographic changes at the state level. *J Adolesc Health.* 2010; 46:517–524. [PubMed: 20472207]
- Yang K, Guan H, Arany E, Hill DJ, Cao X. Neuropeptide Y is produced in visceral adipose tissue and promotes proliferation of adipocyte precursor cells via the Y1 receptor. *FASEB J.* 2008; 22:2452–2464. [PubMed: 18323405]
- Zhang H, Sakharkar AJ, Shi G, Ugale R, Prakash A, Pandey SC. Neuropeptide Y signaling in the central nucleus of amygdala regulates alcohol-drinking and anxiety-like behaviors of alcohol-preferring rats. *Alcohol Clin Exp Res.* 2010; 34:451–461. [PubMed: 20028368]

Fig. 1.

Mean $\pm$ SEM basal and peak plasma ACTH and cortisol and serum NPY and BDNF concentrations prior to and following a public-speaking stressor in alcohol-dependent (*circles*) and control (*squares*) participants. All hormones significantly increased in response to the stressor ($p < 0.01$). Basal, peak, and delta measures did not significantly differ between groups

Table 1

Demographic and clinical measures in healthy control and alcohol-dependent participants (mean±SD)

	Control (n =10)	Alcohol dependent (n=14)	t or χ^2	p Value
Age (years)	37.5±11.9	39.6±10.3	-0.5	NS
Race			2.2	NS
White	6	8		
African-American	3	5		
Hispanic	1	0		
Natural American/Alaskan	0	1		
Employed			3.7	NS
Full time	7	9		
Part time	1	0		
Unemployed	1	1		
Other	1	4		
Education (years)	16.0±1.3	11.9±1.7	6.7	0.001
Nicotine dependent	0	12	17.1	0.001
Cigarette per day	0	16.4±9.8	-6.3	0.001
Lifetime cigarette (years)	0	17.1±11.7	-5.5	0.001
AST (units/l)	21.8±4.8	29.3±17.0	-1.3	NS
ALT (units/l)	22.2±10.7	34.9±32.9	-1.1	NS
BDI	1.8±2.8	9.1±7.0	-3.5	0.001
STAI	26.4±4.3	39.6±12.5	-3.7	0.001
DrInC	6.5±8.7	37.4±5.2	-10.8	0.001
Years problem of drinking	0	18.9±8.7	-8.1	0.001
Days drank in past 90 days	9.6±10.5	76.8±17.7	-10.7	0.001
Number of drinks in past 90 days	20.5±17.3	1,425.2±909.9	-5.8	0.001
Lifetime days of drinks	974.6±999.7	6,962.3±3,596.2	-5.9	0.001
Number of lifetime drinks	4,793.8±6,720.2	88,994.4±45,489.9	-6.8	0.001

ALT aspartate aminotransferase, *AST* alanine aminotransferase

Table 2

Plasma ACTH and cortisol and serum NPY and BDNF measures in healthy control and alcohol-dependent participants (mean±SD)

	Control (n=10)	Alcohol dependent (n=14)	t value ²	df	p Value	Effect size (d)
ACTH (pg/ml)						
Baseline	17.77±7.88	14.16±4.87	1.39	22	0.18	0.5
Peak	37.81±16.98	37.30±31.81	0.05	21	0.96	0.02
Cortisol (µg/dl)						
Baseline	10.12±4.38	15.22±11.88	-1.47	17.51	0.16	0.6
Peak	24.20±23.82	24.82±21.22	-0.07	22	0.95	0.03
NPY (pmol/L)						
Baseline	65.48±20.62	75.32±36.70	-0.76	21	0.46	0.3
Peak	81.17±23.21	84.59±38.66	-0.25	21	0.8	0.1
BDNF (ng/ml)						
Baseline	11.76±5.05	12.69±4.79	-0.43	19	0.68	0.3
Peak	14.78±4.65	16.39±6.22	-0.65	19	0.52	0.3

Table 3

Baseline brief symptom inventory

BSI subscales	Controls		Alcohol dependent		<i>F</i> stat	<i>p</i> Value
	Mean (SD)	Median	Mean (SD)	Median		
Somatization	0.14±0.44	0.00	0.27±0.36	0.14	4.69	0.04
Obsessive–compulsive	0.17±0.31	0.00	0.83±0.84	0.42	5.61	0.03
Interpersonal sensitivity	0.05±0.16	0.00	0.46±0.88	0.00	2.13	0.16
Depression	0.07±0.12	0.00	0.56±0.74	0.25	4.36	0.05
Anxiety	0.07±0.12	0.00	0.51±0.62	0.33	4.94	0.04
Hostility	0.04±0.08	0.00	0.36±0.61	0.20	2.6	0.12
Phobic anxiety	0.06±0.13	0.00	0.14±0.43	0.00	0.35	0.56
Paranoid ideation	0.28±0.36	0.20	0.63±0.80	0.20	1.67	0.21
Psychoticism	0.06±0.10	0.00	0.53±0.66	0.30	4.85	0.04
GSI	0.09±0.11	0.05	0.50±0.55	0.28	5.29	0.03
PST	4.40±5.28	2.50	15.79±14.32	10.50	5.7	0.03
PSDI	0.66±0.46	0.93	0.73±0.24	0.71	0.24	0.62

PSDI Positive Symptom Distress Index, *PST* positive symptom total, *GSI* Global Severity Index