

# Hypercortisolism in Alcohol Dependence and Its Relation to Hippocampal Volume Loss\*

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**ABSTRACT. Objective:** The effects of hypercortisolism on hippocampal volume have not been studied in heavy drinkers. Prior work suggested increased hypothalamic-pituitary-adrenal activity in relation to lowered total hippocampus volume (THV) in heavy-drinking alcohol-dependent (AD) subjects. The present study hypothesized the following: (1) that chronic heavy-drinking subjects would demonstrate significantly higher salivary cortisol concentrations than light-drinking control subjects and (2) that data from the whole sample group would present an inverse relationship between cortisol concentration and THV. **Method:** In carefully selected test and control subject groups matched for age, gender, and ethnicity, we measured salivary cortisol samples at waking, waking + 30 minutes, noon, and 4 PM on the day of magnetic resonance imaging of the brain. We next compared mean cortisol concentrations between groups and assessed the statistical association between cortisol concentration and hippocampus volume measures. **Results:** Comparison of AD test subjects ( $n = 8$ ) and non-AD control subjects ( $n = 8$ ) found significantly higher cortisol concentrations at both

morning sampling times (mean [SD] at waking: 0.49 [0.23] vs 0.24 [0.14]  $\mu\text{g/dl}$ ,  $p = .012$ ; at waking + 30 minutes: 0.57 [0.37] vs 0.28 [0.11]  $\mu\text{g/dl}$ ,  $p = 0.043$ ). Controlling for intracranial volume, there was a significant inverse correlation between waking cortisol concentration and THV ( $p = .007$ ) in the total sample group ( $N = 16$ ). However, when analyzed separately, only the control group maintained a strong, inverse association ( $p = .025$ ). There was no association among the heavy drinking subjects. **Conclusions:** These early data in a small sample support the view that chronic heavy drinking results in high salivary cortisol concentrations. What remains unclear is whether hypercortisolism exerts a selectively injurious effect that results in observed hippocampus volume loss. Further research in larger groups using more frequent, monitored sampling must address the following: (1) whether this finding can be replicated and (2) if replicated, whether the lack of an association between low hippocampal volumes and high cortisol levels may indicate an extent of injury beyond which a normal association of the two may be lost. (*J. Stud. Alcohol* 67: 861-867, 2006)

EARLY STUDIES OF ALCOHOL USE and the hypothalamic-pituitary-adrenal (HPA) axis noted an increase in cortisol production among chronic heavy drinkers. This was manifested clinically by apparent adrenal insufficiency or a pseudo-Cushing's syndrome (Farmer and Fabre, 1975). In experimental conditions, chronic heavy drinkers demonstrated increased cortisol levels with ingestion of ethanol acutely and with greater increases in the early stages of

withdrawal (Mendelson et al., 1971). This finding has been confirmed recently in clinical settings (Adinoff et al., 2003; Frias et al., 2000; Kutscher et al., 2002).

The clinical implications of an alcohol-related cortisol elevation are not clear where the hippocampus is concerned. Research on the rodent hippocampus has shown it to be rich in glucocorticoid receptors, and current thought places these bilateral structures as central in the stress response. In vivo studies have shown that sustained cortisol elevation damages hippocampal neurons selectively, perhaps because of the high concentration of glucocorticoid receptors (McEwen et al., 1992; Martignoni et al., 1992; Sapolsky et al., 1990). This appears to be especially true for the pyramidal neurons of the CA3 region, an observation that has recently been replicated in primates (McMillan et al., 2004). Early animal data suggest that injury to this specific region may play a role in the memory disorders related to the heavy, chronic drinking characteristic of alcohol dependence (AD; Garcia-Moreno et al., 2001; Lukoyanov et al., 1999; Nelson et al., 1999). Additionally, recent research suggests a link between apparent cortisol-mediated hippocampal volume reductions and problems in memory and other cognitive processes (Eriksson et al., 2001; MacLulich et al., 2005; O'Brien et al., 2004; Vythilingam et al., 2004).

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In an early study of hippocampus volume (Beresford et al., 1999), we observed a significant decrease in total hippocampus volume (THV) measures among heavy-drinking subjects compared with light-drinking subjects. The current study sought to demonstrate that heavy chronic alcohol use—a potent stimulus to the HPA axis—(1) is associated with hypercortisolism that (2) may contribute to alcohol-related hippocampal injury. Two hypotheses guided this phase of investigation: (1) morning salivary cortisol concentrations would be higher in chronic, heavy-drinking AD subjects than in light-drinking non-AD control subjects; and (2) observed THV would be negatively associated with cortisol concentrations in the total subject group. We tested these hypotheses in the initial phase of a longitudinal study.

## Method

### *Subjects*

Both AD test subjects and non-AD control subjects were adult male veterans who were matched for age, gender, and ethnicity. AD heavy drinkers qualified for the study if they met all of the following criteria:

(1) chronic heavy drinking: consumed five or more standard drinks daily for at least 3 days weekly and 3 weeks monthly for at least 9 months of the previous year, as established through the Timeline Followback (TLFB; Sobell et al., 1979) interview;

(2) recent continuous heavy drinking: consumed five or more standard drinks daily for at least 3 days weekly for the past 30 days, as established through the TLFB;

(3) AD diagnosis: fulfilled Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV; American Psychiatric Association, 1994), criteria for AD as established through the Structured Clinical Interview for DSM-IV (SCID) Axis I disorders interview (Kessler et al., 2004; Peters et al., 1998; Sbrana et al., 2003; Ventura et al., 1998).

Similarly, non-AD light-drinking comparison subjects presented TLFB histories of the following:

(1) less than two standard drinks daily for no more than 3 days weekly, 4 weeks monthly for 9 months or less during the previous year;

(2) less than two standard drinks daily for no more than 3 days weekly for the previous 30 days; and

(3) none fulfilled DSM-IV criteria, either present or lifetime, for AD at the SCID interview.

Candidates were excluded from the study for SCID-verified psychiatric illnesses: schizophrenia, major depressive disorder, bipolar disorder, posttraumatic stress disorder, or polysubstance dependence. Systemic physical illness excluded those with any liver disease history, bilirubin above 1.2 mg/ml, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) above 200 U/L, alcohol amnestic syn-

drome history, HIV seropositivity, history of head injury resulting in loss of consciousness, seizure-disorder history including those caused by ethanol withdrawal, blood evidence of folate or vitamin B<sub>12</sub> deficiency, dementia of any type, history of endocrine dysfunction (including Addison's disease, Cushing's disease, or exogenous steroid use within the past 5 years), or any history of genetically based reactions to alcohol use (Asian ancestry with history of the ethanol flush response). AD subjects were excluded if withdrawal symptoms at the time of study entry necessitated hospitalization.

This project received prior approval from the Colorado Multiple Institutional Review Board, which services the University of Colorado School of Medicine and its affiliate institutions, as well as from the Research and Development Committee of the Department of Veterans Affairs Medical Center, Denver, where the study was conducted. All subjects provided consent on forms pre-approved by both institutions.

### *Cortisol sampling*

The day before sampling each subject was (1) given salivary collection materials, (2) walked through a practice sampling procedure, and (3) instructed to provide samples at the specified times. Neither preset alarms nor proxy observers were used. Sampling occurred on only one day: at waking, waking + 30 minutes, noon before lunch, and 4 PM before dinner.

### *Cortisol concentration*

Saliva was collected on specially cut filter papers as described elsewhere (Gozansky et al., 2005; Neu et al., in press). Each subject provided saliva samples as above on the day when magnetic resonance imaging (MRI) scan of the brain was done. The filter papers were extracted as described previously (Gozansky et al., 2005; Neu et al., in press) and assayed using a commercially available high sensitivity enzyme immunoassay kit (Salimetrics, LLC, State College, PA). Briefly, 50  $\mu$ l of buffer from the extracted sample was added in duplicate to the wells of a microtiter plate coated with antibody (rabbit anticortisol). The unknowns competed with horseradish peroxidase conjugated cortisol for the binding sites. The substrate tetramethylbenzidine (TMB) was added, and the reaction was stopped with H<sub>2</sub>SO<sub>4</sub> and read at 450 nm on a Dynex microplate reader. Revelation software 4.25 (Dynex, Chantilly, VA) fit a sigmoid function by a four parameter logistic regression for the standard curve and determined unknown concentrations from this curve. The minimal detectable level for this procedure is approximately 0.05  $\mu$ g/dl for the extracted samples. Intra- and interassay coefficients of variation for this assay are less than 5% for undiluted samples.

### *MRI scan*

All study subjects completed a baseline 3T MRI brain scan. For AD subjects, baseline scans were done within 3 days of cessation of alcohol use. Scan data were collected through whole brain-volume acquisition using a 3D inversion-recovery spoiled grass (IR-SPGR) pulse sequence in the coronal plane with an image matrix of  $256 \times 192 \times 124$  on a 3 Tesla MRI scanner (General Electric Company, Milwaukee, WI). The image resolution was  $0.94 \times 0.94 \times 1.7$  mm<sup>3</sup>, with a lower resolution along the anterior/posterior direction. The inversion time (TI) of 450 ms was selected to optimize the gray/white matter contrast. The data acquisition time for the 3D volume was 13 minutes, 11 seconds.

### *Volume measurement*

Hippocampal, total brain, and intracranial volumes were derived using an automated segmentation process on the 3T MRI images of the brain. These were assessed by an imaging analysis research group at the University of Pennsylvania who were supplied scan image data only and were blind to the subjects' study group membership. The steps in image analysis included the following: (1) removal of extracranial tissues (skull stripping); (2) tissue segmentation into gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF); and (3) elastically warping a labeled atlas to all individual subjects to label and measure automatically the regions of interest in the brains. These steps are described briefly.

*Skull stripping.* A seed-based region growing procedure was applied first, which separates the brain parenchyma from extracranial material (Goldszal et al., 1998). Manual editing was then performed on a slice-by-slice basis. The interoperator reliability test revealed nonsignificant differences between the two trained operators (mean [SD] = -0.02% [1.37] for white matter and 0.46% [0.88] for gray matter). Correlations were greater than .99 for both measures: Paired *t* test comparisons yielded no significant differences between raters.

*Tissue segmentation.* The SPGR data were segmented into GM, WM, and CSF (Goldszal et al., 1998). An automated segmentation algorithm (Segal et al., 1995) based on k-means clustering and Markov random fields, which has been validated extensively (Davatzikos and Resnick, 1998; Goldszal et al., 1998), was used at this step. This method also applied correction for magnetic field inhomogeneities.

*Automated measurement of brain structures.* A labeled atlas was transformed spatially into spatial coregistration with each tissue-segmented individual brain scan via an elastic warping algorithm (Davatzikos et al., 2001a,b; Shen and Davatzikos, 2002, 2003), referred to as Hierarchical Attribute Matching Mechanism for Elastic Registration (HAMMER). To achieve this, a finely parcellated brain im-

age was first adopted as the labeled atlas developed by Kabani and colleagues at the Montreal Neurological Institute (Kabani et al., 1998), including 101 regions of interest (ROIs). Left and right hippocampi have separate labels in this atlas. The HAMMER registration algorithm (Shen and Davatzikos, 2002, 2003) then warped this atlas to individual subjects' images, thereby obtaining volumetric measurements of the ROIs for all subjects. The accuracy of the HAMMER registration algorithm has been extensively validated by both real data and simulated data (Shen and Davatzikos, 2002, 2003).

### *Statistical analysis*

First, log transformation of cortisol concentrations allowed for an approximation of normal distribution of these data so that parametric statistical strategies could be employed. The difference between mean salivary cortisol concentrations by drinking status was evaluated using a *t* test. Further, to assess whether inter-subject variations in total brain volume (TBV) and intracranial volume (ICV) measures had an effect on observed cortisol levels, salivary cortisol concentration was also analyzed using multiple linear regression.

Second, to assess the association between mean cortisol concentration and mean hippocampus volumes, we subjected the total sample ( $N = 16$ ) to analysis using partial correlation coefficients (PCC) of volumes and log-transformed cortisol, controlling for ICV. The same analysis procedure was used to test for associations within each subject group.

## **Results**

### *Subjects*

From a total of 54 (35 test and 19 control) screened cases, we recruited a sample of 18 (9 AD test and 9 non-AD control) matched subjects who proceeded to MRI scanning. The most common reasons for not meeting study qualifications included stopping drinking more than 3 days before the study, medical illness exclusion, and presence of Axis I disorders as mentioned in the Method section. Two subjects, one from each group, were removed from final data analysis because of potentially significant anatomic abnormalities on the MRI scans that were not regarded as related to alcohol use and that indicated clinical referral. These abnormalities were evidence of previous anoxic injury and of congenital malformation, respectively. Both subjects were white; dropping them did not affect either age or ethnic distribution. Data were analyzed from the remaining 16 subjects, 8 per group.

Mean subject age was equivalent between groups (AD test group = 47.25 [10.71] years; non-AD control group = 47.75 [10.78] years). There were seven white participants

and one black participant in each group. Within the heavy-drinking group, the subjects reported an average of 392 (259) total standard drinks in the 30 days before study entry. However, the average alone is somewhat misleading. Of the eight heavy drinkers, five drank daily and three drank during heavy-drinking episodes that lasted about 3-4 days weekly. For perspective, all drank an average of 16 (7) standard drinks on drinking days.

Of the eight test subjects, one subject met SCID criteria for current cannabis abuse but not dependence; one met criteria for current stimulant abuse but not dependence; and one met criteria for current cocaine dependence. None of the eight control subjects met criteria for current dependence or abuse of any substance; only one met criteria for a substance-related diagnosis: past alcohol abuse (the abuse occurred more than 30 years before study entry).

#### Baseline cortisol comparison

Morning salivary cortisol concentrations between groups varied in the predicted direction. Mean values were more than twice as high (Figure 1) among heavy-drinking AD subjects than among light-drinking non-AD subjects at waking (0.49 [0.23] vs 0.24 [0.14]  $\mu\text{g}/\text{dl}$ ,  $p = .012$ ) and at waking + 30 minutes (0.57 [0.37] vs 0.28 [0.11]  $\mu\text{g}/\text{dl}$ ,  $p = .043$ ). Mean cortisol concentrations at noon and 4 PM did not differ between the two groups. Viewed as effect sizes, alcohol use strongly increased morning mean cortisol levels both at the waking and the waking + 30 minute time points (Cohen's  $d = 1.3$  and 1.1, respectively [Cohen,

1988]). Neither the total alcohol consumption in the previous 30 days nor the mean number of standard drinks per drinking day bore any statistical relation to cortisol level at any of the time points.

Linear regression demonstrated that neither total brain volume nor intracranial volume significantly contributed to the model as confounding variables affecting salivary cortisol concentration. Because these data were consistent with previous reports on hypercortisolism states (Adinoff et al., 2003), they offered reasonable confidence to seek an association with THV and salivary cortisol level.

#### Hippocampus volume and salivary cortisol association at baseline

Using the whole sample ( $n = 16$ ), THV was significantly associated with waking cortisol level in a negative direction (PCC = -0.641, 12 df,  $p = .007$ ). This significant negative relationship was not seen at the other time points of waking + 30 minutes, noon, and 4 PM. Within their respective drinking groups, however, only the control subjects presented a significant inverse correlation (PCC = -0.82,  $p = .025$ ) between THV and waking cortisol concentration, controlling for ICV (heavy drinking subjects [ $p = .35$ ]). Again, associations were not seen at other cortisol time points or with the magnitude of change between the waking and waking + 30 minute samples. To help explain the differing associations between the two groups, we plotted the mean cortisol data for each as a function of the diurnal collection times (Figure 2). Although both groups

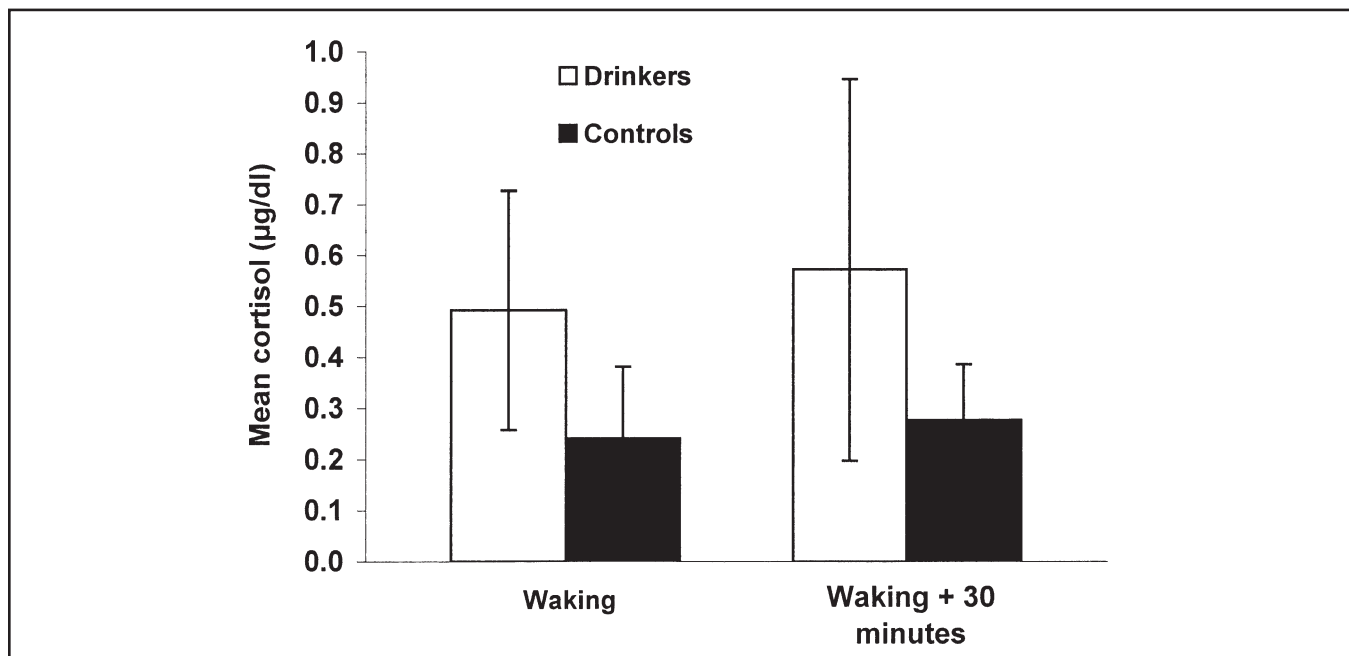


FIGURE 1. Mean cortisol concentrations at waking and at waking + 30 minutes, drinkers versus nondrinkers ( $n = 8$  for each)

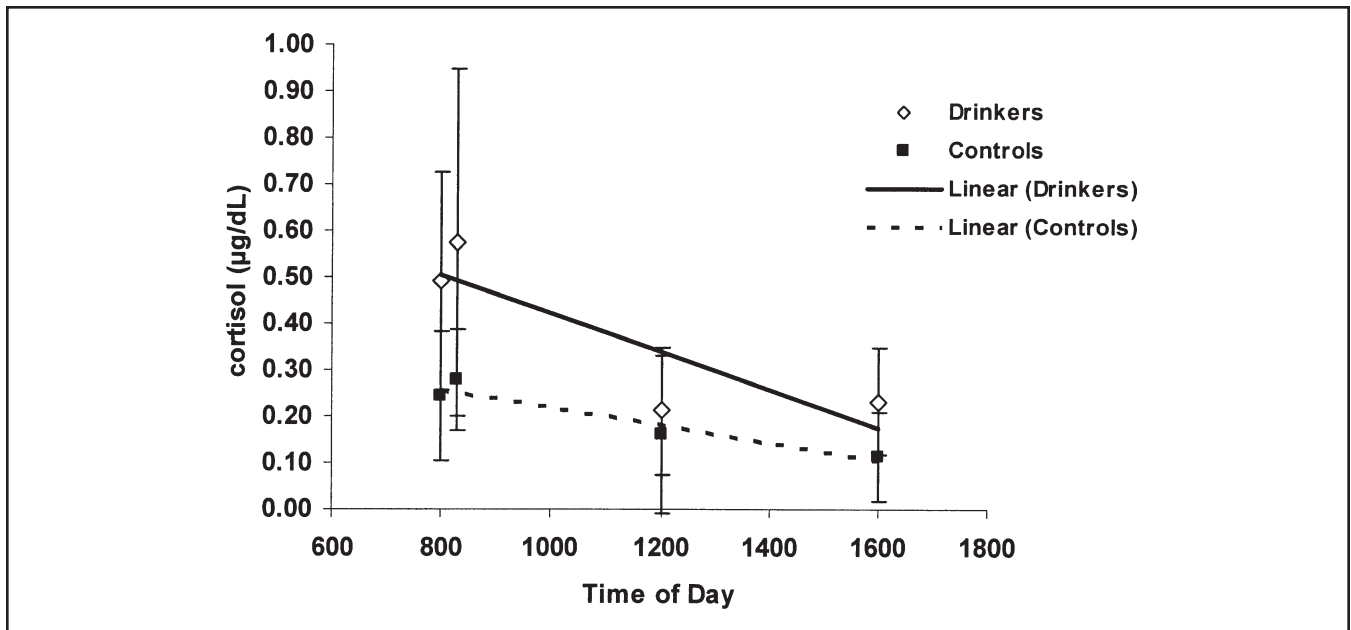


FIGURE 2. Diurnal mean salivary cortisol concentrations, heavy drinkers versus controls ( $n = 8$  for each). Time of day is in military time (i.e., 0600 = 6 AM, 1200 = noon, and 1800 = 6 PM).

demonstrated characteristic diurnal patterns, the downward slope for the drinking group was much steeper than that for the control group. Because the standard deviations among the heavy-drinking group were greater than among controls (Figure 1), two possible explanations exist: variations in sampling among the test group members or a wide individual variation in the HPA effects of chronic heavy drinking.

### Discussion

These results provide early data that support in part our original hypotheses. First, morning salivary cortisol concentrations were significantly higher in the AD subjects whose drinking was chronic and heavy, compared with the light-drinking non-AD control subjects (Beresford et al., 1999; Wand and Dobs, 1991). Second, considering the data from the whole sample, THVs were negatively associated with observed cortisol concentrations in the study subjects. Third, when considering only the heavy drinkers, the statistical association disappeared, whereas in the light drinkers it remained significant.

The lack of an association between cortisol elevations and low THV in the heavy drinkers must be explained. First, it may be due to the single-day cortisol collection method. To address this possibility, future collections must include serial diurnal sampling, watch alarms or other independent reminders, and nondrinking proxy observers. Second, the phenomenon may reflect interindividual variations due to an alcohol interaction in the central nervous systems of the heavy drinkers but not in the control subjects. Be-

cause the diurnal curve of the mean cortisol levels (Figure 2) resembles the expected diurnal pattern of cortisol production, individual variations may indicate underlying differences in the HPA response itself. However, if the lack of an association still persists with improved methodology, it may provide a possible indication of the extent of injury—to THV, to HPA axis functioning, or to both—as compared with an apparently strong association among the control subjects. The data may be pointing toward a more subtle mechanism of injury than originally supposed.

Empirically, sustained heavy drinking has been shown to raise serum cortisol levels, to disrupt the diurnal variations of glucocorticoid secretion, and to expose the brain to twice or more of the total daily cortisol load observed in light-drinking controls (Elias et al., 1982; Eskay et al., 1995; Jenkins, 1981; Lovallo et al., 2000; Menzano and Carlen, 1994; Proto et al., 1985; Rasmussen et al., 2000). Recent research has shown that the hippocampus is intimately involved in the regulation of the HPA axis (Buchanan et al., 2004). Impaired hippocampal function, possibly due to a lack of a hippocampal component of negative feedback, may serve as a mechanism for dysregulated cortisol production and continued injury in the setting of chronic heavy alcohol use. Whether continuously high glucocorticoid levels are directly due to ethanol, to hippocampal injury, or to both—or to other factors—remains unclear (Lovallo et al., 2000; Lukoyanov et al., 1999).

The study data must be considered carefully with respect to generalization to all heavy, sustained users of alcohol. The data derive from early study of a small and highly

selected sample gathered to establish that alcohol-induced hypercortisolism can be reliably observed. The sample included no female subjects and gender may introduce differences not addressed here (Gianoulakis et al., 2003; Pfefferbaum et al., 2001). The subjects were seen in middle age after chronic heavy-drinking histories; the data offer no comment on heavy, sustained drinking at younger ages, for example, in young adults when the course of heavy drinking is still relatively early. Last, these data offer no insights as to whether hypercortisolism or hippocampal volume loss reverse with abstinence from alcohol.

Although the SCID assessment excluded subjects meeting diagnostic criteria for mood and anxiety disorders, it is possible that these disorders may be present but not identifiable using this measure, one of the best available. This argues for serial assessment during observed states of abstinence. Childhood trauma or neglect has more recently been associated with hypercortisolism and must also be assessed in any further study (Nemeroff, 2004; Penza et al., 2003).

Because these data were gathered using a cross-sectional method, they suggest that hypercortisolism associated with the sustained heavy drinking of AD is the direct effect of chronic heavy ethanol exposure. However, an equally plausible line of thinking suggests that continuing daily withdrawal might likewise raise cortisol levels, lower THV, or both. For example, one study reports small hippocampus volumes in association with ethanol withdrawal seizures (Sullivan et al., 1996), but this observation has not been replicated (Bleich et al., 2003).

Much of the published data suffers from the one-time-only observation of the cross-section method. Followed longitudinally, and in the absence of both alcohol use and frequent withdrawal episodes, comparisons with baseline may shed light on the effect of chronic heavy alcohol use. In this way, longitudinal study may illuminate the course of healing with respect to both the neuroendocrine system and the hippocampus.

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