INTRODUCTION

Several lines of evidence indicate that disturbances in the central noradrenergic (NA) and serotonergic (5-HT) systems may mediate excessive alcohol consumption. For example, animal experiments have shown that acute alcohol exposure increases NA turnover and release, and downregulates central $\alpha_2$ adrenergic receptors (Wang et al., 1999; Thiele et al., 2000). Similarly, acute administration of alcohol is known to stimulate 5-HT turnover, while chronic alcohol intake is associated neurobiological changes have attracted interest, due to the possibility that they may contribute to relapse. Alcohol withdrawal has been linked to increased metabolism and release of NA (Fujimoto et al., 1983; Hawley et al., 1985), reduced $\alpha_2$ adrenoceptor function (Nutt et al., 1988; Berggren et al., 2000), reduced 5-HT function (Ballenger et al., 1979), and alterations in neuroendocrine responsivity to challenge with NA and 5-HT agents (Krystal et al., 1996). Most of the data have come from determinations at one or two time points and very few studies have attempted to longitudinally study the changes in NA and 5-HT during the withdrawal period. Another clinical symptom, craving, has been increasingly recognized to contribute to continued drinking and is believed to play an important role in relapse (Petrikas et al., 1999). There is evidence that selective serotonin reuptake inhibitors (SSRI) may reduce craving for alcohol (Naranjo and Knoke, 2001), though contrary findings have also been reported (Petrikas et al., 2001). Since craving is a prominent symptom in alcohol withdrawal it seems reasonable to explore whether it may be related to neurobiological changes.

A major methodological issue in studying peripheral levels of NA and 5-HT and their relationship to central mechanisms, such as craving, is that it is unclear whether plasma NA and 5-HT levels reflect corresponding changes in human brain. 

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Certain metabolic differences are present between periphery and the brain in humans that may need to be considered during interpretation of data [e.g. high-affinity alcohol dehydrogenase enzyme is absent in the brain (Linnoila et al., 1986)]. However, human studies have found correlations between brain and plasma levels of 2-methoxy-4-hydroxy-phenylglycol (MHPG) and NA, and plasma NA and its metabolites are believed to be a major determinant of levels of NA in the cerebrospinal fluid (Kopin et al., 1983). Also, similarities have been observed in the uptake, storage and release of 5-HT in neurons and blood platelets (Da Prada et al., 1988). Since repeated central measurements of neurotransmitters in humans are expensive, invasive and technically difficult, we examined plasma NA and 5-HT levels in our study.

Extending our previous work investigating 5-HT function in alcohol dependence (Patkar et al., 1995), we examined the role of NA and 5-HT systems in alcohol withdrawal. The objectives were to examine longitudinal differences in plasma NA and 5-HT levels during alcohol withdrawal, to compare these measures between withdrawing alcoholics and controls and to determine whether craving is associated with NA and 5-HT levels during withdrawal. We also explored whether the type I and type II subgroups of alcoholic patients differ from each other and from controls in measures of NA, 5-HT and craving.

SUBJECTS AND METHODS

Subjects

The sample consisted of 26 alcohol-dependent Caucasian men, who were hospitalized for detoxification from alcohol, and 28 race- and gender-matched healthy volunteers, who served as the control population. The alcohol-dependent subjects were recruited from a university-affiliated in-patient addiction unit in Nottingham, England. The inclusion criteria were: (1) DSM-IV diagnosis of alcohol dependence (American Psychiatric Association, 1994); (2) ability to give informed consent; (3) no interruption of alcohol intake in the previous 72 h as determined by self-reports of alcohol consumption. This permitted us to obtain a baseline blood sample at the beginning of the withdrawal process. Individuals with major depression, bipolar disorders or psychotic disorders, serious medical disorders, current illicit drug misuse, a positive urine drug screen (except benzodiazepines), and those receiving psychotropic medication (except benzodiazepines) were excluded from the study. Smoking status was determined by self-reports (Do you consider yourself as a current smoker, non-smoker or former smoker?) and coded as a dichotomous variable (current smoker or non-smoker/former smoker). Alcohol-dependent subjects were typically recruited within 24 h of admission to the in-patient unit. The detoxification regime consisted of tapering doses of benzodiazepines over a 15-day period along with multivitamins. A fixed dose schedule was used; the starting dose was chlordiazepoxide 20 mg every 6 h, which was reduced by 20% every 3 days. Additional doses were used as clinically needed. The average daily dose of chlordiazepoxide was 44 mg.

Controls were hospital employees or their relatives. They were excluded if they had a positive response to items on the CAGE questionnaire (Ewing, 1984), a positive breathalyser reading [reported greater than 21 units of alcohol consumption per week (one unit is equivalent to 10 ml or 8 g of pure alcohol and corresponds to 4 fluid ounces of table wine, 1.5 fluid ounces of spirits or 8 fluid ounces of beer) in the previous month], a major psychiatric or medical disorder, current or past illicit drug misuse, or were receiving psychotropic medication. Family history of a psychiatric illness or substance misuse was not an exclusion criterion.

Behavioural assessments

The study protocol was approved by the ethics committee of the hospital. After a description of the study, written informed consent was obtained. Alcohol-dependent individuals who volunteered for the study underwent a psychiatric interview and had their medical health assessed by medical history and physical examination. Eligible subjects were tested for breath alcohol levels. This was part of the routine admission procedure of the unit. Subjects then completed a visual analogue scale (VAS) for craving (McCormack et al., 1988), and a questionnaire to classify subjects into type I and type II subtypes of alcoholism (Cloninger, 1987).

The VAS has been frequently used to quantify craving for drugs and alcohol and has been used as a subjective measure of alcohol craving (McCormack et al., 1988). It can be completed in 2 min. The VAS consisted of a 100 mm horizontal line anchored on the left with 0 (‘no craving’) and on the right with 100 (‘most craving ever felt for alcohol’). Subjects were asked to place a mark on the VAS based on their perceived intensity of craving at that time. The scores ranged from 0 to 100. A single VAS was administered at four time points during withdrawal. Studies have found a high correlation between craving scores obtained on the VAS and those obtained using a multi-item scale in substance misusers (Mezinskis et al., 1999). The type I/type II classification was based on procedures outlined by Gilligan et al. (1988). The questionnaire included 12 variables, which were categorized as representing a type I characteristic (e.g. first dependence symptom after age 25) or a type II characteristic (e.g. family history of alcoholism). All positive responses to type I items (i.e. yes) were weighted positively (+) and all positive responses to type II items were weighted negatively (–). A total score was obtained for each subject by summing scores for all items. Subjects with scores in the positive range were classified as type I; those with negative scores were classified as type II. A score of 0 represented ‘unclassified’ subjects. The questionnaire required 15 min to complete.

Consenting controls were screened using the CAGE questionnaire (Ewing, 1984), a clinical interview, a physical examination, and breath alcohol readings. The CAGE is a 4-item questionnaire that takes about 1 min to complete. It has been extensively employed as a screening tool to detect alcoholism in clinical settings (Soderstrom et al., 1997).

The NA and 5-HT assay

Twenty millilitres of venous blood were collected in glass tubes from subjects and controls. For controls a single blood sample was obtained. For alcoholic subjects, blood was drawn on the day of admission (day 0), the next day (day 1), 7 days after admission (day 7) and 14 days after admission (day 14). Patients who could not produce at least two blood samples for any reason were not included in the study. Blood samples were collected in the morning and transported to the laboratory on...
ice within 2 h. The samples were centrifuged within 4 h of arrival at the laboratory. The plasma was separated into platelet-rich and platelet-poor fractions, platelet counts were performed on both fractions to ensure optimal separation, and the plasma was stored at –80°C. For the 5-HT assay, a good blood sampling technique, adequate platelet stabilization in the test tubes and rapid processing after collection reduced the possibility of platelet aggregation and activation that may stimulate 5-HT release and inflate the measured values (Beck et al., 1993). The NA and 5-HT assay was performed on the platelet-poor fraction using high-performance liquid chromatography (HPLC) (Beckman 110 system) with electrochemical detection (Warwick Instruments) optimized for the detection of NA and 5-HT. Details of the techniques for the NA and 5-HT assay have been previously described in published studies from our laboratory (Marsden, 1987; Fulford and Marsden, 1997). Briefly, after preparation of test samples, a solvent delivery pump was used to circulate the mobile phase. The samples were injected onto a column via a Rheodyne 7125 injector. NA and 5-HT were measured electrochemically using a dual glassy carbon electrode maintained by a silver/silver chloride reference electrode and a platinum auxiliary electrode. Changes in potential were measured by an amperometric detector linked to an integrator for the display of chromatograms. The HPLC system was calibrated using standard solutions to give a linear response over a range of NA and 5-HT concentrations. The protein assay was performed by the methods described by Lowry et al. (1951). The intra-assay and inter-assay coefficients of variation for NA were 1.2 and 3.9%, respectively, and for 5-HT were 1.8 and 3.7%, respectively. The NA and 5-HT concentrations of variation for NA were 1.2 and 3.9%, respectively, and for 5-HT were 1.8 and 3.7%, respectively. The NA and 5-HT levels in controls were expressed in picomoles per millilitre (pmol/ml) and nanomoles per litre (nmol/l) of plasma, respectively. The mean and standard deviation values for plasma NA and 5-HT levels were 2.34 ± 0.18 pmol/ml (Forster et al., 1991) and 2.9 ± 1.8 mmol/l (Hindberg and Naeh, 1992), respectively. The laboratory personnel were blind to the clinical data.

Blood samples from alcoholic subjects were also analysed for serum γ-glutamyl transpeptidase (GGT) and mean corpuscular volume (MCV) values. Elevated GGT and MCV are both indicative of excessive alcohol consumption and reported to be elevated in 50–75% of alcoholic individuals (Allemann, 2000).

Statistical analysis

Two-tailed t-tests and chi-squared tests were employed to compare subjects and controls on continuous and dichotomous variables respectively. Changes in NA and 5-HT levels and craving during withdrawal were evaluated using repeated measures analysis of variance (ANOVA). For this purpose, a Huynh-Feldt correction was used if the Mauchley test indicated that the data did not meet the assumption of sphericity (Gagnon et al., 1989). Relationships between clinical variables and NA or 5-HT levels were examined using Pearson or point biserial correlations. When appropriate, the data were analysed using analyses of covariance (ANCOVA) to control for the effects of other dependent variables on the biological measures. Differences in group sizes for biological measures reflect data lost due to missed blood draws or laboratory-related problems. Power calculations were based on our previous study that found a significant difference in platelet tritiated imipramine binding between 29 alcoholic patients and 29 controls (P < 0.005); moreover, platelet imipramine binding was significantly increased during alcohol withdrawal (P < 0.01) (Patkar et al., 1995). Assuming a similar effect size (f = 0.34), the sample in the current study had a power of 0.85 to detect differences between alcoholics and controls and a power of 0.87 to detect changes in biological measures over the four time points during withdrawal.

RESULTS

Subjects

A total of 54 male, Caucasian, individuals were studied: 26 were alcohol-dependent and 28 were controls. Table 1 summarizes the demographic and clinical characteristics of the sample.

As seen in Table 1, the two groups did not differ significantly in age; however alcohol-dependent individuals were significantly more likely to be single, unemployed and tobacco smokers, than controls. No individuals tested positive for HIV; however, eight alcohol-dependent individuals had a history of medical diseases or were taking medications for their medical conditions. Five alcohol-dependent patients had a history of illicit drug misuse; however, none of these was currently misusing drugs. Five patients were taking benzodiazepines at the time of admission. Thirteen alcohol-dependent patients were classified as type I, 12 as type II and one individual was unclassified. The two subtypes did not differ in age, employment or marital status, duration of drinking, breathalysers readings of alcohol, or MCV values (all t values < 1.72, df = 23, P > 0.05; χ² < 0.84, df = 1, P > 0.05 in each case). However, type II patients had higher GGT values (231.15 ± 238.76 IU/l) than type I alcoholics (64.16 ± 44.03 IU/l) (t = 2.38, df = 23, P < 0.05). Four of the 12 (33.3%) type II patients had a history of illicit drug use, compared with one of the 13 (7.7%) type I patients. The two subtypes did not differ in tobacco use (χ² = 1.35, P > 0.05).

Plasma NA levels during withdrawal

The plasma NA levels in controls were 3.03 ± 1.30 pmol/ml. During alcohol withdrawal the NA levels were: 5.19 ± 2.37 pmol/ml on day 0, 5.38 ± 2.67 pmol/ml on day 1, 2.51 ± 1.22 pmol/ml on day 7, and 2.46 ± 1.05 pmol/ml on day 14. As summarized in Fig. 1, a repeated measure ANOVA that compared all four test days revealed a significant effect of time on plasma NA levels during alcohol withdrawal (F(3,75) = 25.55, P < 0.001).

The ANOVA analyses comparing two test days at a time (i.e. day 0 with day 1; day 7 with day 1) showed that NA levels were similar on days 0 and 1 [F(1,25) = 0.41, P = 0.52]; however, there was a significant reduction in NA concentrations on day 7 compared with day 1 [F(1,23) = 30.43, P < 0.001]. The NA levels on days 7 and 14 were not significantly different [F(1,12) = 0.23, P = 0.89]. When NA levels in controls were compared with those during alcohol withdrawal, the control levels were significantly lower than day 0 (t = 4.25, P < 0.001) and day 1 (t = 4.22, P < 0.001) values; however, control values were similar to day 7 (t = –1.51, P = 0.14) and day 14 (t = –1.73, P = 0.09) values. We also re-analysed the data controlling for benzodiazepines prescribed for detoxification. For this purpose, the mean daily total benzodiazepine dose in diazepam
equivalents was used as covariate in an ANCOVA analysis. This showed that the effect of NA during withdrawal continued to remain significant after adjusting for benzodiazepine use [$F(3,75) = 22.70$, $P < 0.001$]. We then examined whether there were differences in NA levels during withdrawal between type I and type II alcoholics. No significant interaction was observed between the two subtypes and NA levels during withdrawal [$F(3,72) = 0.49$, $P = 0.68$].

**Plasma 5-HT levels during withdrawal**

The plasma 5-HT levels in controls were $3.73 \pm 1.81$ nmol/l. During withdrawal the 5-HT values were: $2.29 \pm 1.88$ nmol/l on day 0, $1.96 \pm 1.58$ nmol/l on day 1, $1.35 \pm 0.87$ nmol/l on day 7, and $1.34 \pm 0.85$ nmol/l on day 14. Similar to NA, a significant effect of time on plasma 5-HT levels was observed during withdrawal [$F(3,75) = 5.47$, $P < 0.05$]. The 5-HT levels during withdrawal are summarized in Fig. 2.

ANOVA comparing two test days at a time showed that there was a significant decrease in 5-HT levels on day 1 compared with day 0 [$F(1,25) = 6.41$, $P < 0.05$], and day 7 compared with day 1 [$F(1,23) = 5.69$, $P < 0.05$], whereas the day 7 and day 14 values were not significantly different [$F(1,21) = 0.31$, $P = 0.83$]. The plasma 5-HT levels in controls were significantly higher than the day 1 ($t = -2.12$, $P < 0.05$), day 7 ($t = -2.86$, $P < 0.01$) and day 14 ($t = -2.28$, $P < 0.05$) values in alcoholic patients; however, the control 5-HT levels were similar to the day 0 values during withdrawal ($t = -0.85$, $P = 0.40$).

ANOVA analysis using the benzodiazepine dose as a covariate did not change the direction or significance of findings [$F(3,75) = 5.56$ and $F(3,75) = 5.47$, respectively, $P < 0.05$]. Type I and type II alcoholic patients did not significantly differ in plasma 5-HT levels during withdrawal [$F(3,72) = 1.43$, $P = 0.31$].

**Craving during withdrawal**

As summarized in Fig. 3, a significant effect of time on craving was found during withdrawal [$F(3,75) = 25.11$, $P < 0.001$]. The VAS craving scores on day 0 (71 ± 30) were significantly higher than those on day 1 (49 ± 28) [$F(1,25) = 18.08$, $P < 0.001$], whereas the day 1 scores were higher than the day 7 scores.

### Table 1. Clinical characteristics of alcoholic subjects and controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Alcoholic ($n = 26$)</th>
<th>Controls ($n = 28$)</th>
<th>$t/\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>$39.4 \pm 10.1$</td>
<td>$36.6 \pm 6.4$</td>
<td>$t = 1.69$</td>
</tr>
<tr>
<td>Employed (%)</td>
<td>27.5</td>
<td>93.1</td>
<td>$\chi^2 = 14.22^{**}$</td>
</tr>
<tr>
<td>Single (%)</td>
<td>68.9</td>
<td>44.8</td>
<td>$\chi^2 = 8.26^{*}$</td>
</tr>
<tr>
<td>Tobacco smokers (%)</td>
<td>89.6</td>
<td>34.4</td>
<td>$\chi^2 = 11.31^{**}$</td>
</tr>
<tr>
<td>Duration of drinking (years)</td>
<td>$19.2 \pm 10.4$</td>
<td>Not applicable</td>
<td></td>
</tr>
<tr>
<td>Admission breath alcohol (mg %)</td>
<td>128 ± 83</td>
<td>Not applicable</td>
<td></td>
</tr>
<tr>
<td>Craving (VAS scores)</td>
<td>54.2 ± 32.1</td>
<td>Not applicable</td>
<td></td>
</tr>
<tr>
<td>MCV* (fl)</td>
<td>96 ± 6</td>
<td>Not applicable</td>
<td></td>
</tr>
<tr>
<td>GGT* (IU/l)</td>
<td>249 ± 507</td>
<td>Not applicable</td>
<td></td>
</tr>
<tr>
<td>Benzodiazepines* (%)</td>
<td>35.7</td>
<td>Not applicable</td>
<td></td>
</tr>
</tbody>
</table>

* $P < 0.05$, ** $P < 0.01$, for $t$-tests df = 55, for $\chi^2$ df = 1.

* Normal mean corpuscular volume (MCV) values ranged from 76 to 97 fl.

* Normal $\gamma$-glutamyl transpeptidase (GGT) values ranged from 10 to 50 IU/l.

* Prescribed or present in the urine.

### Fig. 1. Changes in noradrenaline levels during alcohol withdrawal ($n = 26$).

$F(3,75) = 25.55$, $P < 0.01$; vertical bars, ± 1 SD; ■■, mean NA concentration.

### Fig. 2. Changes in 5-HT levels during alcohol withdrawal ($n = 26$).

$F(3,75) = 5.47$, $P < 0.05$; ■■, mean 5-HT concentration; vertical bars, ± 1 SD.
alcoholic subjects and controls, we examined whether these variables were related to plasma NA or 5-HT levels. We found that NA or 5-HT levels were not significantly correlated with employment, marital status or smoking status ($\chi^2$ ranged from 0.48 to 1.87, df = 1, $P > 0.05$ in each case), or with GGT values or breath alcohol levels. Smoking status was also not related to craving scores ($\chi^2 = 1.64$, df = 1, $P > 0.05$).

DISCUSSION

There were four main findings of interest from this study. First, both plasma NA and 5-HT levels changed significantly during the withdrawal period. Secondly, NA levels were higher than controls at the beginning of withdrawal, but subsequently declined and resembled those in controls within 7 days of abstinence. In contrast, 5-HT levels were similar to controls at the beginning of withdrawal, then declined and remained reduced compared with controls even after 14 days of abstinence. Thirdly, the alterations in NA or 5-HT activity were not specific for type I or type II alcoholism. Finally, craving was not related to alterations in NA or 5-HT during withdrawal.

**Plasma NA levels during withdrawal**

Our findings that plasma NA levels were significantly elevated in early withdrawal are consistent with reports of changes in plasma NA (Smith et al., 1990) and CSF 2-methoxy-4-hydroxyphenylglycol (MHPG) (Hawley et al., 1985). Elevated levels of circulating catecholamines can lead to down-regulation of $\alpha_2$-adrenergic receptors. Consistent with our results, Nutt et al. (1988) documented blunting of neuroendocrine responses to intravenous clonidine, an $\alpha_2$ agonist in withdrawing alcoholics, compared with controls. In contrast, other studies have yielded negative results (Bylund et al., 1984). A possible reason for these discrepant findings could be that most studies have examined NA levels at one or two time points during withdrawal. Since we found that NA levels declined from early to late withdrawal, the stage of withdrawal during which data were obtained could have affected the results. It is also noteworthy that clinical signs and symptoms of alcohol withdrawal peak by 48–72 h and then diminish progressively over the next 10–14 days (Stockwell, 1994). This parallels the change in NA levels found in our study, suggesting that at least a part of the alcohol withdrawal syndrome may be mediated by the NA system (Linnola et al., 1986; Lubman et al., 1983; Üzüey et al., 1998). An excellent review of the possible mechanisms for increased NA activity during early withdrawal has been published by Linnola et al. (1987). Since the NA levels resembled control values by 7 days of abstinence, it appears that NA changes during withdrawal may be reversible and temporary. Data in this area remain inconsistent with both normal and disturbed NA function having been reported in the literature (Muller et al., 1989; Berggren et al., 2000). Further studies, incorporating longitudinal designs, are needed to clarify whether changes in NA activity during various stages of alcohol dependence are state-dependent phenomena or represent long-term consequences of alcohol consumption.

**Plasma 5-HT levels during withdrawal**

There could be two possible explanations for the decline in plasma 5-HT levels throughout the withdrawal period. Since alcohol consumption has been known to stimulate serotonin...
turnover (Tollefson, 1989), it is possible that alcohol consumption may be an attempt to compensate for an underlying serotonin-deficit state that is unmasked during withdrawal. Alternatively, the withdrawal process may lead to reduction in the firing rates of serotonergic neurons resulting in decreased 5-HT release (Bailey et al., 2000). Consistent with several reports (Bailly et al., 1990; Arranz et al., 1999), 5-HT levels did not return to normal values at the end of withdrawal. It was beyond the scope of this study to determine whether the reduced 5-HT function would recover with extended abstinence; however, there is increasing evidence that central serotonergic function may be decreased in alcoholics (Krystal et al., 1996; Heinz et al., 1998). Considering the variability in response to treatment with 5-HT agents in alcoholism (Naranjo and Knoke, 2001), it may be timely to examine whether biological variables such as low 5-HT levels may help to predict response to treatment with selective serotonin reuptake inhibitors.

NA, 5-HT and craving in alcohol withdrawal

The patterns of changes in NA and 5-HT during withdrawal were different and there were no significant relationship between the two systems. This suggests that the disturbances in the NA and 5-HT systems during alcohol withdrawal may be independent of each other and possibly mediate distinct clinical symptoms. Consistent with studies of the course of withdrawal symptoms (Skinner and Allen, 1982; Stuppaec et al., 1994), craving declined over the withdrawal period; however, it was not associated with changes in NA or 5-HT levels. This suggests that self-reports of urges to drink during withdrawal may not be extensively modulated by changes in plasma NA or 5-HT turnover. Also, craving is a multidimensional phenomenon having cognitive, affective and motivational components; a VAS does not reflect all these attributes. Because we did not measure withdrawal symptoms, it was not possible to determine whether symptoms such as anxiety that overlap with craving, could have been related to plasma NA or 5-HT. Our results support findings from studies that found no relationship between NA measures (Petakis et al., 1999) or serotonergic manipulations (Petakis et al., 2001) and cue-induced craving in alcohol misusers. However, some researchers have suggested that craving studies in alcoholics may require larger sample sizes than studies of other substance misusers (Tiffany et al., 2000). Although the current study was sufficiently powered to detect changes in NA (effect size = 0.58) and craving (effect size = 0.55), the relationship between the two may be less strong and larger sample sizes may be necessary to detect such small effects.

Type I and type II alcoholism

Contrary to our earlier findings that type I and II alcohol-dependent individuals differ in platelet tritiated imipramine binding (Patkar et al., 1995), the two subtypes did not differ in plasma 5-HT or NA levels during withdrawal. While the data on differences in NA function between the two subtypes are not robust, substantial evidence indicates that type I and type II alcoholics may differ in 5-HT measures (Roy et al., 1987; Virkunen et al., 1994). The discrepant findings may be related to studying alcoholics during a state of withdrawal, a period characterized by significant perturbations in neurobiological systems, as opposed to chronic drinking or prolonged abstinence. Also, it must be noted that stratifying alcoholics in the two subtypes almost halved the group sizes and reduced the power to detect between-subject differences. With advances in neuroimaging, it seems feasible to examine central measures of NA and 5-HT, which may clarify the neurobiological differences between the two subtypes.

Limitations

Our data should be interpreted in the light of certain inherent limitations, given the clinical nature of the sample. First, patients received a regular dose of benzodiazepines, which may suppress NA or 5-HT activity (Finlay et al., 1995). While we statistically controlled for this influence, we cannot completely exclude the confounding effects of medications. Secondly, we considered day 0 as the day of admission, which may not have corresponded to beginning of withdrawal for all patients. Nevertheless, based on breathalyser values and clinical evaluations, we felt that the four time points represented early as well as late withdrawal. Thirdly, we used a single blood sample for the NA and 5-HT measurements in controls as opposed to four samples in alcohol-dependent patients. Fourthly, because we excluded patients with current substance misuse, this may have disproportionately excluded severe type II alcoholic individuals, who frequently misuse illicit substances. Also, it is unclear the extent to which blood levels of NA and 5-HT reflect changes in function in the brain, although central and peripheral measures of NA and 5-HT have been correlated in some studies (Kopin et al., 1983; Da Prada et al., 1988). Finally, since the sample was restricted to Caucasian men, our findings may not be generalizable to individuals from other ethnic backgrounds and women.

We conclude that the present study demonstrates that plasma NA and 5-HT levels change significantly during alcohol withdrawal in humans. The normalization of NA activity with brief abstinence suggests that the withdrawal-related NA changes may be a state-dependent phenomena. In contrast, the clinical significance of low 5-HT levels at the end of withdrawal need to be explored. Although NA or 5-HT measures do not appear to influence craving for alcohol, it is possible they may affect other factors such as negative mood states that may be important contributors to relapse. Examining the relationships between NA and 5-HT mechanisms during different stages of alcohol dependence may clarify the importance of these systems in mediating the addictive properties of alcohol.

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