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Alterations of BDNF and GDNF serum levels in alcohol-addicted patients during alcohol withdrawal

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ABSTRACT – Background and Objectives: Brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) are neurotrophic neuropeptides that play important roles in the synaptic plasticity, neuronal growth, survival and function. A possible neuroprotective role of neurotrophic factors against alcohol-induced cell damage has been suggested, and dysregulations in neurotrophic factors may be involved in the vulnerability to addiction. The aim of this study was to investigate the alterations of BDNF and GDNF serum levels in alcohol-addicted patients during alcohol withdrawal compared to healthy controls.

Methods: BDNF and GDNF serum levels of 34 male inpatients diagnosed with alcohol addiction according to DSM-IV-TR were investigated during alcohol withdrawal (day 1, 7 and 14) in comparison to 32 healthy controls using an enzyme-linked immunosorbent assay (ELISA). Severity of alcohol withdrawal was measured by Clinical Institute Withdrawal Assessment for Alcohol (CIWA-Ar), and intensity of alcohol craving was measured by Penn Alcohol Craving Scale (PACS) during alcohol withdrawal (day 1, 7 and 14).

Results: BDNF serum levels increased significantly during alcohol withdrawal ($p = 0.020$). They were negatively correlated to the severity of alcohol withdrawal, and the correlation was close to being statistically significant ($p = 0.058$). BDNF and GDNF serum levels did not differ significantly between the patient and control groups. GDNF serum levels did not change significantly during alcohol withdrawal.

Conclusions: Our results may provide support for the previously hypothesized role of BDNF in the neuroadaptation during alcohol withdrawal.

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Introduction

Alcohol addiction is a chronic relapsing disorder characterized by repetitive and uncontrolled alcohol drinking patterns. Brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) are neurotrophic neuropeptides that play important roles in the synaptic plasticity, neuronal growth, survival and function¹⁻³. A possible neuroprotective role of neurotrophic factors against alcohol-induced cell damage has been suggested, and dysregulations in neurotrophic factors may be involved in the vulnerability to addiction and in the brain damage caused by long-term alcohol exposure¹⁻⁷.

Animal studies have shown that acute alcohol consumption results in increased BDNF expression, whereas prolonged alcohol consumption is associated with decreased BDNF expression^{8,9}. Acute releases in BDNF during initial exposure to alcohol tends to degrade during chronic alcohol consumption, which may increase the severity of withdrawal symptoms^{8,10,11}. Moreover, it has been reported that increased behavioral responses to alcohol in rats is associated with decreased BDNF expression⁹. BDNF is one of the most abundant neurotrophic factors expressed in the central nervous system, and it is also present in human peripheral blood^{7,12}. Clinical studies have reported contradictory results including decreased, increased and nonaltered BDNF serum levels of alcohol-addicted patients^{1,3,5,7,13-15}. Recent studies have suggested the association between acute withdrawal severity and BDNF serum levels^{3,16,17}.

GDNF is an essential growth factor for the development, survival of midbrain dopamine neurons, and it may play a neuroprotective role of neurotrophic factors against alcohol-induced cell damage^{2,18}. Animal studies have suggested that GDNF may be a negative reg-

ulator of biochemical and behavioral adaptations in alcohol addiction, and it suppresses self-administration of alcohol¹⁸⁻²⁰. There are a few clinical study results that show alterations of GDNF serum levels in alcohol addiction or abuse. It has been reported that GDNF serum levels are reduced during chronic alcohol consumption and withdrawal period³, and young individuals with alcohol abuse have increased GDNF serum levels⁶.

To validate recent study results and to better clarify the putative dysregulations of neurotrophic factors in alcohol addiction, we investigated the alterations of BDNF and GDNF serum levels in alcohol-addicted patients during alcohol withdrawal compared to healthy controls and the possible associations between BDNF and GDNF serum levels and clinical features related to drinking behavior.

Materials and methods

The present study was a part of the research project (Evaluation of the levels of biochemical markers and neurotrophic factors in patients with alcohol use disorder) approved by the local Ethics Committee of the Trakya University Faculty of Medicine (Project No: 2012-116). All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study. We investigated BDNF and GDNF serum levels of 34 male inpatients who were diagnosed with alcohol addiction according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-TR) and were admitted for detoxification treatment from June 2013 to September 2014 (the Alcohol and

Substance Addiction Treatment and Rehabilitation Center, Trakya University Faculty of Medicine, Edirne, Turkey). Exclusion criteria were axis one psychiatric diagnoses apart from alcohol and nicotine use disorders according to the DSM-IV-TR, delirium tremens, severe neurological diseases such as epilepsy and cerebrovascular diseases, significant physical illnesses such as cardiovascular, hepatic and renal diseases, and intake of psychopharmacological medication.

We assessed the patients with an initial clinical interview to ascertain their DSM-IV-TR diagnoses. All patients underwent a detailed physical examination, routine laboratory testing and urine drug screening. Alcohol consumption was stopped immediately and completely at admission. Patients were treated by various doses of diazepam, which were adjusted by the severity of their withdrawal symptoms and tapered gradually during alcohol withdrawal. The cumulative benzodiazepine dosage used during detoxification was recorded and documented as milligrams of diazepam (minimum: 115 mg, maximum: 740 mg, mean: 318.09 ± 161.49 mg). Oral vitamin B complex (including B1, B6, B12) supplementation were also given to every participant. There was no further psychopharmacological treatment during alcohol withdrawal. Measurements of breath alcohol concentration were performed on admission and during alcohol withdrawal using the alcohol breath analyzer.

The control group included 32 healthy male subjects without known psychiatric and physical illnesses and with normal results for routine laboratory tests. Controls were assessed with an initial clinical interview and screened with the Alcohol Use Disorder Identification Test (AUDIT)^{21,22}. They were negative for alcohol use disorders and any other axis one diagnosis according to DSM-IV-TR. A score below 7 points in the AUDIT was re-

quired for inclusion in the control group. They were not receiving any therapeutic treatment.

BDNF and GDNF serum levels were investigated on the next morning of admission for detoxification (day 1), day 7 and day 14 of alcohol withdrawal and were compared to the serum levels of the healthy control group. Fasting blood samples were taken on admission between 8 and 10 am. All blood samples were centrifuged and stored at -80°C immediately after collection until they were studied. BDNF and GDNF serum levels were assessed using the enzyme-linked immunosorbent assay (ELISA) kits (Boster Biological Technologies, CA, USA). All the assays were performed according to the manufacturer's directions. The BDNF and GDNF values were defined as ng/ml and pg/ml, respectively. Determination of further blood parameters (including aspartate transaminase [AST], alanine transaminase [ALT], gamma-glutamyl transferase [GGT] and complete blood count) was performed using routine clinical laboratory methods.

Additional data (such as sociodemographic data, body mass index [BMI], history of alcohol drinking and smoking behavior) were obtained in a structured interview. Intensity of alcohol craving was measured by the Penn Alcohol Craving Scale (PACS)^{23,24}. Severity of alcohol withdrawal was measured by the Clinical Institute Withdrawal Assessment for Alcohol (CIWA-Ar)²⁵. These measurements were taken once a day on day 1, day 7 and day 14.

Statistical analysis

Descriptive statistics for continuous variables were shown as mean \pm standard deviation (SD), categorical variables were expressed as number of cases (n) and (%). According to the normality of distribution, group differ-

ences were investigated using the Student's *t*-test or the Mann Whitney U test, and alterations of the serum levels during alcohol withdrawal were assessed using the one-way analysis of variance (ANOVA) with repeated measures or the Friedman test. Categorical variables were evaluated by the Pearson's Chi square test. Correlations between the BDNF and GDNF levels and other variables were analyzed by the Pearson's correlation coefficient or the Spearman's rank correlation coefficient. Analyses were carried out using the statistical package for the social sciences (SPSS) version 20. Statistical significance was defined as a two-sided *p* value of < 0.05 .

Results

The mean age \pm SD and the age range of the alcohol-addicted patients were 45.44 ± 8.98 years and 28 to 61 years, respectively, and no difference was observed from those of the control group (41.78 ± 13.15 years and 27 to 64 years). Characteristics of the alcohol-addicted patients compared with the healthy controls are shown in table 1. There were significant differences between the two groups with regard to BMI, average alcohol consumption amount in the past month, smoker percentage and smoking amount. 29.41% of the patients and 28.13% of the controls reported a positive family history of alcohol addiction. BDNF and GDNF serum levels of the alcohol-addicted patients were not significantly correlated with age (Pearson's $r = -0.186$, $p = 0.291$ [BDNF, day 1]; $r = 0.195$, $p = 0.269$ [GDNF, day 1]), BMI ($r = 0.167$, $p = 0.396$ [BDNF, day 1]; $r = -0.147$, $p = 0.446$ [GDNF, day 1]), duration of regular alcohol consumption ($r = 0.056$, $p = 0.755$ [BDNF,

day 1]; $r = 0.250$, $p = 0.154$ [GDNF, day 1]), drinking amount in the past month ($r = -0.160$, $p = 0.366$ [BDNF, day 1]; $r = -0.056$, $p = 0.755$ [GDNF, day 1]), smoking amount (cigarettes/day) (Spearman's $\rho = 0.029$, $p = 0.874$ [BDNF, day 1]; $\rho = 0.021$, $p = 0.908$ [GDNF, day 1]) and smoking amount (pack-years) ($r = -0.194$, $p = 0.280$ [BDNF, day 1]; $r = -0.138$, $p = 0.445$ [GDNF, day 1]).

BDNF and GDNF serum levels of alcohol-addicted patients and healthy controls

Blood parameters of alcohol-addicted patients and healthy controls, and clinical features of alcohol withdrawal are shown in table 2. BDNF serum levels of the patient group did not differ significantly from BDNF serum levels of the healthy control group ($t = -1.212$, $p = 0.230$ [day 1]; $t = 0.538$, $p = 0.592$ [day 7]; $t = 1.578$, $p = 0.121$ [day 14]). BDNF serum levels increased significantly during alcohol withdrawal ($F = 4.221$, $p = 0.020$). In pairwise comparisons, the BDNF serum levels on day 14 were significantly higher than those on day 1 ($p = 0.016$). There was no significant difference in other pairwise comparisons (between day 1 and day 7, $p = 0.363$; between day 7 and day 14, $p = 0.677$).

GDNF serum levels of the patient group did not differ significantly from GDNF serum levels of the healthy control group ($z = -1.585$, $p = 0.113$ [day 1]; $z = -0.436$, $p = 0.663$ [day 7]; $z = -0.630$, $p = 0.529$ [day 14]). GDNF serum levels did not change significantly during alcohol withdrawal ($\chi^2 = 0.500$, $p = 0.779$). BDNF and GDNF serum levels of the alcohol-addicted patients during alcohol withdrawal compared with the healthy controls are shown in figure 1.

Table 1

Characteristics of alcohol-addicted patients compared with healthy controls

	Patients (n = 34)	Controls (n = 32)	Test	p
Age (years)	45.44 ± 8.98	41.78 ± 13.15	t = 1.327	0.189
Body mass index	25.03 ± 4.22	26.98 ± 2.98	t = -2.088	0.041
Regular alcohol consumption (years)	16.32 ± 8.82	–	–	–
Drinking amount (g of ethanol/last month)	6231.33 ± 2304.27	166.09 ± 215.81	z = -7,020	<0.001
Positive family history (%)	29.41 (n = 10)	28.13 (n = 9)	$\chi^2 = 0.013$	0.908
Smokers (%)	97.06 (n = 33)	56.25 (n = 18)	$\chi^2 = 15.632$	<0.001
Smoking amount (cigarettes/day)	25.46 ± 9.63	6.47 ± 9.05	z = -5.832	<0.001
Smoking amount (pack-years)	26.85 ± 12.85	12.25 ± 17.55	t = 3.835	<0.001

Table 2

Blood parameters of alcohol-addicted patients and healthy controls, and clinical features of alcohol withdrawal

	Patients (mean ± SD)			Controls (n = 32) (mean ± SD)
	Day 1 (n = 34)	Day 7 (n = 34)	Day 14 (n = 28)	
BDNF (ng/ml)	39.56 ± 17.09	47.02 ± 17.25	51.47 ± 15.46	44.72 ± 17.52
GDNF (pg/ml)	26.82 ± 25.96	38.46 ± 39.99	37.32 ± 35.87	43.84 ± 42.33
GGT (U/l)	226.41 ± 253.81	151.88 ± 162.91	86.24 ± 64.52	32.41 ± 49.23
MCV (fl)	94.94 ± 7.36	95.77 ± 6.03	94.95 ± 5.96	86.34 ± 5.38
ALT(U/l)	46.79 ± 30.79	43.41 ± 23.77	31.00 ± 12.14	22.88 ± 11.47
AST(U/l)	58.71 ± 35.29	38.15 ± 18.41	30.69 ± 10.61	20.25 ± 4.73
TC (x10 ³ /mm ³)	218.47 ± 60.87	260.63 ± 77.49	272.44 ± 70.58	221.72 ± 59.42
AUDIT Score	31.68 ± 5.30	–	–	–
PACS Score	21.74 ± 7.94	10.94 ± 8.61	6.75 ± 6.19	–
CIWA-Ar Score	9.79 ± 5.64	4.41 ± 3.62	1.47 ± 1.54	–

BDNF: brain derived neuronal factor, GDNF: glial cell-line derived neurotrophic factor, GGT: gamma-glutamyl transferase, MCV: mean corpuscular volume, ALT: alanine transaminase, AST: aspartate transaminase, TC: thrombocyte count, AUDIT: Alcohol Use Disorder Identification Test, PACS: Penn Alcohol Craving Scale, CIWA-Ar: Clinical Institute Withdrawal Assessment for Alcohol.

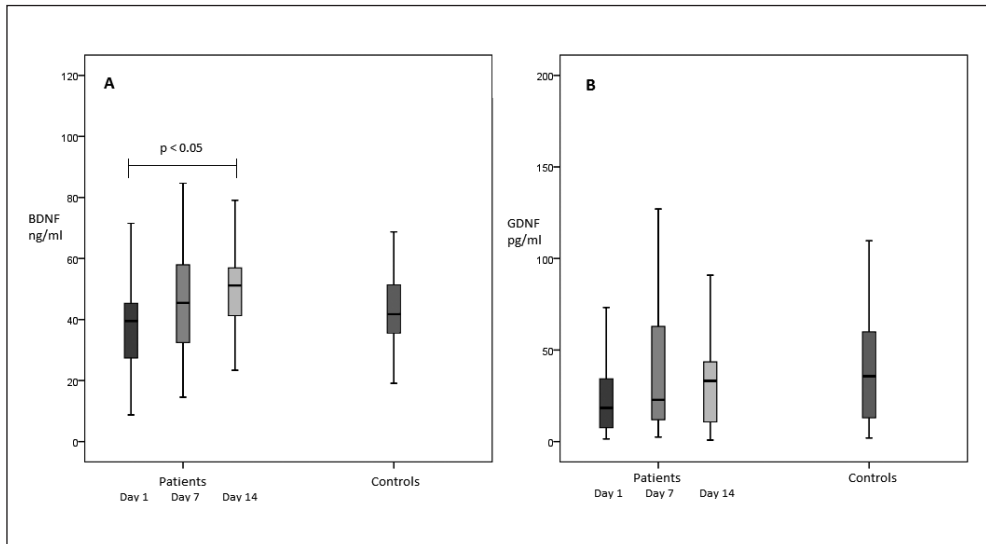


Figure 1. BDNF and GDNF serum levels of alcohol-addicted patients during alcohol withdrawal compared with healthy controls.

(A) BDNF serum levels increased significantly during alcohol withdrawal ($F = 4.221$, $p = 0.020$).

BDNF serum levels on day 14 were significantly higher than those on day 1 ($p = 0.016$).

BDNF serum levels of the patient group did not differ significantly from those of the control group ($t = -1.212$, $p = 0.230$ [day 1]; $t = 0.538$, $p = 0.592$ [day 7]; $t = 1.578$, $p = 0.121$ [day 14]).

(B) GDNF serum levels of the patient group did not differ significantly from those of the control group ($z = -1.585$, $p = 0.113$ [day 1]; $z = -0.436$, $p = 0.663$ [day 7]; $z = -0.630$, $p = 0.529$ [day 14]).

GDNF serum levels did not change significantly during alcohol withdrawal ($\chi^2 = 0.500$, $p = 0.779$).

Correlations between BDNF, GDNF and other variables in alcohol-addicted patients

BDNF serum levels were significantly correlated with thrombocyte count (TC) ($r = 0.494$, $p = 0.004$ [day 1]) and negatively correlated with GGT ($\rho = -0.402$, $p = 0.022$ [day 1]). BDNF was not significantly correlated with GDNF ($r = 0.364$, $p = 0.480$ [day 1]), MCV ($r = -0.122$, $p = 0.500$ [day 1]), ALT ($r = 0.229$, $p = 0.193$ [day 1]) and AST ($r = -0.065$, $p = 0.716$ [day 1]).

GDNF serum levels were not significantly correlated with GGT ($\rho = -0.140$, $p = 0.445$ [day 1]), MCV ($r = -0.086$, $p = 0.636$ [day 1]), ALT ($r = 0.028$, $p = 0.873$ [day 1]),

AST ($r = -0.169$, $p = 0.340$ [day 1]) and TC ($r = -0.015$, $p = 0.932$ [day 1]).

BDNF serum levels were negatively correlated to the severity of alcohol withdrawal measured by the CIWA-Ar, and the correlation was close to being statistically significant ($r = -0.329$, $p = 0.058$ [day 1]). BDNF was not significantly correlated with AUDIT scores ($r = 0.049$, $p = 0.784$ [day 1]) and PACS scores ($r = -0.236$, $p = 0.179$ [day 1]).

GDNF serum levels were not significantly correlated with AUDIT scores ($r = -0.129$, $p = 0.468$ [day 1]), CIWA-Ar scores ($r = -0.114$, $p = 0.521$ [day 1]) and PACS scores ($r = -0.212$, $p = 0.228$ [day 1]). BDNF and GDNF serum levels were not significantly correlated with the cumulative benzodiazepine dosage

used during alcohol withdrawal ($r = -0.083$, $p = 0.641$ [BDNF, day 1]; $r = 0.084$, $p = 0.636$ [GDNF, day 1]).

BDNF and GDNF serum levels were not significantly correlated with the further blood parameters (GGT, MCV, ALT, AST and TC), CIWA-Ar and PACS scores on day 7 and day 14 (data not shown).

Discussion

To the best of our knowledge, this is the second clinical study to investigate the alterations of BDNF and GDNF serum levels in alcohol-addicted patients during alcohol withdrawal compared to healthy controls and the possible associations between these serum levels and clinical features related to drinking behavior. Our main findings are as follows: (a) BDNF and GDNF serum levels did not differ significantly between the patient and control groups; (b) BDNF serum levels increased significantly during alcohol withdrawal; (c) BDNF serum levels were negatively correlated to the severity of alcohol withdrawal, and the correlation was nearly statistically significant; and (d) GDNF serum levels did not change significantly during alcohol withdrawal.

Preclinical studies have shown that acute alcohol consumption results in increased BDNF expression, whereas prolonged alcohol consumption is associated with decreased BDNF expression^{8,9}. The acute release of BDNF during initial exposure to alcohol tends to degrade during chronic alcohol consumption, which may increase the severity of withdrawal symptoms^{8,10,11}. Preclinical studies have suggested that GDNF may be a negative regulator of biochemical and behavioral adaptations in alcohol addiction, and it sup-

presses the self-administration of alcohol¹⁸⁻²⁰. Neurotrophic factors may exert a possible neuroprotective role against alcohol consumption and neurotoxicity in the early stages, and their levels and protective effects may decrease along with the development of alcohol addiction^{3,6}.

Our GDNF-related results are contrary to those of a previous clinical study, which investigated the alterations of BDNF and GDNF serum levels in alcohol-addicted patients during alcohol withdrawal compared to healthy controls. Heberlein *et al*³ reported reduced GDNF serum levels during chronic alcohol consumption and the withdrawal period and a negative association of its serum levels with alcohol tolerance. These findings supported the opinion that reduced GDNF serum levels may indicate the reduced capability of neuronal repair of neuronal damage, as is frequently observed in alcohol-addicted patients³. Our results do not confirm this assumed role of the GDNF serum levels. Because the knowledge about peripheral GDNF levels in alcohol-addicted patients is limited, further studies are warranted to better clarify the alterations in GDNF serum levels during acute/chronic alcohol consumption and the withdrawal period.

In the literature, there are studies that report lower peripheral BDNF levels in alcohol-addicted patients^{5,7,13,26} along with studies that report similar levels to those in healthy controls^{1,3,14,16}. We found no significant alterations between the BDNF serum levels of the patient and control groups. Our results are consistent with previous clinical studies that reported that BDNF levels increase during abstinence^{1,15,16,26}; however, some studies indicate no change in serum BDNF levels during alcohol withdrawal³ or a tendency to decrease in the early stages of abstinence¹³.

The differences between the results obtained in these studies may be partially ex-

plained by different sample sizes, clinically heterogeneous cohorts of patients, different strategies of alcohol detoxification treatment, and genetic differences in the BDNF system^{3,27}. We investigated BDNF and GDNF serum levels in a sample limited to male inpatients that included small number of participants. BDNF and GDNF serum levels did not differ significantly between the patient and control groups, even though the male inpatients might be in a relatively severe stage of alcohol addiction. This might be due to the limited number of subjects. Patients in our study were treated by various doses of diazepam, which were adjusted by the severity of their withdrawal symptoms and tapered gradually during alcohol withdrawal. BDNF and GDNF serum levels were not correlated with the cumulative benzodiazepine dosage, but we could not rule out the effect of the medication used in alcohol detoxification. We investigated the alterations of BDNF and GDNF levels for 2 weeks among the patients undergoing withdrawal. It has been reported that patients abstaining for at least 30 days have lower BDNF levels than controls⁵, and patients who remained abstinent during the 6 months following alcohol detoxification have higher BDNF levels than non-abstinent patients and controls¹. The difference between patients and controls with regard to smoker percentage and smoking amount could have confounded the results. Although BDNF and GDNF serum levels were not correlated with the degree of smoking (cigarettes/day and pack-years), we could not rule out the potential effect of smoking on neurotrophin levels. The difference with regard to BMI between patients and controls could influence the results, even though BDNF and GDNF serum levels were not correlated with BMI. Furthermore, the psychological stress associated with the hospitalization for detoxification treatment may affect BDNF and GDNF serum levels, and measuring concomitant cor-

tisol levels as a possible stress indicator could be helpful to clarify this issue^{7,28}.

Recent studies have suggested the association between serum BDNF levels and acute withdrawal severity^{3,16,17}. In our study, BDNF serum levels were negatively correlated with the severity of alcohol withdrawal measured by the CIWA-Ar, and the correlation was close to being statistically significant. Moreover, BDNF serum levels were significantly correlated with TC and negatively correlated with GGT. It has been reported that circulating BDNF is released by thrombocytes²⁹. While we observed a tendency to increase in TC during alcohol withdrawal, BDNF serum levels were not significantly correlated with TC on day 7 and day 14. TC variations may contribute to the differences in BDNF serum levels, but it is widely accepted that peripheral BDNF levels reflect the BDNF levels in the brain³⁰⁻³². We observed a decreasing tendency in GGT levels during alcohol withdrawal, but BDNF serum levels were not significantly correlated with GGT on day 7 and day 14. BDNF expression is also detectable in the liver, which is often damaged during chronic alcohol consumption³³. If the increased BDNF levels during alcohol withdrawal were caused by liver regeneration, they should have correlated with GGT levels¹.

In conclusion, the significant increase of BDNF serum levels during alcohol withdrawal and the negative correlation of these serum levels with the severity of alcohol withdrawal may provide support for the previously hypothesized role of BDNF in neuroadaptation during alcohol withdrawal. Our results do not support the opinion that reduced GDNF serum levels may indicate the reduced capability of neuronal repair of neuronal damage, as is frequently observed in alcohol-addicted patients. The small number of participants used to generate the results of the study is a severe limitation of this study. Further

studies including larger samples and the use of a standardized medication schedule during alcohol withdrawal are required to better determine the profile of neurotrophic factors in different stages of alcohol addiction and during early/late periods of alcohol abstinence.

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