Effects of Chronic Alcoholic Disease on Magnocellular and Parvocellular Hypothalamic Neurons in Men

E. V. Sivukhina[1, 2*]
A. A. Dolzhikov[2]
Iu. E. Morozov[3]
G. F. Jirikowski[1]
V. Grinevich[4, 5**]

Abstract

Although numerous data showing severe morphological impairment of magnocellular and parvocellular hypothalamic neurons due to chronic alcoholic consumption have been gathered from animal experiments, only one study (Harding et al., 1996) was performed on *post mortem* human brain. This study showed a reduction in the number of vasopressin (VP)-immunoreactive neurons in the supraoptic (SON) and paraventricular (PVN) nuclei, but did not provide any data regarding the effect of chronic alcohol intake on human parvocellular neurons. In order to assess whether the changes observed in the animal model also occur in humans and provide a structural basis for the results of clinical tests, we performed immunohistochemical and morphometric analysis of magnocellular (VP and oxytocin, OT) and parvocellular (corticotropin-releasing hormone, CRH) neurons in post-mortem brains of patients afflicted with chronic alcoholic disease. We analyzed 26-male alcoholics and 22 age-matched controls divided into two age groups – "young" (< 40 yr) and "old" (> 40 yr). Hypothalamic sections were stained for OT, VP, and CRH. The analysis revealed: 1) decrease in VP-immunoreactivity in the SON and PVN as well as OT-immunoreactivity in the SON in alcoholic patients; 2) increase in OT-immunoreactivity in the PVN; 3) increase in CRH-immunoreactivity in parvocellular neurons in the PVN. Furthermore, the proportion of cells containing CRH and VP was increased in alcoholics. These findings indicate that chronic alcohol consumption does indeed impair the morphology of magnocellular neurons. The enhancement of CRH-immunoreactivity and increased co-production of CRH and VP in parvocellular neurons may be due to a decline in glucocorticoid production, implied by the hypoplasic impairment of adrenal cortex we observed in alcoholics during the course of this study.

Key words
Vasopressin · oxytocin · corticotrophin-releasing hormone · human · alcoholic disease

Introduction

Chronic alcohol exposure profoundly modifies the morphology and function of certain brain structures [1], including hypothalamic neuroendocrine centers. Due of the side effects of alcohol intake – dehydration and change in serum osmolarity – many studies focus on the hypothalamic neurohormone vasopressin (VP), which is synthesized in magnocellular hypothalamic nuclei, supraoptic (SON) and paraventricular (PVN) nuclei, and released into peripheral blood circuitry from the posterior pituitary lobe. A number of studies in rodents demonstrated the inhibition of VP synthesis by chronic alcohol intake [2,3]. In the rat model, prolonged ethanol exposure leads to a selective loss of VP-ergic neurons in the SON [4] and PVN [5], and an elevation in the VP mRNA levels in the remaining magnocellular neurons [4,6]. In the only study on the effects of chronic alcohol abuse in humans, Harding and colleagues [7] demonstrated a considerable loss of VP-immunoreactive neurons in magnocellular nuclei of alcoholics. After prolonged alcohol intake, plasma levels of VP remained unchanged in both humans [8–10] and rodents [4], but in humans, VP response to an osmotic challenge was reduced [8]. The effects of alcohol on the second population of magnocellular neurons, which produce oxytocin (OT), are much less known. No changes in OT mRNA have been reported in rats subjected to prolonged ethanol exposure [5]. In contrast, plasma levels of OT are elevated in human alcoholics [10]. Post-mortem morphological analysis, however, was not performed.

Ethanol also has an effect on parvocellular hypothalamic neurons. Of particular interest are those that produce corticotropin-releasing hormone (CRH) [11,12]. As a central neurohormone of the hypothalamic-pituitary-adrenal (HPA) axis, CRH triggers adrenocorticotropic hormone (ACTH) and glucocorticoid secretion in response to a variety of acute and chronic stimuli [13]. CRH is co-produced with VP, which potentiates effects of CRH on pituitary corticotrophs, especially under chronic stress paradigms [14–16]. Prolonged alcohol intake in rats blunts the expression of both CRH and VP mRNAs in the parvocellular PVN [17]. In actively drinking men, lower basal plasma levels of stress hormones were detected when compared to non-alcoholics, while alcoholics showed a blunted ACTH response in the CRH stimulation test [18]. However, there is no evidence that ethanol induces death or damage in CRH neurons [17].

In order to provide a structural basis for the results of clinical tests and to compare animal models with changes in humans, we performed a morphological analysis of magnocellular (VP and OT) and parvocellular (CRH) neurons in post-mortem brains of patients afflicted with chronic alcoholic disease. We studied VP- and OT-immunoreactivity in magnocellular neurons from the hypothalamic nuclei and in the posterior pituitary. To characterize the activity of the HPA axis in alcoholics, we analyzed the content of CRH-immunoreactive product and its co-localization with VP in the hypothalamic PVN, as well as the morphology of the adrenal gland.

Materials and Methods

Tissue collection

Brains from patients that had given their informed consent for brain autopsy and the use of brain tissue and medical files for research purposes were obtained at autopsy from the Department of Pathology, Emergency Hospital, Kursk, Russia. The study was approved by the Local Ethics Committee of the Kursk State Medical University Council. Brains from 26 male patients that had died of alcoholic disease (alcoholic encephalopathy) ranging in age from 25 to 64 years (41.6 ± 2.2) were obtained at autopsy. In all cases, alcoholic disease was verified by several pathological criteria – liver fat degeneration, hepatocirrhosis, alcoholic cardiomyopathy and encephalopathy (fibrosis of pia mater, atrophic changes of a brain matter, injuries of cerebral blood vessels with an increase in their penetration and diapedesis; [19,20]). Patients died of brain edema, acute heart failure or respiratory failure. Retrospective analysis of the patients' drinking history revealed an alcoholic binge of three weeks to six months in duration, which ended in admission to the hospital one to three days prior to death.

The control group contained samples of brains from 22 male individuals (mean age: 53.4 ± 3.9) without alcoholic pathology or primary neurological diseases; these patients had died of non-alcohol-related causes – acute brain stroke (6), acute or recurrent myocardial infarction (5), diseases of the respiratory system (3), acute surgical pathology (2), and others (6). Postmortem time in all cases was 4–12 hours. The control and the alcoholic patients were divided into two groups: younger than 40 yrs (30 ± 2.8 and 32 ± 1.3 correspondingly) termed the "young" group and older than 40 yrs (59.4 ± 2.7 and 49.9 ± 1.9 correspondingly) termed the "old" group.

Hypothalami were dissected from the optic chiasm to the posterior surface of the mammillary bodies and fixed by immersion in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS, pH 7.4) at room temperature for about two months. The pituitaries were dissected and fixed separately. In some cases (n = 5, young alcoholic group; n = 3, old alcoholic group; n = 8, age-matched controls) the left and right adrenal glands were collected from each autopsy and fixed in 4% saline-buffered PFA for about two months. All tissue samples were dehydrated in graded ethanol, embedded in paraffin and cut into serial 6 µm sections. Hypothalami were sectioned serially in coronal plane from prechiasmatic part to mammillary bodies. One series of sections was stained with hematoxylin and eosin for evaluation of changes in hypothalamic neurons induced by chronic alcohol consumption. Consecutive unstained sections were used for further immunohistochemical staining. Pituitaries were sectioned in horizontal plane from the top to bottom. Sections of the middle part of the pituitary containing the posterior lobe were selected for immunohistochemistry. Adrenal glands were sectioned in frontal plane. Sections containing well-appeared medulla were stained with hematoxylin and eosin for analysis of morphological changes in alcoholic patients.

Hematoxylin and Eosin Staining

Sections of hypothalami and adrenal glands were deparaffinized in xylene and rehydrated through a graded ethanol series. The sections were than immersed in Ehrlich's hematoxylin for 10 min, differentiated in 70% ethanol and rinsed in distilled water. After that, the sections were counterstained in alcoholic eosin solution containing 1% eosin for 3 min. Following washing under running water, sections were dehydrated, cleared in xylene and cover-slipped.

Immunohistochemistry

Consecutive sections of pituitaries and hypothalami containing the dorsolateral part of the SON and PVN from each subject were stained with antisera to VP, OT, or CRH. After deparaffinization and rehydration, slides were preincubated for an hour at room temperature (RT) in 1% Triton X 100-phosphate buffered saline containing 5% normal goat serum (PBS-NGS). Overnight incubation with one of the primary rabbit polyclonal antibodies – anti-VP or anti-OT (Chemicon International, CA; 1 : 1,000), or anti-CRH (Peninsula Laboratories Inc., Division of Bachem, CA, USA; 1 : 1,000) in working solution (1% NGS, 0.3% Triton on PBS, pH 7.4) was performed in humid chambers at 4 C. After that, sections were washed in PBS for an hour and incubated in working solution with biotinylated goat anti-rabbit IgG (Vector Elite kit, Vector Laboratories, Inc., Burlingame, CA; 1 : 500) for two hours at RT. After washing in PBS for an hour, ABC complex (Vector Laboratories, CA) in PBS was applied (1 h, RT). Finally, after washing in PBS, the reaction was visualized with freshly prepared glucose-oxidase-diaminobenzidine (GOD-DAB) as described by Zaborsky and Heimer [21]. After rinsing in distilled water, sections were dehydrated through ascending concentrations of ethanol, cleared in xylene and mounted with embedding medium (OTC, Germany). For control incubations we used either normal rabbit serum, diluted 1 : 1,000 in PBS-Triton instead of the primary antibodies or anti-OT (anti-VP or anti-CRH), preabsorbed with synthetic OT (VP or CRH) (Sigma).

Double immunostaining for CRH and VP was performed as previously described [22]. Briefly, sections were incubated with the rabbit polyclonal anti-CRH overnight at 4 C. After that, biotinylated goat anti-rabbit IgG was applied followed by avidin-horseradish peroxidase complex (Vector Laboratories, 1 : 100). Immunoprecipitates were visualized by DAB (Sigma). Next, sections were treated for 15 min in 0.1 M glycine buffer at pH 3.4 in order to remove the immunocomplexes. Subsequently, sections were incubated overnight in the rabbit polyclonal anti-VP and developed by Cy3-labeled goat anti-rabbit Fab fragment (Dianova) at 1 : 200. After washing, sections were mounted onto glass slides in the mixture of veronal-glycerol (2 : 8). We counted the total number of CRH-positive neurons and the number of CRH neurons in the VP-immunoproduct from the PVN. Then the percentage of single-labeled and double-labeled CRH-positive cells was calculated. Sections were examined with an Olympus BX50 microscope. An Olympus DP10 digital camera with DP-Soft 3.0 software was used for microphotography.

Quantification of immunoreactivity in neurons of hypothalamic nuclei

Areas and spectrum of the optical density of DAB-immunoreactive products in the neurons and posterior pituitary lobe were defined in immunocytochemically stained VP, OT, or CRH sections with the ImageTool (University of Texas Health Science Center, USA) and ImageJ (NIH) software packages. Four sections of each sample that were stained either with VP, OT or CRH antibodies were processed for semiquantitative analysis. Correlation of measured area (in pixels) and average density of the gray level were calculated (in arbitrary units, a.u.) for an estimation of the relative optical density of immunoreactive products. On the sections stained for VP, OT or CRH, areas of the cell somata and the nuclei of the neurons were also measured using ImageTool. After that, absolute and relative (in %) areas of immunoreactive products were calculated.

Statistics

The distinctions in the mean all measured parameters between the subjects as a whole and among the different age groups were tested using MS Excel 7.0. *t*-test was used for statistical analysis; $p < 0.05$ was considered significant. Data are presented as the mean ± SE of values as indicated.

Results

Effects of chronic alcoholic disease on magnocellular neurons of the SON and PVN

Histological examination of the SON and PVN revealed pyknosis, cytokaryolysis (Fig. **1 a**) and pericellular edema (Fig. **1 b**) in a fraction of magnocellular neurons.

After immunohistochemical staining for OT and VP, similar cellular distribution within the SON and PVN was observed in both control and alcoholic groups. The largest part of the SON – the dorsolateral (prechiasmatic) part – contained predominantly VP-positive neurons (Fig. **1 c**); only few OT-positive neurons were present in the cap of this part (Fig. **1 e**). In the PVN, the proportion of OT and VP-immunoreactive neurons was relatively equal (Fig. **1 g, i**).

In controls, a clear reduction of VP- and OT- immunoreactivity in the SON and PVN was found in patients in the old group (Table **1**). Analysis of the SON in the young alcoholic group revealed no changes in OT and VP immunoreactivity (Table **1**). However, profound reduction of OT and VP-positive profiles was observed in the SON of the old group (Fig. **1 d, f**; Table **1**). In the PVN from alcoholics, VP-immunoproduct was significantly reduced in both age groups (Fig. **1 h**; Table **1**). In contrast, pronounced increase in OT immunoreactivity was observed in elder patients (Fig. **1 j**; Table **1**).

Effects of chronic alcoholic disease on the posterior pituitary

Relatively equal and moderate distribution of VP and OT immunoproducts was observed in controls (Fig. **2 a, c**), with no difference in the young and old groups in optic immunoreactivity density (Fig. **2 e**). In alcoholics, irregular distribution of VP signal with "lump-like" accumulations in terminals occurred in both groups (Fig. **2 b**). General reduction of optic density of VP-immunoreactivity was found in the young group (Fig. **2 e**). In spite of this, OT

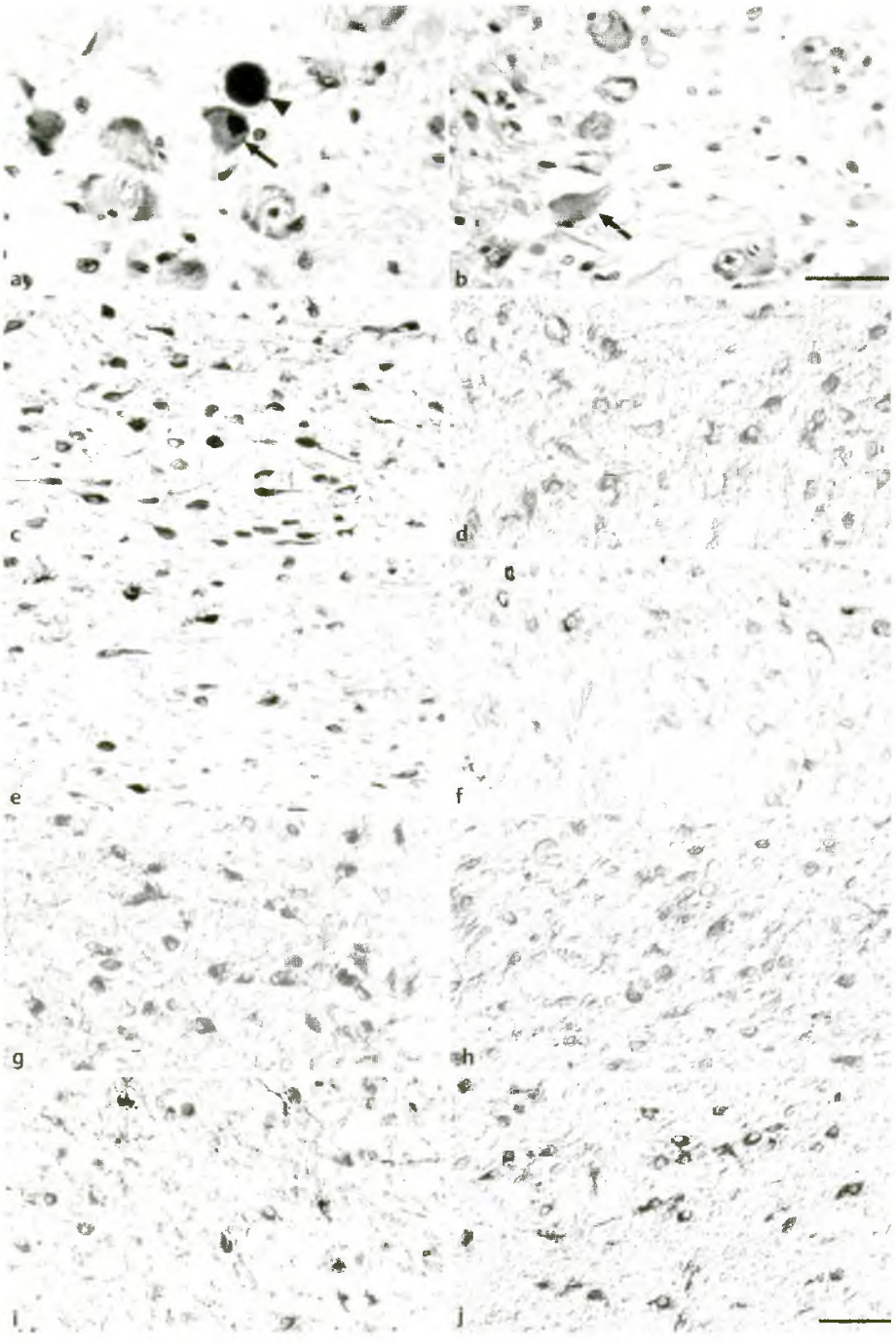


Fig. 1 Morphology of magnocellular neurons and VP- and OT-immunoreactivity in the SON and PVN in alcoholics. **a** Normal-appearing neurons are present near to pyknotic (arrow) and cytokaryolytic (arrowhead) neurons in the SON of a 42-year-old alcoholic. **b** A neuron with pericellular edema (arrow) is located near normal-shaped neurons in the same nucleus. Staining with hematoxylin-eosin. Scale bars = 50 µm (**a, b**). **c – f** The SON of control (**C, E**) and alcoholic (**D, F**) patients. The VP (**D**) and OT (**F**) signals are ubiquitously decreased in the 47-year-old alcoholic patient compared to the 51-year control. **g – j** The PVN of a 51-year old control (**g, i**) and a 47-year old alcoholic (**h, j**) patients. Whereas VP-immunostaining in the alcoholic (**h**) is weaker than in the control (**g**), OT-positive cells are heavily stained in the alcoholic (**j**). Immunohistochemical staining with anti-VP or anti-OT antibodies, developed by GOD-DAB method. Scale bars = 100 µm (**c – j**).

content remained unchanged (Fig. **2e**), but "coarse-like" distribution of OT-immunoreactivity was often observed (Fig. **2d**).

CRH neurons of the PVN in alcoholics

Small and medium-sized CRH-positive neurons were located along the third ventricle in the PVN from non-alcoholic patients (Fig. **3a**). The intensity of CRH-staining varied from poor to very strong; weakly and moderately stained cells prevailed (Fig. **3b**).

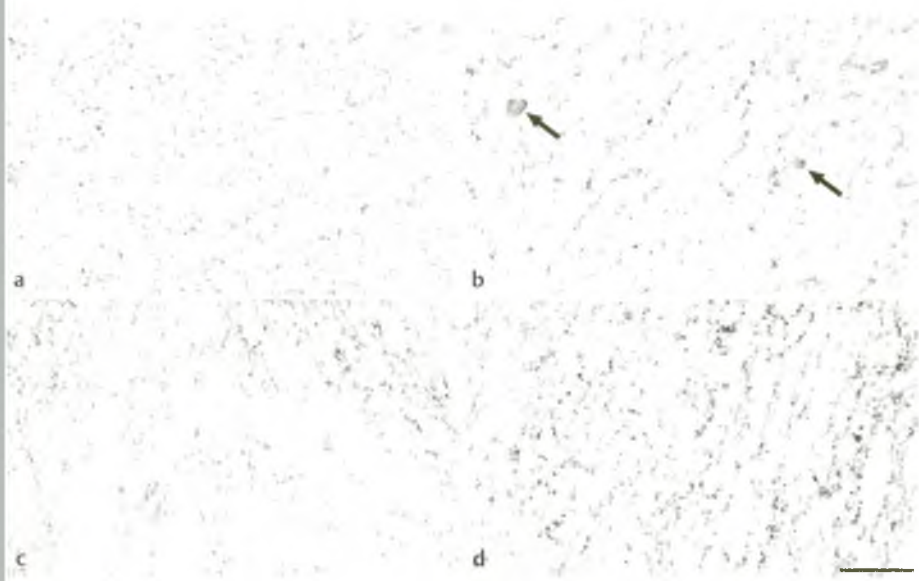
In controls, the number of CRH-positive neurons and the intensity of the signal were clearly increased in elder patients (Table **1**). In alcoholics, the population of CRH-positive neurons in the PVN was much larger in both age-matched groups (Fig. **3c**). At the same time, CRH-positive perikarya and processes were heavily stained (Fig. **3d**) and cellular profiles were enlarged (Table **1**).

We performed double-immunostaining to evaluate co-expression of CRH and VP in the same parvocellular neurons. In controls, VP-immunoreactivity was found in 11.2 ± 1.74 % CRH-positive neurons from young patients (Fig. **3e**, **f**) and in 13.21 ± 1.65 % CRH-positive neurons in the old group. In alcoholics, the proportion of double-labeled neurons was significantly increased in both age groups; 18.38 ± 3.54 % in young patients ($p < 0.05$; Fig. **3g**, **h**) and to 19.4 ± 2.41 % in elder patients ($p < 0.05$).

Table 1 Relative areas (in %) of vasopressin (VP)-, oxytocin (OT)- and corticotropin-releasing hormone (CRH)- immunoreactive products in neurons of the supraoptic (SON) and paraventricular (PVN) nuclei in alcoholics

Nucleus/neurohormone	*Age ≤ 40 yr*		*Age > 40 yr*	
	Control group	*Alcoholics*	*Control group*	*Alcoholics*
SON				
VP	81.54 ± 0.69	81.55 ± 0.45	78.52 ± 0.54[b]	77.93 ± 0.45[b]
OT	77.46 ± 0.61	76.54 ± 0.65	75.09 ± 0.72[b]	73.37 ± 0.76[a, b]
PVN				
VP	82.99 ± 0.67	80.54 ± 0.64[a]	81.13 ± 0.61[b]	78.27 ± 0.67[a, b]
OT	78.66 ± 0.76	79.18 ± 0.56	74.14 ± 0.87[b]	76.31 ± 0.68[a, b]
CRH	66.28 ± 0.41	73.85 ± 0.41[a]	68.31 ± 0.37[b]	69.23 ± 0.32[a, b]

[a] $p < 0.05$ in the same age group; [b] $p < 0.05$ between different age groups.



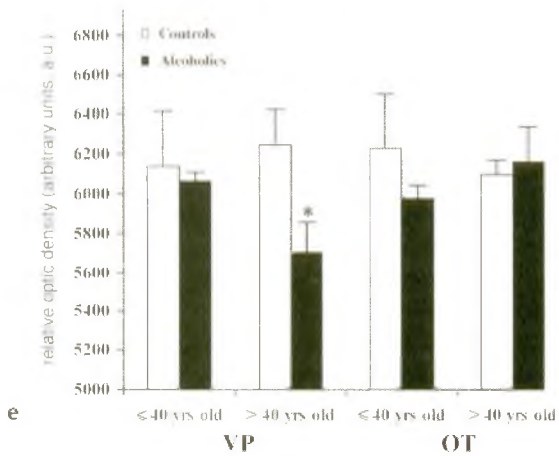


Fig. 2 VP- and OT-immunoreactivity in the posterior pituitary in alcoholics. **a–d** The posterior pituitary of a 42-year old control (**a, c**) and a 47-year old alcoholic (**b, d**) patients. Relatively equal and moderate distribution of VP- (**a**) and OT- (**c**) immunoreactivity is observed in the control patients. In the alcoholic patient, VP- (**b**) and OT- (**d**) immunoreactivity is distributed irregularly, with an accumulation in "coarse-grain" or "lump-like" (**b**, arrows) axonal terminals. Immunohistochemical staining with anti-VP and anti-OT antibodies, developed by GOD-DAB method. Scale bar for all panels = 100 µm. **e** Mean of relative optical density (OD) of immunoreactive products in posterior pituitaries of control and alcoholic patients. Significant reduction of OD for VP ($P < 0.05$) occurred in the elder alcoholic group.

Effects of chronic alcoholic disease on the adrenal gland

In alcoholic patients, a dramatic reduction in the width of adrenal cortex with alteration in zonation was observed (Fig. **4c**). Detailed examination revealed pyknosis (Fig. **4d, e**) and vacuolated dystrophy (Fig. **4f**) of corticocytes in zona fasciculata and zona glomerulosa.

Discussion

In agreement with Harding and colleagues [7], who reported neuronal death in the SON and PVN, we found signs of neuronal degeneration – pyknosis, cytokaryolysis, and pericellular edema – in those brain regions. Neuronal loss of VP and OT-ergic neurons with compensatory up-regulation of VP and OT mRNAs expression in surviving neurons was also reported in animal mod-

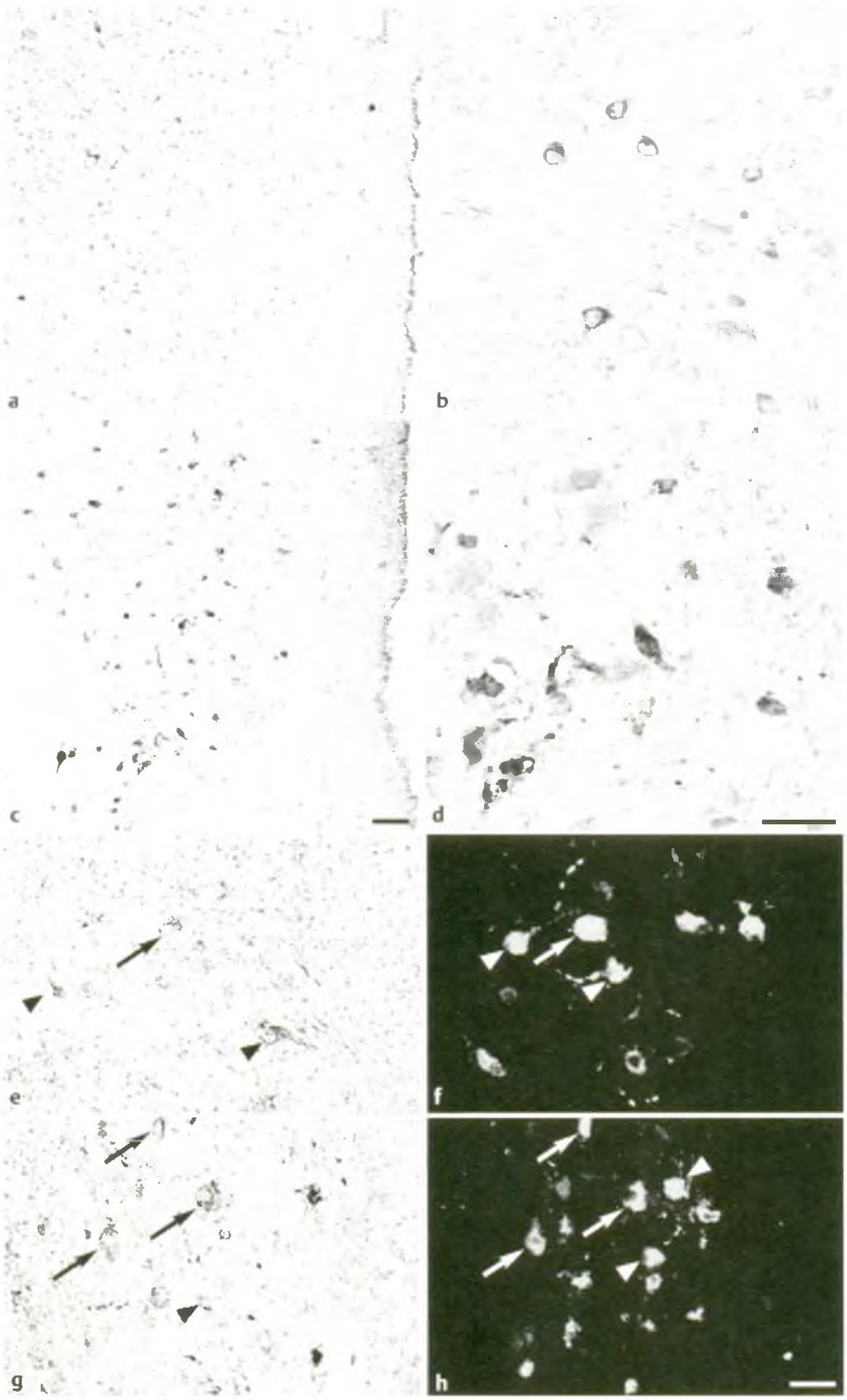


Fig. 3 CRH-immunoreactivity in parvocellular neurons of the PVN in control and alcoholic patients. **a, b** CRH-positive neurons in a 42-year-old control patient, at small (**a**) and big (**b**) magnifications. **c, d** Intensively stained CRH-positive neurons and their processes in the PVN of a 47-year-old alcoholic patient, at small (**c**) and big (**d**) magnifications. Immunohistochemical staining with anti-CRH antibodies, developed by GOD-DAB method. Scale bars = 100 µm (**a, c**), 50 µm (**b, d**). **e, f** Double immunostaining for CRH (**e**) and VP (**f**) in the hypothalamic PVN of the 40-year-old control. Arrows indicate singular cells containing both CRH- and VP-immunoreactivities. Single-labelled neurons are indicated by arrowheads. **g, h** An increased number of cells showed colocalized CRH- (**g**) and VP- (**h**) immunostaining in the 37-year-old alcoholic. Immunohistochemical staining with anti-CRH antibodies developed by GOD-DAB method and with anti-VP antibodies visualised with fluorescent (CY3) secondary antibodies. Scale bar = 50 µm (**e – h**).

els [4]. We observed substantial decrease in VP immunoreactivity in neurons of the hypothalamic nuclei and accumulation of VP-immunoproduct in "coarse-grained" and "lump-like" structures in the posterior pituitary. These observations, suggesting inhibition of VP synthesis and impairment of VP release, are in line with the blunted response of VP to osmotic challenges in alcoholic patients [8]. Similar changes have been found in OT-ergic population of the SON. However, clear elevation of OT-immunoreactivity was found in the PVN, especially in elder patients. Activation of OT synthesis in the PVN may contribute to abnormal elevation of plasma OT in alcoholics during periods of abstinence lasting 4 to 28 days [10]. The abnormal increase in OT may be due to alcohol withdrawal, as OT is typically released in response to acute stress paradigms [23 – 25]. However, it is unlikely that the OT rebound would last a whole month. Enhanced OT production may contribute to compensation of water loss [26, 27] and development of alcohol tolerance [28].

According to clinical studies, chronic alcohol consumption suppresses cortisol plasma levels [18], which is consistent with the

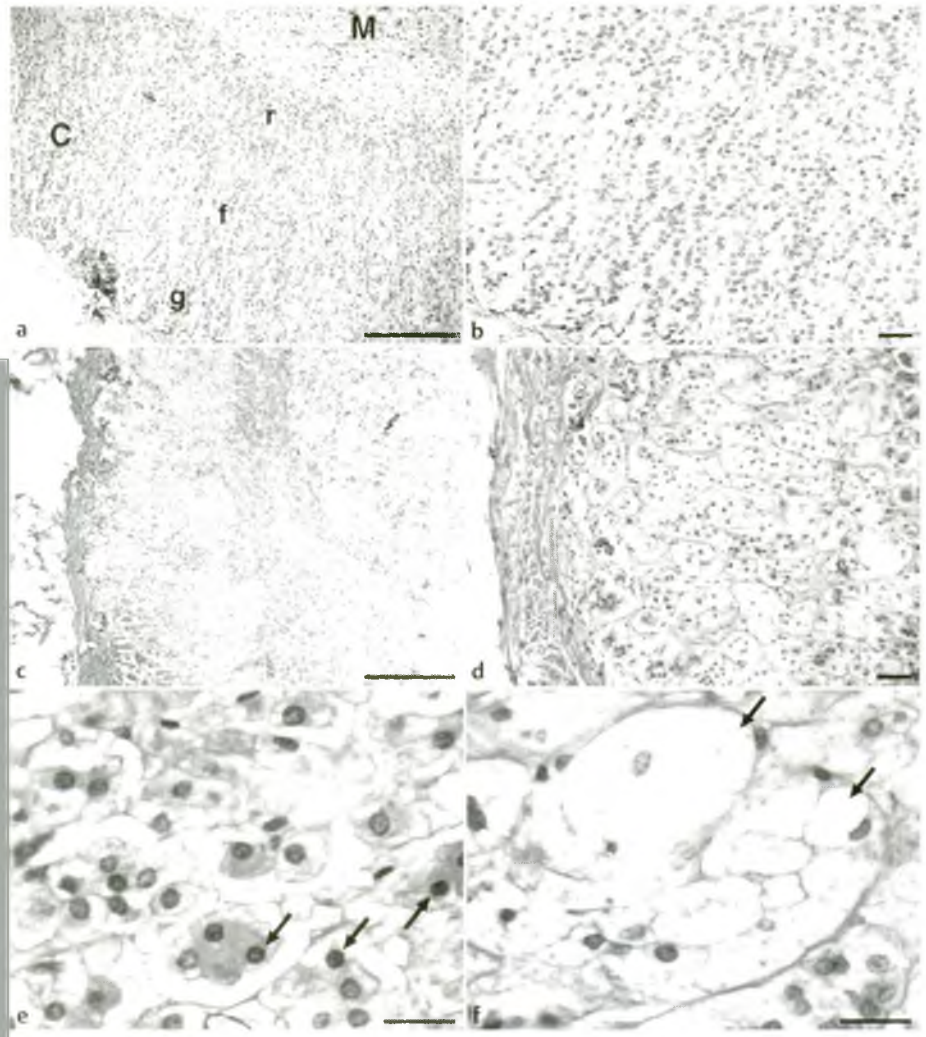


Fig. **4** Morphology of the adrenal gland of a 52-year-old control and a 48-year-old alcoholic. **a** The adrenal gland of a control patient. C = cortex; g = zona glomerulosa; f = zona fasciculata; r = zona reticularis; M = medulla. **b** Magnified fragment of **a**. **c–f** Pathomorphological changes in the adrenal cortex in the alcoholic: A reduction of the width (< 1 mm) of adrenal cortex (**c**), an alteration in the zonation of the adrenal cortex (**d**), pyknotic (arrows) and vacuolar changes in corticocytes in zona glomerulosa and zona fasciculata (**e**), a vacuolated dystrophy (arrows) of corticocytes in zona fasciculata (**f**). Staining with hematoxylin-eosin. Scale bars = 500 μm (**a, c**), 100 μm (**b, d**), 50 μm (**e, f**).

adrenal cortex hypoplasia observed by us and reported by other groups in humans and animal models [29–31]. Interestingly, while prolonged ethanol intake leads to decrease in CRH mRNA levels in parvocellular neurons in rats [17, 32], we observed a profound increase in CRH immunoreactivity in parvocellular neurons from the PVN of alcoholic patients. This increase may be explained by several mechanisms. First, low levels of circulating glucocorticoids may tonically upregulate CRH expression; second, ethanol withdrawal (the patients were admitted to hospital after long-term drinking) may exaggerate CRH production and release [33–35]; and third, depression (25–50% of actively drinking alcoholics are depressed [36]) may activate CRH synthesis [37–40].

Co-expression of CRH and VP in parvocellular neurons of the PVN is well-documented in rodents [13] and reported in humans [41,42]. Chronic alcohol consumption and alcohol withdrawal do not affect VP mRNA levels in parvocellular neurons in rodents [17]. In alcoholic patients, we found an increase in the number of double-labeled cells in both, the young and the old groups. Upregulation of VP expression usually occurs in situations of insufficient feed-back of glucocorticoid signaling, such as after adrenalectomy or prolonged stress [14–16]. Hypoplasia of adrenal cortex in chronic alcoholics observed in our study suggests that silencing of negative glucocorticoid feed-back signaling in chronic alcohol consumption can lead to tonic activation of VP expression in CRH-ergic neurons.

The changes observed in magnocellular neurons were age-dependent. In the control group, VP and OT content in the SON and PVN was ubiquitously lower in old patients (> 40 years). Consistently, decrease in VP and OT content in both nuclei (except OT in the PVN) was much more drastic in alcoholics in the old group than in younger patients. We observed an elevation in CRH immunoreactivity in the PVN of the control old group in agreement with reported activation of CRH expression during ageing in rodents and humans [43,44]). However, elevation of CRH immunoreactivity was more profound in the young alcoholic patients.

In conclusion, the analysis of magnocellular hypothalamic nuclei in alcoholic patients revealed a decrease in VP content in SON and the PVN, and OT content in the SON; all three findings were more pronounced in the elder patients (> 40 years). In spite of this, content of OT was elevated in the PVN of the old group. We found an increase in CRH immunoreactivity in individual cells in parvocellular neurons as well as an increase in the number of CRH-positive neurons co-expressing VP. These changes are likely induced by insufficient levels of plasma glucocorticoids due to hypoplastic impairments of the adrenal cortex.

Acknowledgements

We thank Dr. Pavel Osten for comments on manuscript. We thank Julia Kuhl for carefully reading and correcting the manuscript. E. S. was a recipient of a DAAD fellowship.

References

[1] Madeira MD, Paula-Barbosa MM. Effects of alcohol on the synthesis and expression of hypothalamic peptides. Brain Res Bull 1999; 48: 3 – 22

[2] Gulya K, Dave JR, Hoffman PL. Chronic ethanol ingestion decreases vasopressin mRNA in hypothalamic and extrahypothalamic nuclei of mouse brain. Brain Res 1991; 557: 129 – 135

[3] Sanna PP, Folsom DP, Barizo MJ, Hirsch MD, Melia KR, Maciejewski-Lenoir D, Bloom FE. Chronic ethanol intake decreases vasopressin mRNA content in the rat hypothalamus: a PCR study. Brain Res Mol Brain Res 1993; 19: 241 – 245

[4] Madeira MD, Sousa N, Lieberman AR, Paula-Barbosa MM. Effects of chronic alcohol consumption and of dehydration on the supraoptic nucleus of adult male and female rats. Neuroscience 1993; 56: 657 – 672

[5] Silva SM, Madeira MD, Ruela C, Paula-Barbosa MM. Prolonged alcohol intake leads to irreversible loss of vasopressin and oxytocin neurons in the paraventricular nucleus of the hypothalamus. Brain Res 2002; 925: 76 – 88

[6] Ruela C, Sousa N, Madeira MD, Paula-Barbosa MM. Stereological study of the ultrastructural changes induced by chronic alcohol consumption and rehydration in the supraoptic nucleus of the rat hypothalamus. J Neurocytol 1994; 23: 410 – 421

[7] Harding AJ, Halliday GM, Ng JLF, Harper CG, Kril JJ. Loss of vasopressin-immunoreactive neurons in alcoholics in dose-related and time-dependent. Neuroscience 1996; 72: 699 – 708

[8] Collins GB, Brosnihan B, Zuti RA, Messina M, Gupta MK. Neuroendocrine, fluide balance, and thirst response to alcohol in alcoholics. Alcohol Clin Exp Res 1992; 16: 228 – 233

[9] Legros JJ, Deconinck I, Willems D, Roth B, Pelc I, Brauman J, Verbank M. Increase of neurophysin II serum levels in chronic alcoholic patients: relationship with alcohol consumption and alcoholism blood markers during withdrawal therapy. J Clin Endocrinol Metab 1983; 56: 871 – 875

[10] Marchesi C, Chiodera P, Brusamonti E, Volpi R, Coiro V. Abnormal plasma oxytocin and beta-endorphin levels in alcoholics after short and long term abstinence. Progr Neuro-Psychopharmacol Biol Psychiat 1997; 21: 797 – 807

[11] Haddad JJ. Alcoholism and neuro-immune-endocrine interactions: physiochemical aspects. Bichem Biophys Res Commun 2004; 323: 361 – 371

[12] Sillaber I, Henninger MSH. Stress and alcohol drinking. Ann Med 2004; 36: 596 – 605

[13] Aguilera G. Regulation of pituitary ACTH secretion during chronic stress. Front Neuroendocrinol 1994; 15: 321 – 350

[14] Grinevich V, Ma X-M, Herman J, Jezova D, Akmayev I, Aguilera G. Effect of repeated lipopolysaccharide administration on tissue cytokine expression and hypothalamic-pituitary-adrenal axis activity in rats. J Neuroendocrinol 2001; 13: 711 – 723

[15] Grinevich V, Harbuz M, Ma X-M, Jessop D, Tilders FJH, Lightman SL, Aguilera G. Hypothalamic pituitary adrenal axis and immune responses to endotoxin in rats with chronic adjuvant-induced arthritis. Exp Neurol 2002; 178: 112 – 123

[16] Lightman SL, Windle RJ, Ma XM, Harbuz MS, Shanks NM, Julian MD, Wood SA, Kershaw YM, Ingram CD. Hypothalamic-pituitary-adrenal function. Arch Physiol Biochem 2002; 110: 90 – 93

[17] Silva SM, Paula-Barbosa MM, Madeira MD. Prolonged alcohol intake leads to reversible depression of corticotropin-releasing hormone and vasopressin immunoreactivity and mRNA levels in the parvocellular neurons of the paraventricular nucleus. Brain Res 2002; 954: 82 – 93

[18] Wand GS, Dobs AS. Alterations in the hypothalamic-pituitary-adrenal axis in actively drinking alcoholics. J Clin Endocrinol Metab 1991; 72: 1290 – 1295

[19] Halliday G, Ellis J, Heard R, Caine D, Harper C. Brainsteam serotoninergic neurons in chronic alcoholics with and without the memory impairment Korsakoff's psychosys. J Neuropath Exp Neurol 1993; 52: 567 – 579

[20] Victor M, Adams RD, Collins GH. The Wernicke-Kosakoff syndrome and related neurological disorders due to alcoholism and malnutrition. Philadelphia: F. A. Devis Company, 1989

[21] Zaborsky L, Heimer L. Combination of tracer techniques, especially HRP and PHA-L, with transmitter identification for correlated light and electron microscopic studies. In: Heimer L, Zaborsy L (eds). Neuroanatomical tract tracing methods 2. New York: Plenum Press, 1989: 49 – 96

[22] Telleria-Diaz A, Grinevich VV, Jirikowski GF. Colocalization of vasopressin and oxytocin in hypothalamic magnocellular neurons in water-deprived rats. Neuropeptides 2001; 3 – 4: 162 – 167

[23] Jezova D, Skultetyova I, Tokarev DI, Bakos P, Vigas M. Vasopressin and oxytocin in stress. Ann NY Acad Sci 1995; 771: 192 – 203

[24] Engelmann M, Landgraf R, Wotjak CT. The hypothalamic-neurohypophyseal system regulates the hypothalamic-pituitary adrenal axis under stress: an old concept revisited. Front Neuroendocrinol 2004; 25: 132 – 149

[25] Laguna-Abreu MT, Koenigkam-Santos M, Colleta AM, Elias PC, Moreira AC, Antunes-Rodrigues J, Elias LL, Castro M. Time course of vasopressin and oxytocin secretion after stress in adrenalectomized rats. Horm Metab Res 2005; 37: 84 – 88

[26] Chou CL, DiGiovanni SR, Mejia R, Nielsen S, Knepper MA. Oxytocin as an antidiuretic hormone. Amer J Physiol 1995; 269 (1 Pt 2): F70 – 77, F78 – 85

[27] Haanwinckel MA, Elias LK, Favaretto AL, Gutkowska J, McCann SM, Antunes-Rodrigues J. Oxytocin mediates atrial natriuretic peptide release and natriuresis after volume expansion in the rat. Proc Natl Acad Sci USA 1995; 92: 7902 – 7906

[28] Kovacs GL, Sarnyai Z, Szabo G. Oxytocin and addiction: a review. Psychoneuroendocrinology 1998; 23: 945 – 962

[29] Chu I, Poon R, Valli V, Yagminas A, Bowers WJ, Seegal R, Vincent R. Effects of an ethanol-gasoline mixture: results of a 4-week inhalation study in rats. J Appl Toxicol 2005; 25: 193 – 199

[30] Jedrzejewska EA. Morphochemical and statistical studies of the adrenal cortex in albino rats with chronic alcoholic intoxication. I. Histologic and morphometric studies. Ann Univ Mariae Curie Sklodowska 1984; 39: 293 – 301

[31] Paukov VC, Erokhin IuA. Endocrine glands in alcohol drinkers and patients with alcoholism. Arkh Patol 2001; 63: 21 – 26

[32] Silva TP, Silverira GA, Fior-Chadi DR, Chadi G. Effects of ethanol consumption on vasopressin and neuropeptide Y immunoreactivity and mRNA expression in peripheral and central areas related to cardiovascular regulation. Alcohol 2004; 32: 213 – 222

[33] Hundt W, Zimmermann U, Poettig M, Spring K, Holsboer F. The combined dexamethasone-suppression/CRH-stimulation test in alcoholics during and after acute withdrawal. Alcohol Clin Exp Res 2001; 25: 687 – 691

[34] Von Bardeleben U, Heuser I, Holsboer F. Human CRH stimulation response during acute withdrawal and after medium-term abstention from alcohol abuse. Psychoneuroendocrinology 1989; 14: 441 – 449

[35] Zimmermann U, Hundt W, Spring K, Grabner A, Holsboer F. Hypothalamic-pituitary-adrenal system adaptation to detoxification in alcohol-dependent patients is affected by family history of alcoholism. Biol Psychiatry 2003; 53: 75 – 84

[36] Dackis C, Stuckley R, Gold M, Pottash A. Dexametasone suppression testing of depressed alcoholics. Alcohol Clin Exp Res 1986; 10: 59 – 60

[37] Bailly D, Dewailly D, Beuscart R, Couplet G, Dumont P, Racadot A, Fossati P, Parquet PJ. Adrenocorticotropin and cortisol responses to ovine corticotropin-releasing factor in alcohol dependence disorder. Preliminary report. Horm Res 1989; 31: 72 – 75

[38] Majumdar SK, Shaw GK, Bridges PK. The dexamethasone suppression test in chronic alcoholics with and without depression and its relationship to their hepatic status. Drug Alcohol Depend 1988; 21: 231 – 235

[39] Raadsheer FC, Hoogendijk WJ, Stam FC, Tilders FJ, Swaab DF. Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. Neuroendocrinology 1994; 60: 436 – 444

[40] Targum SD, Wheadon DE, Chastek CT, McCabe WJ, Advani MT. Dysregulation of hypothalamic-pituitary-adrenal axis function in depressed alcoholic patients. J Affect Disord 1982; 4: 347 – 353

[41] Huitinga I, Erkut ZA, van Beurden D, Swaab DF. Impaired hypothalamus-pituitary-adrenal axis activity and more severe multiple sclerosis with hypothalamic lesions. Ann Neurol 2004; 55: 37 – 45
[42] Raadscheer FC, Sluiter AA, Ravid R, Tilders FJ, Swaab DF. Localization of corticotropin-releasing hormone (CRH) neurons in the paraventricular nucleus of the human hypothalamus: age-dependent colocalization with vasopressin. Brain Res 1993; 615: 50 – 62
[43] Mejer OC, Topic B, Steenbergen PJ, Jochan G, Huston JP, Oitzl MS. Correlations between hypothalamus-pituitary-adrenal axis parameters depend on age and learning capacity. Endocrinology 2005; 146: 1372 – 1381
[44] Zhuo JN, Swaab DF. Activation and degeneration during aging: a morphometric study of the human hypothalamus. Microsc Res Tech 1999; 44: 36 – 48