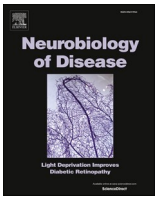
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Increased pituitary adenylate cyclase-activating polypeptide in the central bed nucleus of the stria terminalis in mood disorders in men

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ABSTRACT

The mood disorders major depressive disorder (MDD) and bipolar disorder (BD) are highly prevalent worldwide. Women are more vulnerable to these psychopathologies than men. The bed nucleus of the stria terminalis (BNST), the amygdala, and the hypothalamus are the crucial interconnected structures involved in the stress response. In mood disorders, stress systems in the brain are put into a higher gear. The BNST is implicated in mood, anxiety, and depression. The stress-related neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) is highly abundant in the central BNST (cBNST). In this study, we investigated alterations in PACAP in the cBNST of patients with mood disorders. Immunohistochemical (IHC) staining of PACAP and in situ hybridization (ISH) of PACAP mRNA were performed on the cBNST of post-mortem human brain samples. Quantitative IHC revealed elevated PACAP levels in the cBNST in both mood disorders, MDD and BD, but only in men, not in women. The PACAP ISH was negative, indicating that PACAP is not produced in the cBNST. The results support the possibility that PACAP innervation of the cBNST plays a role in mood disorder pathophysiology in men.

1. Introduction

Major depressive disorder (MDD) and bipolar disorder (BD), also referred to as mood disorders, present a major public health problem. The life-time prevalence of MDD is estimated between 2% and 21% (Gutiérrez-Rojas et al., 2020), while the prevalence of BD is between 1.5% and 2.4% (Carvalho et al., 2020). Women are more vulnerable to these psychopathologies than men (Swaab and Bao, 2020). Although MDD and BD show distinct symptoms, they are both characterized by overlapping hyperactive stress-related neuro-circuits (Swaab and Bao, 2020; Bao and Swaab, 2018; Belvederi Murri et al., 2016; Sigitova et al., 2017), which often show sex differences (Swaab and Bao, 2020).

The brain structures particularly involved in mood disorders are the hypothalamus, amygdala, prefrontal cortex, and hippocampus (Bao and Swaab, 2018; Pandya et al., 2012). The bed nucleus of the stria terminalis (BNST) has extensive connections with these brain areas, and is a central hub in the regulation of these stress related brain areas. In this way the BNST is implicated in stress regulation, mood, anxiety, and depression (Hulsman et al., 2021). The stress-related neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) is highly abundant in the rodent oval nucleus (ovBST), the homologue of the human central BNST (cBNST), (Hu et al., 2020a) and in other stress and

Abbreviations: MDD, major depressive disorder; BD, bipolar disorder; BNST, bed nucleus of the stria terminalis; PACAP, pituitary adenylate cyclase activating polypeptide; ovBST, oval bed nucleus of the stria terminalis; cBNST, central nucleus of the bed nucleus of the stria terminalis; PVN, paraventricular nucleus; PTSD, post-traumatic stress disorder; BDNF, brain-derived neurotrophic factor; PAC1, pituitary adenylate cyclase-activating polypeptide type I receptor; SOM, somatostatin; VIP, vasoactive intestinal peptide; NBB, Netherlands Brain Bank; PMD, post-mortem delay; FT, fixation time; CTD, clock time of death; MOD, month of death; CSF, cerebrospinal fluid; BW, brain weight; SOMAAR, anti-somatostatin; LNA, locked nucleic acid; FAM, fluorescein group; IR, immunoreactivity; OD, optical density; cIOD, corrected integrated optical density.

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anxiety related areas in the human brain such as the paraventricular nucleus (PVN) and the amygdala (Palkovits et al., 1995). PACAP is presumed to play a role in mood disorders, post-traumatic stress disorder (PTSD) and schizophrenia (Ressler et al., 2011; Katayama et al., 2009). Increased PACAP expression was found in the ovBST in chronically variate-stressed rodents, a model for depression (Hu et al., 2020b). In addition, PACAP knock-out mice showed reduced anxiety-like behaviour, while intra-BST infusion of PACAP in rats has been found to enhance long-lasting anxiety-like behaviour. Moreover, BST administration of PACAP was shown to induce the anxiety-like behaviour during ethanol abuse withdrawal in rats (Ferragud et al., 2021), suggesting that BNST PACAP is anxiogenic (Roman et al., 2014; Hammack et al., 2009). Moreover, PACAP regulates the release of brain-derived neurotrophic factor (BDNF), while dysregulation of BDNF is associated with mood disorders (Martinowich et al., 2007; Pinhasov et al., 2011). In this way chronic stress/PACAP-induced neuroplasticity of the BNST can contribute to the over-activation of the stress response (Pittenger and Duman, 2008; Hammack et al., 2021). In support of this idea is the observation that chronic variate stress increased BDNF in the BNST of rats, while the effect of BDNF was diminished when the pituitary adenylate cyclase-activating polypeptide type I receptor (PAC1) was blocked (Hammack et al., 2009).

Women are twice as vulnerable to mood disorders and less responsive to existing treatments than men (Swaab and Bao, 2020; Lebow and Chen, 2016). In young adults, the BNST is shown to be sexually dimorphic in structure in both humans and rodents (Zhou et al., 1995; Kruijver et al., 2000; Stefanova and Ovtscharoff, 2000; Chung et al., 2002). The central BNST (cBNST) of women was shown to be 40% smaller (Chung et al., 2002; Zhou et al., 1995), and has 76% greater structural connectivity with regions connected with the BNST in comparison to the cBNST of men (Lebow and Chen, 2016). Therefore, in this study special attention was paid to the presence of possible sex differences.

In the present study we quantitatively determined alterations in PACAP in the cBNST of mood disorder patients and well-matched controls in clinically and neuropathologically well-documented human post-mortem material. The cBNST was, as in earlier studies, delineated by somatostatin (SOM) (Kruijver et al., 2000). In addition, we determined whether PACAP was produced in the cBNST by in situ hybridization (ISH) in the control patients.

2. Methods

2.1. Human brain samples

Post-mortem human hypothalami containing the adjacent BNST were obtained from the Netherlands Brain Bank (NBB, Director Prof. Inge Huitinga). Informed consent was provided by the donors or their next of kin for a brain autopsy and the use of the tissue and the clinical information for research purposes. Following the autopsy, the hypothalamic region was fixed in 4% formaldehyde (pH 7.4) for about one month at room temperature (RT) and subsequently embedded in paraffin. Serial 6 μm sections were made using a Leitz microtome (Leitz, Wetzlar, Germany).

The cohort for the immunohistochemical (IHC) staining procedures was composed of 17 patients (age range 55–93 years) with mood disorders. The cohort consisted of major depressive disorder patients (MDD $n = 10$, 5 women, 5 men) and bipolar disorder patients (BD $n = 7$, 1 woman, 6 men) and 17 controls (6 women, 11 men) (Supplementary Material SM Table A.1–2). The control patients did not have any neuropsychiatric disorders and did not receive corticosteroids in the last three months of their life, unless stated otherwise (SM Table A.2). All the patients and controls were well matched for sex, age, post-mortem delay (PMD), fixation time (FT), clock time of death (CTD), month of death (MOD), cerebrospinal fluid (CSF) pH, which is a measure of the agonal state (Swaab and Bao, 2021), brain weight (BW), and Braak stage for Alzheimer neuropathology (Braak and Braak, 1991) (SM Table A.1 and A.2). Matching was statistically analysed using the non-parametric Mann-Whitney *U* test (two-tailed) (GraphPad Software, 2021). No significant differences were observed in matching between the (sub)-groups (SM Table A.3). The group of control patients for the ISH consisted of 9 women (aged 32–85 years) and 8 men (aged 44–92 years) (SM Table A.4).

Diagnosis of MDD or BD at any time during life was made by qualified psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders (DSM)-III-R/DSM-IV criteria (Guze, 1995). The absence of neuropathological changes, both in the patients with mood disorders and in the controls, was confirmed by systematic neuropathological investigation (van de Nes et al., 1998).

2.2. Immunohistochemical (IHC) staining

From each patient, 1 out of 100 sections were mounted across the rostral-caudal axis, after which IHC staining was performed with rabbit anti-somatostatin (SOMAAR, 8–2-89, RRID: AB_2732878) (for details and specificity see (Kruijver et al., 2000) (Kruijver et al., 2000). Somatostatin (SOM) stained sections were used to determine the volume of the cBNST (Kruijver et al., 2000), and the location of the cBNST peak, i.e. the largest surface. To confirm that the distribution of PACAP throughout the cBNST was similar to SOM, two patients were immunohistochemically stained from rostral to caudal for SOM and PACAP in alternating 1:100 sections. Subsequently an IHC staining of PACAP was performed on three sections per patient in the cBNST peak.

2.3. In situ hybridization PACAP-mRNA probe

A locked nucleic acid probe was used, which is a 19-mer locked nucleic acid (LNA) probe consisting of three locked bases within a − 2-*O*-methylRNA backbone, designed using the SiDESIGN tool and targeted against bases 541–523 of the human PACAP mRNA sequence (Accession Number NM_001099733.2). The probe was tagged at the 5′ end with a fluorescein (FAM) group and the sequence was as follows:

FAM 5' LTmAmG mCmUmG LTmCmC mGmUmG LAmAmG mAmUmC mC 3′(L and m denote locked and methyl RNA bases, respectively). The sequence was run through BLAST to check for cross alignments with other transcripts. A scrambled probe and an irrelevant probe targeting a gene not found in the human genome were used as negative controls. The irrelevant probe was directed against beta lactamase, the gene that confers ampicillin resistance in Lactococcus bacteria. All the probes were custom ordered (Eurogentec, Seraing, Belgium). More than five replications were carried out. To ensure adequate sensitivity, we also tested the probe at 50 nM, higher than the standard used 20 nM The ISH procedure was done according to earlier publications of our group (Bin et al., 2016).

2.4. Cross-reactivity of anti-PACAP

Cross-reactivity of the anti-PACAP antibody with the vasoactive intestinal peptide (VIP) is a major point of concern, since the two precursor molecules and the two peptides have quite some amino-acid sequences in common. Interestingly, the fact sheet of the polyclonal rabbit-anti-PACAP antibody (anti-PACAP-38, T-4473, BMA, Peninsula Labs) that was used in the present study, stated that the cross-reactivity with VIP in a radioimmunoassay was 0%. In addition, cross-reactivity with VIP was tested in the present study by a Western blot (for the protocol see SM D), where despite the 68% sequence homology between PACAP-38 and VIP, this anti-PACAP-38 antibody clearly reacted solely with PACAP-38 and not with VIP (for results see SM D). In addition, we performed a solid phase adsorption to remove potential VIP cross-reacting antibodies from the anti-PACAP antibody, following three solid phase adsorptions with VIP (Van der Sluis et al., 1987; Garcia-Falgueras et al., 2011). cBNST sections were subsequently stained using

the adsorbed antibody solution (SM fig. E.1 panel A) and compared to the original non-adsorbed anti-PACAP antiserum (SM fig. E.1 panel B). The staining that remained after adsorption was quite similar to the original staining, indicating that potential VIP-cross reacting antibodies do not add significantly to the immunohistochemical signal produced by the anti-PACAP antibody.

2.5. Quantification

Every 100th section along the rostral-caudal axis of the cBNST, immunohistochemically stained for SOM, was used for cBNST volume measurement (Kruijver et al., 2000). The volume of the cBNST was calculated by multiplying the sum of the surfaces of each section, with the section's thickness (6 μm) considering the distances between the measured sections, as derived from Cavalieri's Principle (Alkemade et al., 2022).

Images were captured using the Zeiss Axiovert 200 M inverted microscope with 10× Neoplan Fluar objective and an EXi Aqua Bio-Imaging Microscopy Camera (Teledyne QImaging, Surrey, Canada) and Image Pro software Version 6.3 (Media Cybernetics, Inc., Rockville, Maryland) using identical settings for each slide. The images were captured using a black and white camera with a green filter (band pass filter 560 nm; band width 40 nm). A shading correction was carried out to correct for non-uniform illumination. Quantification was made using the same software, by manually outlining a background area and the cBNST area on the basis of the borders of an adjacent SOM-stained section.

To determine PACAP-immunoreactivity (ir), the collected black and white images were transformed into optical density (OD) images. All the signal that was over two times the background has been considered as a positive signal and was included into a mask.

The total amount of PACAP-ir in the BNST was quantified by first calculating the average PACAP staining from three sections in the cBNST peak. This was done by multiplying the average area of the mask by the section thickness (e.g. 6 μm) and by the average OD (PACAP OD minus the background OD), which gave the corrected integrated OD (cIOD). Then a multiplication factor was calculated by dividing the volume of the total cBNST with the volume of a signal section of the cBNST. The total amount of PACAP staining was computed by multiplying the PACAP cIOD of the cBNST peak by this multiplication factor.

2.6. Statistics

Since the data were sometimes not normally distributed, distribution

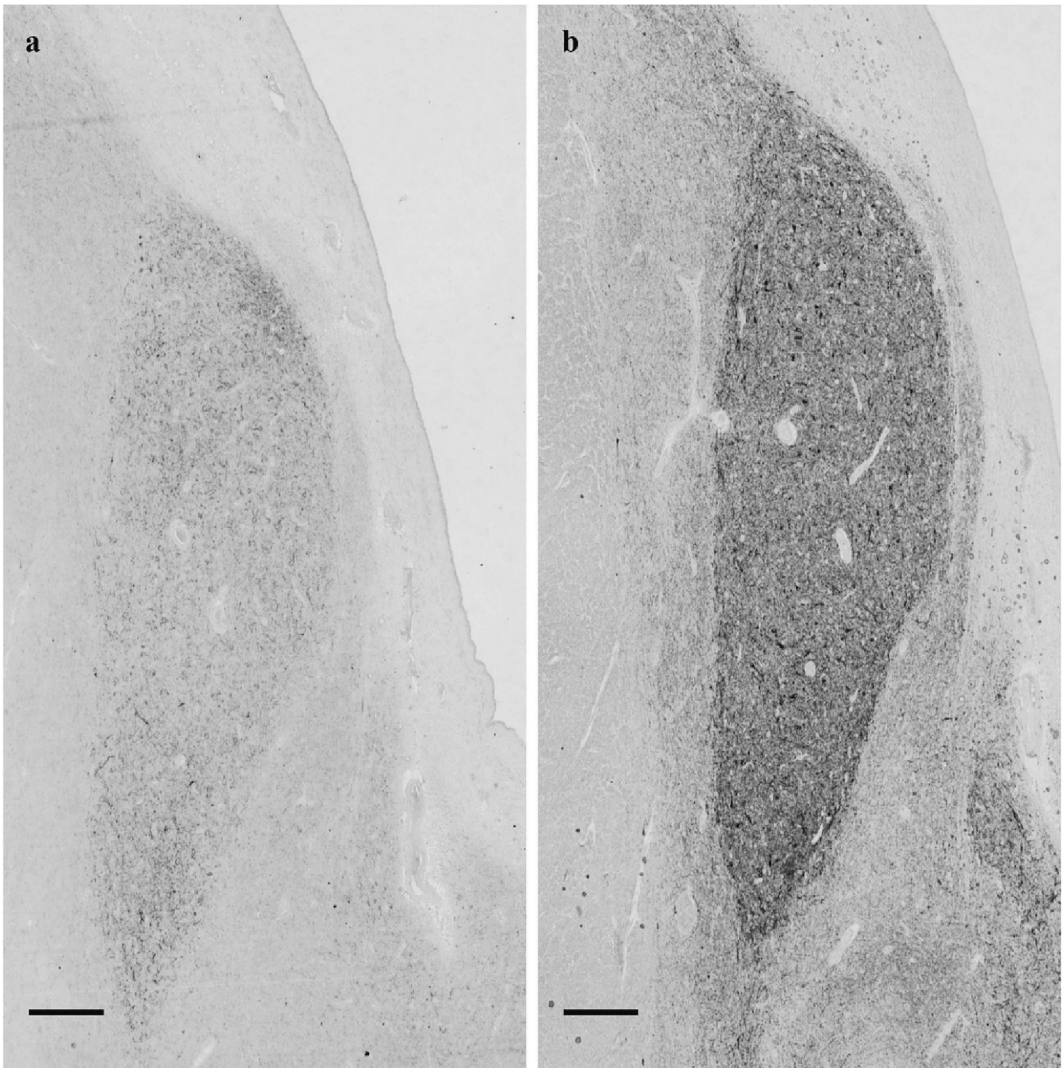


Fig. 1. Immunohistochemical staining of cBNST with PACAP and SOM. The images were taken with a B&W camera and green filter (objective 10×), coronal view. Pituitary adenylate cyclase activating polypeptide (PACAP) staining (a) is more heterogeneously distributed across the cBNST compared to the stronger somatostatin (SOM) staining (b). PACAP staining clustered more at the edges of the cBNST, while the centre showed relatively lighter staining. The bars represent 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

free tests were used. The differences between the groups were statistically analysed using the two-tailed nonparametric Mann-Whitney *U* test (GraphPad Prism 9.2) (GraphPad Software, 2021). $P < 0.05$ was considered to be significant in all statistical tests.

A correlation analysis was carried out using a Spearman rank correlations test ($p < 0.05$) (GraphPad Prism 9.2) (GraphPad Software, 2021).

3. Results

3.1. PACAP in the cBNST

Similar moderate PACAP-ir was observed throughout the entire cBNST along the rostral-caudal axis with the same borders as the stronger SOM staining. However, PACAP-ir was less homogeneously distributed over the section than SOM, as clustering was observed near the edges of the cBNST while the centre showed moderate staining (Fig. 1 – a and b). Consequently, SOM staining could be used for determining the cBNST volume.

No PACAP-mRNA staining was observed in the cBNST by ISH. Moreover, no cytoplasmic PACAP-ir was observed in the cBNST, only fibres and basket-like structures around non-staining neurons (Fig. 2 – a and b).

However, we observed PACAP-mRNA cell bodies in the same sections in hypothalamic nuclei such as the PVN and the supraoptic nucleus (SON) (Fig. 2 – c and d). The scrambled and a non-relevant negative control probe displayed no staining in ISH in the cBNST or other nuclei (Fig. 2 – e and f).

3.2. cBNST PACAP in mood disorders

The pooled mood disorder (MDD + BD) group had a significantly

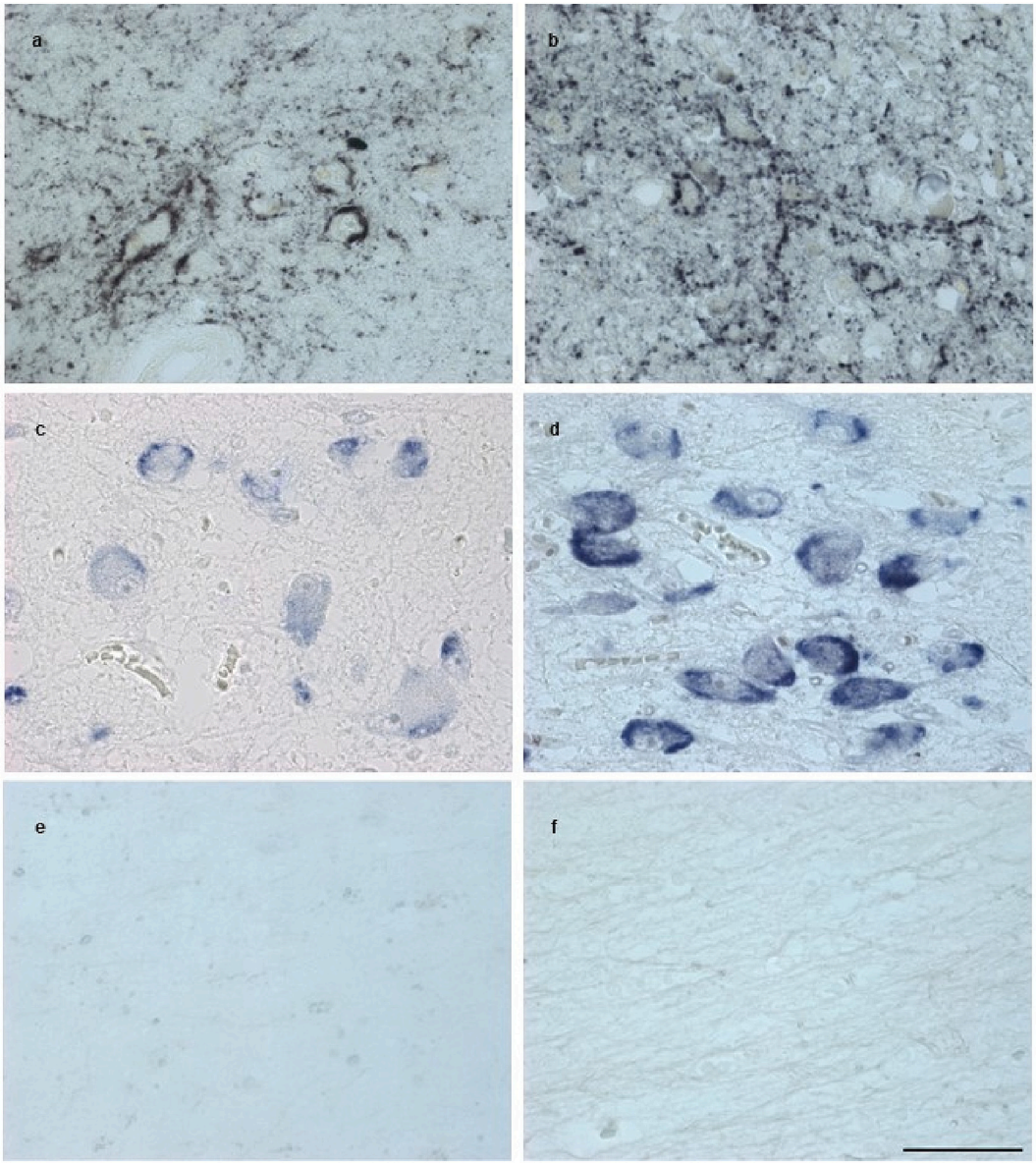


Fig. 2. Representative pictures of immunohistochemical staining and in situ hybridization of PACAP in the cBNST. Immunohistochemical staining (IHC) of the cBNST of a man with bipolar disorder (BD) (a), and a IHC staining of the cBNST of a man with major depressive disorder (MDD) (b). The PACAP IHC staining was present in fibres and basket-like structures around unstained neurons, and not in the cytoplasm of cells. A positive PACAP in situ hybridization (ISH) in the PVN (c) and in the SON (d). In addition, a negative staining of PACAP mRNA in an ISH in the human cBNST (e), and a negative staining of the scrambled probe in the cBNST (f). The bar represents 0.05 mm.

higher PACAP-ir cIOD compared to the control group (p = 0.02) (Fig. 3a).

Significantly higher PACAP-ir levels were found in the cBNST of mood disorder (MDD + BD) men compared to the matched CTR men (p = 0.002) and between the male MDD patients and matched CTR men (p = 0.01). No significant difference was detected between the MDD and BD men (p = 0.176) (Fig. 4).

No significant differences were observed in cBNST PACAP-ir expression between the subgroups consisting of only women (SM fig. B.1). No sex differences were found in cBNST PACAP staining (SM fig. B.2).

Possible outliers in our data were statistically analysed with the Grubbs's test ($p < 0.05$). We found a CTR woman with a relatively high cIOD which was detected as a significant outlier. Removing this value from our data did not change the significance of our results. Therefore, it was decided to not exclude this value from our data.

3.3. Correlations between PACAP cIOD and putative confounding factors

Age negatively correlated with the PACAP-ir cIOD values in the combined MDD + BD group and the MDD group (p_{MDD+BD} = 0.04, rho = −0.5; p_{MDD} = 0.038, rho = −0.67) (SM table A.5).

Positive correlations were found between CSF pH and PACAP-ir cIOD values in men with mood disorders (MDD + BD) (p = 0.04, rho = 0.71), BD men (p = 0.03, rho = 0.89) and the total BD group (p = 0.03, rho = 0.89) (SM table A.5).

3.4. Correlations between cBNST volume and confounding factors

Significant negative correlations were found between Braak stage and cBNST volume in MDD (p = 0.03, rho = −0.93), and between volume in MDD women and CTD (p = 0.03, rho = −0.97). Significant positive correlations were found between volume in CTR and fixation time (FT) (p = 0.04, rho = 0.50), volume in CTR and brain weight (p =

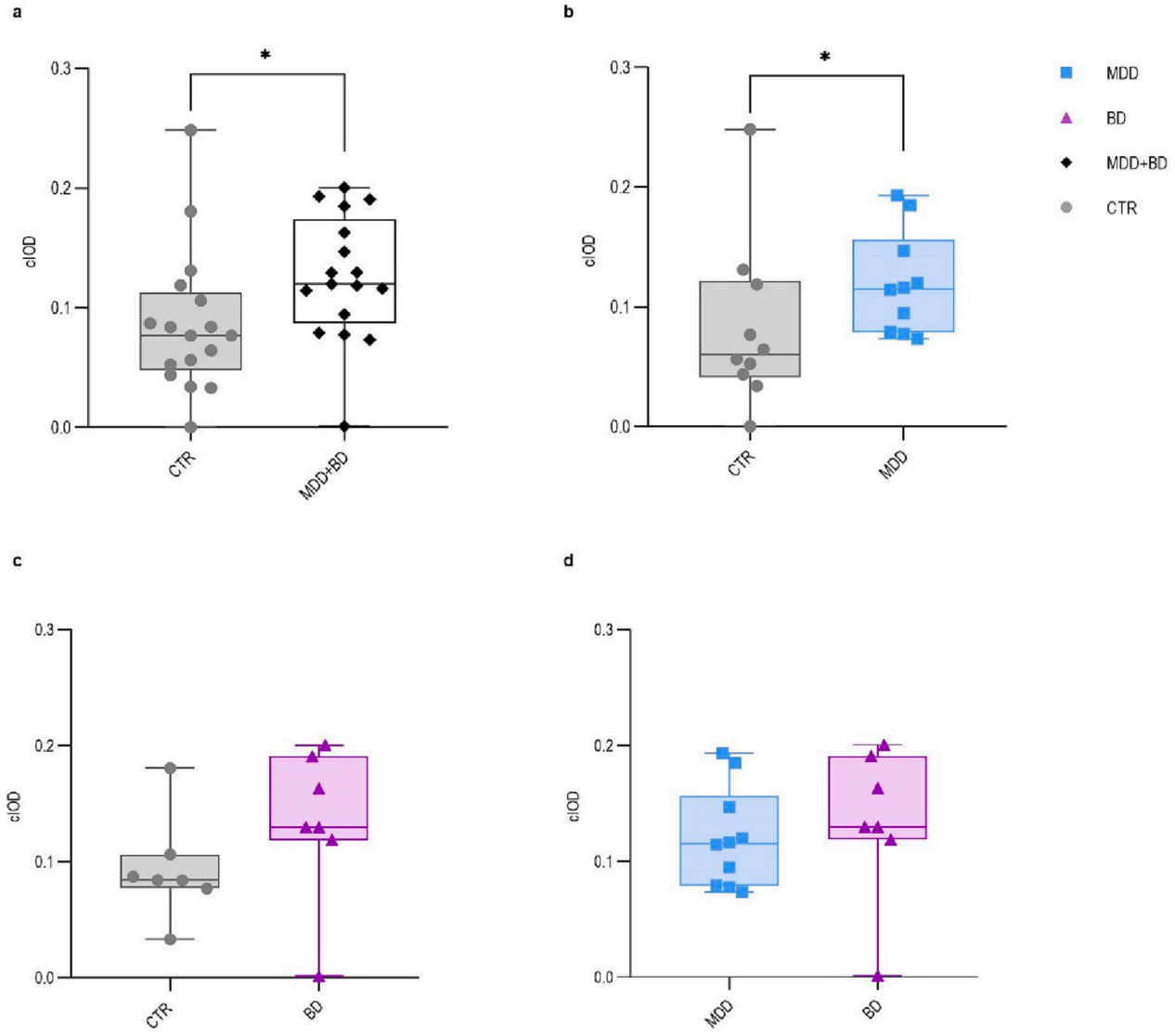


Fig. 3. PACAP IHC staining in the cBNST of the mood disorder groups. The corrected integrated optical density (cIOD) values of the immunohistochemical (IHC) staining of pituitary adenylate cyclase activating polypeptide (PACAP) in the central bed nucleus of the stria terminalis (cBNST). The data are shown as box plots with individual values, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. A significant difference was found between the cIOD values of the pooled mood disorder group (MDD + BD) (n = 17, mean = 0.13, standard deviation (SD) = 0.05), and the control group CTR (n = 17, mean = 0.09, SD = 0.06, p = 0.02*) (a). Significantly higher levels were also observed between MDD (n = 10, mean = 0.12, SD = 0.04) and matched CTR (n = 10, mean = 0.08, SD = 0.07, p = 0.05*) (b), and a trend towards significance was found between BD (n = 7, mean = 0.13, SD = 0.07) and matched CTR (n = 7, mean = 0.09, SD = 0.04, p = 0.10) (c). No significant differences were found between MDD and BD (p = 0.27) (d).

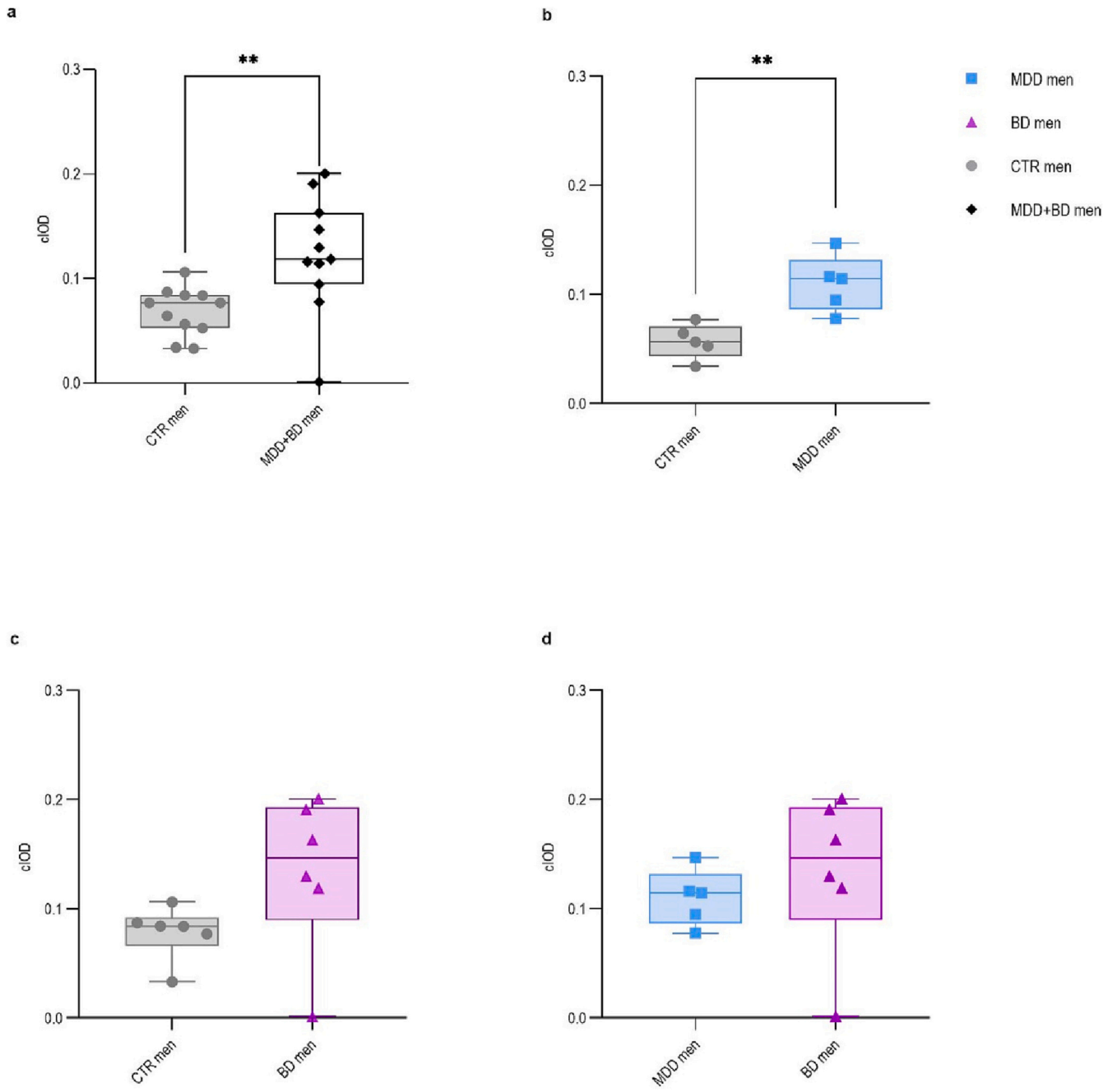


Fig. 4. PACAP IHC staining in the cBNST of men. The corrected integrated optical density (cIOD) values of the immunohistochemical (IHC) staining of pituitary adenylate cyclase activating polypeptide (PACAP) in the central bed nucleus of the stria terminalis (cBNST) of men. The data were analysed by the Mann Whitney *U* test (two-tailed). Data are shown as box plots with individual values, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. Significant differences were observed between the mood disorder (MDD + BD) men ($n = 11$, mean = 0.12, SD = 0.06) and control (CTR) men (n = 11, mean = 0.07, SD = 0.02) ($p = 0.002$**) (a), and between the major depressive disorder (MDD) men ($n = 5$, mean = 0.11, SD = 0.03) and matched CTR men (n = 5, mean = 0.06, SD = 0.02) ($p = 0.01$**) (b). A trend towards significance was observed between the bipolar disorder (BD) men ($n = 6$, mean = 0.13, SD = 0.07) and CTR men (n = 6, mean = 0.08, SD = 0.02) ($p = 0.06$) (c). No significant difference was observed between the MDD and BD male groups ($p = 0.18$) (d).

0.54, rho = 0.5) and between volume in CTR women and brain weight (p = 0.03, rho = 0.89) (SM table A.6).

3.5. Results volume measurements

No significant volume differences were found in the cBNST in any of the comparisons (SM figs. C.1-C.4).

4. Discussion

This study provides evidence for a role of cBNST PACAP in mood disorders in men, since increased PACAP levels were observed in this structure in both MDD and the combined mood disorder groups (MDD + BD). Interestingly, only men with mood disorders showed significantly higher PACAP levels in the cBNST compared to controls, while such a difference was not observed in women. The lack of an increased PACAP staining in the cBNST in women indicates that PACAP in the cBNST may not play a major role in the higher vulnerability of women to the

development of mood disorders (Swaab and Bao, 2020; Lebow and Chen, 2016).

Our results are in line with earlier observations in the ovBST in rodents, as Roman et al. (2014) (Roman et al., 2014), Hammack et al. (2009) (Hammack et al., 2009) and Hu et al. (2020) (Hu et al., 2020b) previously found PACAP levels to be elevated in the ovBST of chronically (variate) stressed male rats and mice compared to controls. BNST PACAP has previously been shown to modulate in rodents anxiety-like behaviour, one of the major symptoms of mood disorders (Carvalho et al., 2020; Pinhasov et al., 2011). Our results, together with these data from animal models for depression, indicate that the increase in BNST PACAP may be directly related to the symptom of anxiety in mood disorders.

The present study also investigated whether PACAP is produced in the cBNST. A strength of our paper is that we observed positive PACAP mRNA containing cell bodies throughout the hypothalamus by ISH, indicating the sensitivity of our procedure, while there was no positive ISH signal in the cBNST. It should be noted that, in agreement with this observation, we did not find cytoplasmic PACAP-ir in the human cBNST either. In contrast, Hammack et al. (2009) (Hammack et al., 2009), Maita et al. (2021) (Maita et al., 2021) and Hu et al. (2020) (Hu et al., 2020a; Hu et al., 2020b) did report cytoplasmic PACAP-ir staining in the rat ovBST, indicating a possible species difference. Consequently, PACAP-ir fibres in the human cBNST seem to originate from elsewhere. PACAP positive neurons have previously been observed in the rodent parabrachial nucleus, from which PACAP-ergic fibres seem to project to the ovBST and the central nucleus of the amygdala (CeA) (Hammack and May, 2015; Seiglie et al., 2019). Interestingly, CeA PACAP was also shown to be increased after chronic stress in rats (Seiglie et al., 2022). Consequently, CeA PACAP is also proposed to have a role in mediating anxiety-like behaviour due to chronic stress (Seiglie et al., 2022).

No sex differences were found in the volume of the cBNST immunohistochemically stained with SOM, in contrast to our earlier studies in which it was determined that the cBNST of men is 40% larger than the cBNST of women (Zhou et al., 1995; Kruijver et al., 2000; Chung et al., 2002). The best explanation for this discrepancy is the age of the present cohort (mean 79 ± 2 years). The subjects in the previous studies were of a considerably younger age: 37 ± 2 years (Zhou et al., 1995; Kruijver et al., 2000) and 36 ± 2 years (Chung et al., 2002). In an independent pilot study from our group, we measured cBNST volume in elderly subjects (74 ± 2 years) in a different cohort and also did not find sex differences (D. Velzeboer, 2004, unpublished) (Velseboer, 2004).

A negative correlation was found between age and PACAP cIOD values in mood disorders. This was based upon OD values that decreased with age. Since the patients and controls were well matched for age, this correlation did not influence our conclusions.

Interestingly, the PACAP-ir cIOD in men with a mood disorder and in the BD group showed a positive correlation with CSF pH. The CSF pH is a measure of the agonal state and may change enzyme activity (Swaab and Bao, 2021). However, since the CSF pH in patients and controls was well matched, this correlation did not influence our conclusions.

No significant correlation was found between cBNST volume and age in any of the sub-groups.

Moreover, different brain areas show different PACAP-ir changes in relation to depression and suicide. We previously found significantly higher PACAP expression in BD patients in PVN, while in both DLPFC and ACC, PACAP was downregulated in this mood disorder (Z. Slabe et al., submitted). Therefore, the effects of the administration of PACAP agonists or antagonists to patients are difficult to predict.

4.1. Limitations

The present study has some limitations. There may be more sex differences, since only one woman with BD was included. An unavoidable factor for post-mortem research is the effect of medication, which can influence the expression of neuropeptides in human tissue. There is no information on the effect of the many medicines the patients used on PACAP in the cBNST. We excluded patients who had received corticosteroid therapy in the three months prior to death because of its effect on the hypothalamic stress systems (Erkut et al., 1998).

Considering the results of the present study, PACAP could potentially become a biomarker for stress-related disorders, at least in men. PACAP may be transported across the blood-brain barrier (BBB) (Amin and Schytz, 2018) since it is detectable in blood samples, and increased PACAP blood levels have previously been detected in female patients with post-traumatic stress disorder (PTSD) (Ressler et al., 2011). However, whether alterations in blood levels of PACAP do indeed reflect alterations in the cBNST still remains to be established.

PACAP antagonists were proposed to serve as a novel therapeutic treatment for mood disorders (Katayama et al., 2009; Pinhasov et al., 2011). However, PACAP is widely distributed across the brain (Zhang et al., 2021) and is changing in different directions in different brain areas in mood disorders. Since PACAP is a highly multifunctional peptide involved in many processes, the effects of administration on patients cannot be predicted and many side effects of blocking PACAP can be expected (Katayama et al., 2009).

A systematic study of the sites of origin of PACAP innervation of the human cBNST, comparing the human brain with rodents, is warranted because of possible presence of species differences.

5. Conclusions

PACAP densely innervates neurons of the cBNST, often with basket-like structures. However, PACAP is not produced in the human cBNST, as appeared from our ISH results. The present study shows increased PACAP levels in the cBNST in mood disorders, but only in men, and not in women. Considering the results of the present study, PACAP could potentially become a biomarker for stress-related disorders, at least in men.

Ethics declaration

Post-mortem human hypothalami containing the adjacent BNST were obtained from the Netherlands Brain Bank (NBB, Director Prof. Inge Huitinga). Informed consent was provided by the donors or their next of kin for a brain autopsy and the use of the tissue and the clinical information for research purposes.

Credit authorship contribution statement

Zala Slabe: Conceptualization, methodology, executing experiments, data curation, formal analysis, writing – original draft. Gwyneth A. Pechler: Executing experiments, data collection, writing-original draft, analysis, graphics. Joop van Heerikhuize: technical support, review & editing of the final manuscript. Benjamin A. Samuels: review & editing of the final manuscript. Maja Zorović: technical support, editing of the final manuscript. Marko Živin: review & editing of the final manuscript. Dick F. Swaab: Planning study, supervision, writing – review & editing all versions, funding acquisition.

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Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2023.106191.

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