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Brain Serotonergic and Noradrenergic Deficiencies in Behavioral Variant Frontotemporal Dementia Compared to Early-Onset Alzheimer's Disease

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Abstract. Routinely prescribed psychoactive drugs in behavioral variant frontotemporal dementia (FTD) for improvement of (non)cognitive symptoms are primarily based on monoamine replacement or augmentation strategies. These were, however, initially intended to symptomatically treat other degenerative, behavioral, or personality disorders, and thus lack disease specificity. Moreover, current knowledge on brain monoaminergic neurotransmitter deficiencies in this presenile disorder is scarce, particularly with reference to changes in Alzheimer's disease (AD). The latter hence favors neurochemical comparison studies in order to elucidate the monoaminergic underpinnings of FTD compared to early-onset AD, which may contribute to better pharmacotherapy. Therefore, frozen brain samples, i.e., Brodmann area (BA) 6/8/9/10/11/12/22/24/46, amygdala, and hippocampus, of 10 neuropathologically confirmed FTD, AD, and control subjects were analyzed by means of reversed-phase high-performance liquid chromatography. Levels of serotonergic, dopaminergic, and noradrenergic compounds were measured. In nine brain areas, serotonin (5-HT) concentrations were significantly increased in FTD compared to AD patients, while 5-hydroxyindoleacetic acid/5-HT ratios were decreased in eight regions, also compared to controls. Furthermore, in all regions, noradrenaline (NA) levels were significantly higher, and 3-methoxy-4-hydroxyphenylglycol/NA ratios were significantly lower in FTD than in AD and controls. Contrarily, significantly higher dopamine (DA) levels and reduced homovanillic acid/DA ratios were only found in BA12 and BA46. Results indicate that FTD is defined by distinct

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serotonergic and noradrenergic deficiencies. Additional research regarding the interactions between both monoaminergic networks is required. Similarly, clinical trials investigating the effects of 5-HT_{1A} receptor antagonists or NA-modulating agents, such as $\alpha_{1/2}/\beta_1$-blockers, seem to have a rationale and should be considered.

Keywords: Alzheimer's disease, brain tissue, frontotemporal dementia, monoamines, neurochemistry, neuropsychiatric symptoms, noradrenaline, prefrontal cortex, RP-HPLC-ECD, serotonin

INTRODUCTION

Inasmuch as remarkable progress in diagnostic, molecular, genetic, and neuropathological aspects of frontotemporal dementia (FTD) has been made in recent years, efficient neurotransmitter-based pharmacotherapies for substantial improvement of cognitive and noncognitive symptoms, however, are still lacking, with no Food and Drug Administration (FDA)-approved treatment at the moment [1]. In addition, routinely prescribed psychoactive drugs in FTD are primarily based on monoamine replacement or augmentation strategies, which, conversely, have been developed to symptomatically treat other neurodegenerative, behavioral or personality disorders, such as Alzheimer's disease (AD), Parkinson's disease, major depressive disorder, obsessive-compulsive disorder, and schizophrenia [2, 3]. Moreover, current knowledge on brain monoaminergic neurotransmitter deficiencies in this devastating presenile disorder is scarce, particularly with reference to changes in AD [4], still the most common form of primary early-onset dementia [5]. The latter thus favors neurochemical comparison studies in order to fully clarify the monoaminergic underpinnings of FTD as opposed to early-onset AD, which could contribute to the development of more dementia subtype-specific pharmacological treatments in the long term, and, provide more insight into which combinations of currently administered psychotropic drugs may be more effective.

In this context, Bowen et al. [4] evidenced that there might be a severely imbalanced serotonergic system in frontal and temporal cortex of FTD compared to AD patients, whereas there are no similar reports in literature of studies that examined noradrenergic and dopaminergic brain changes. On the other hand, several cerebrospinal fluid (CSF) studies have been performed previously, even though results were found to be inconsistent. More specifically, some studies observed no alterations in CSF 5-hydroxyindoleacetic acid (5-HIAA; metabolite of serotonin (5-HT; 5-hydroxytryptamine)) content between FTD and AD [2, 6, 7], whereas Engelborghs et al. [8] demonstrated higher CSF homovanillic acid (HVA; metabolite of dopamine (DA)) to 5-HIAA ratios in FTD, which was interpreted as a reflection of the inhibitory modulation of the serotonergic system on dopaminergic functioning [9, 10]. As for the dopaminergic neurotransmitter system, Sjögren et al. [6] concluded that only CSF HVA levels in FTD compared to healthy controls were reduced, while the study of Francis et al. [11] did not report such alterations. Interestingly, the same holds true for the comparison with AD patients [7, 8]. Additionally, Engelborghs et al. [8] also revealed a strong association between physically nonaggressive behavior and CSF levels of 3,4-dihydroxyphenylacetic acid (DOPAC; metabolite of DA) in a subgroup of FTD subjects, which indicates that monoaminergic neurotransmitter alterations might certainly underlie dementia-specific neuropsychiatric symptoms (NPS) [12, 13]. Finally, there is little evidence on noradrenergic deficiencies in FTD compared to AD, with only one CSF study that showed significantly higher 3-methoxy-4-hydroxyphenylglycol (MHPG; metabolite of (nor)adrenaline ((N)A)) levels in FTD [6]. Engelborghs et al. [8] and Vermeiren et al. [7], however, could not corroborate these findings. Sjögren et al. [6] also suggested that CSF MHPG might be useful to differentiate between both neurodegenerative conditions. Correspondingly, the addition of CSF MHPG to the traditional set of AD biomarkers (amyloid-$\beta_{1\text{-}42}$ peptide, total-, and phosphorylated tau protein) has shown to improve the discrimination of dementia with Lewy bodies from AD, but not FTD, whereas the differentiation of AD from FTD only improved marginally [14].

Besides neurochemical studies quantitatively measuring CSF or brain tissue monoamine levels, neuroimaging, receptor, and transporter binding studies also found strongly decreased 5-HT_{1A} and 5-$HT_{2(A)}$ receptor bindings, a decreased presynaptic striatal DA transporter binding, and an unchanged to decreased postsynaptic striatal D_2 receptor binding in FTD patients compared to healthy elderly [2, 15, 16]. The authors further implied that selective serotonin reuptake inhibitors (SSRIs) as antidepressants may be

used as a first-line treatment to reduce NPS in FTD, even though most related clinical trials were small and uncontrolled [2]. As a result, future neurochemical FTD studies have become essential.

Altogether, more insight into the distribution of brain monoamines and metabolites in FTD compared to AD could subsequently not only contribute to a better monoaminergic-based therapeutic approach and understanding of disease-related pathophysiological mechanisms, but might even enable the appraisal of their added biomarker value. Therefore, the present study determined the levels of eight monoamines and metabolites in various postmortem brain regions of age- and gender-matched neuropathologically defined patients with FTD, AD, and, a healthy control group. Except for the intergroup comparisons, the relationship between antemortem NPS and the analyzed neurochemical compounds were examined in both dementia subtypes as well. On the whole and based on aforementioned studies, we mainly expect to find a severely impaired serotonergic and dopaminergic neurotransmission in FTD compared to AD brain with significantly altered 5-HT, DA, DOPAC, and HVA levels, and, to a lesser extent, 5-HIAA levels, whereas the noradrenergic system might have remained relatively preserved (except for MHPG levels).

MATERIALS AND METHODS

Study population and protocol

Frontotemporal lobar degeneration (FTLD) is an umbrella term encompassing a group of heterogeneous pathological disorders which are characterized by relatively selective frontotemporal atrophy and disease onset before the age of 65 in 75–80% of patients. Two main clinical phenotypes exist, including the behavioral variant FTD, which accounts for more than 50% of patients (in the current study is also referred to as 'FTD') and primary progressive aphasia, which can be further categorized into primary nonfluent aphasia and semantic dementia [5, 17]. Primary characteristics of FTD are impairment of executive functions and severe behavioral changes, while in the initial stages of the disease, memory and perceptuospatial skills remain intact [18].

Besides neuropathologically confirmed FTD patients ($n = 10$), ten neuropathologically confirmed AD patients and ten age-matched control subjects were included in our study population as well. All samples were retrospectively selected from the Biobank of the Institute Born-Bunge (University of Antwerp, Antwerp, Belgium). Initially, patients with probable AD according to the NINCDS-ADRDA criteria of McKhann et al. [19, 20] were recruited at the Memory Clinic of the Hospital Network Antwerp (ZNA-Middelheim and ZNA-Hoge Beuken, Antwerp, Belgium) for inclusion in a prospective, longitudinal study on NPS [21]. The latter also applies to all included FTD and control subjects. Probable FTD was diagnosed using the criteria of Neary et al. [22]. All patients also fulfilled the DSM-IV-TR criteria for dementia [23]. The ten age- and gender-matched included AD subjects were part of a larger group of 40 on which neurochemical NPS-related brain research had been conducted previously [24, 25].

Apart from general physical and neurological examinations, blood screening tests, structural neuroimaging by CT, MRI or SPECT, neuropsychological examination (Mini-Mental State Examination scores, MMSE), and optional CSF/blood sampling for biomarker and/or DNA analyses, a baseline behavioral assessment was routinely performed as well. Follow-up behavioral ratings of AD and FTD patients were performed, if possible. Age-matched control subjects were hospitalized in the Middelheim General Hospital (Antwerp, Belgium) and gave brain donation autopsy consent shortly before death. Moreover, clinical neurological examination and a retrospective review of the clinical history, neuropsychological examination, and hospital records did not demonstrate any evidence of dementia, psychiatric antecedents, or cognitive decline. Furthermore, none of the control subjects suffered from central nervous system pathology which was neuropathologically confirmed. Death causes were carcinoma (esophageal ($n = 1$); cervical ($n = 1$); lung ($n = 2$); neuroendocrine ($n = 2$)), multiple myeloma ($n = 1$)), liver cirrhosis ($n = 1$), cardiovascular disease/metabolic syndrome ($n = 1$) and Burkitt's lymphoma ($n = 1$). Written informed consents concerning autopsy and subsequent use of brain tissue, clinical documentation and behavioral information for research purposes were obtained from all participants. The study was approved by the Medical Ethical Committee of the Middelheim General Hospital (Antwerp, Belgium) and conducted in compliance with the Helsinki Declaration.

In case AD, FTD, or control subjects who gave brain donation consent died, brain autopsy was performed within 8 h postmortem, followed by freezing of the left hemisphere at –80°C for

neurochemical analysis, and fixation of the right hemisphere in paraformaldehyde (12%) for neuropathological examination.

Behavioral assessment

Behavior of AD and FTD patients was assessed together with relatives and/or nursing staff using a battery of behavioral assessment scales, including: Behavioral Pathology in Alzheimer's Disease Rating Scale (Behave-AD) [26]; Middelheim Frontality Score (MFS) [18]; Cohen-Mansfield Agitation Inventory (CMAI) [27]; and Cornell Scale for Depression in Dementia (CSDD) [28]. Dementia staging was based on the Global Deterioration Scale (GDS) with a range varying from 1 (nondemented) to 7 (terminal stage of dementia) [29]. During each NPS rating, only the behavioral phenomena covering the last two weeks prior to the assessment were included and rated. Behavioral assessments were repeated during each neurological follow-up examination in the hospital, if possible ($n = 2$ for AD with one ($n = 1$) and two ($n = 1$) follow-up ratings; $n = 4$ for FTD with one ($n = 1$), three ($n = 2$) and four ($n = 1$) follow-up ratings). A final retrospective behavioral scoring was performed in case patients died approximately more than two weeks after the last follow-up visit. However, in total, eight AD and six FTD patients underwent only one rating close to death, given the short amount of time which was left since they entered our study protocol. No behavioral scores were available for the control group. For this study, behavioral scores of the final assessment as close as possible to date of death were used.

Neuropathological evaluation

Neuropathological diagnosis was performed on the formaldehyde-fixated right hemisphere. A standard selection of 10 to 13 regionally dissected brain regions, including frontal, temporal and occipital blocks of the neocortex, amygdala, hippocampus (at the level of the posterior part of the amygdala and the lateral geniculate body), basal ganglia, thalamus, brain stem, substantia nigra (SN), pons at the level of the locus coeruleus (LC) and cerebellum, was embedded in paraffin and routinely stained with hematoxylin-eosin, cresyl violet and Bodian's technique. Furthermore, routinely applied immunostains were 4G8 (amyloid) and AT8 (P-tau$_{181\text{-P}}$), as well as staining against hyperphosphorylated TAR DNA-binding protein (TDP)-43 and ubiquitin. When the presence of Lewy bodies was suspected on hematoxylin-eosin and ubiquitin immunoreactivity, an anti-α-synuclein staining was applied.

AD patients were neuropathologically diagnosed according to the criteria of Braak and Braak [30], Braak et al. [31], and, Jellinger and Bancher [32] to decide on definite AD. Additionally, the 'ABC' scoring method of Montine et al. [33] was applied to all AD brains collected after May 2011 ($n = 2$). FTD patients, on the other hand, were diagnosed using the criteria by Cairns et al. [34] and Mackenzie et al. [35–37], which propose a new terminology for FTLD-subtypes and a classification of TDP-43 proteinopathies into types A-D [37]. The overall histopathological diagnoses of the included FTD subjects were FTLD-tau/Pick's disease ($n = 3$) and FTLD with ubiquitin-positive inclusions (FTLD-U) ($n = 7$), of which the FTLD-U patients could be further categorized into FTLD-TDP-43 type A ($n = 3$), FTLD-TDP-43 type B ($n = 3$) and FTLD-ubiquitin proteasome system (UPS) ($n = 1$).

Regional brain dissection

Regional brain dissection of the left frozen hemisphere was performed according to a standard procedure [24, 25] during which 21 brain regions are dissected. With regard to this study design, a total of 11 behaviorally and neurochemically relevant brain areas were ultimately analyzed by reversed-phase high-performance liquid chromatography (RP-HPLC) with electrochemical detection (ECD), i.e., Brodmann area (BA) 6, 8, 9, and 10 (medial and prefrontal cortex), BA11 and 12 (orbitofrontal cortex), BA22 (temporal cortex), BA24 (cingulate gyrus), BA46 (dorsolateral prefrontal cortex), amygdala, and hippocampus.

Neurochemical analysis sample preparation procedure and pH measurement

A recently optimized and validated RP-HPLC-ECD system for the fast and simultaneous detection of monoaminergic compounds in human brain tissue was used to simultaneously measure the concentrations of 5-HT, (N)A, DA, and their respective metabolites, i.e., 5-HIAA, MHPG, and DOPAC/HVA [39]. In short, sample analysis was performed using an Alexys™ Dual Monoamines Analyzer (Antec Leyden BV, Zoeterwoude, The Netherlands) by which each brain tissue sample was directly analyzed in duplicate. Output ranges were 500 pA and

1 nA with two electrochemical VT03 flow cells each containing a glassy carbon working electrode of 0.7 mm and an *in situ* Ag/AgCl reference electrode at 670 mV placed in a Decade II electrochemical detector (Antec Leyden BV, Zoeterwoude, The Netherlands). An isocratic flow rate of 40 μL of mobile phase per minute was set for both LC 110 pumps. The optimal conditions for separation of the monoaminergic compounds were obtained using a mobile phase comprising 13 % methanol combined with a mixture of phosphoric (50 mM) and citric acid (50 mM), octane-1-sulfonic acid sodium salt (1.8 mM), KCl (8 mM), and ethylenediaminetetraacetic acid (EDTA; 0.1 mM; pH 3.6). Samples were loaded onto two microbore ALF-125 columns (250 mm × 1.0 mm, 3 μm particle size) filled with a porous C18 silica stationary phase. Separation of the monoamines and metabolites was achieved at a stable column and VT03 flow cell temperature of 36°C with a total runtime of approximately 45 min per sample. Levels of the monoaminergic compounds were calculated using Clarity™ Software (DataApex Ltd, 2008, Prague, The Czech Republic). All purchased chemicals were of analytical grade. The brain sample preparation procedure prior to RP-HPLC-ECD analysis was fast and simple, and performed as described in Van Dam et al. [39].

Samples need to be nonacidotic (i.e., pH > 6.1) [40, 41] in order to guarantee high-quality brain tissue, since acidosis may induce alterations in neurotransmitter concentrations and enzyme activities. Several factors such as a prolonged death struggle, antemortem stroke, and a long postmortem delay could acidify brain tissue [42]. For this study, pH values of the cerebellar cortex were measured since the cerebellar pH has previously been shown to be most representative for the entire brain hemisphere [43]. The accompanying analytical procedure was performed as described by Stan et al. [43].

Statistical analysis

Nonparametric statistics were applied due to the limited number of patients and ordinal variables (behavioral scores). A Shapiro Wilk test of normality was first performed to test whether our obtained data complied with a normally distributed study population. Fisher's Exact test was applied to compare male/female ratios and patients taking/not taking psychotropic medication across groups. Kruskal-Wallis analyses with *post hoc* Mann-Whitney U tests were used for comparison of all demographic, clinical, behavioral, pH, and monoaminergic data between AD, FTD, and control subjects. In all cases, only data remaining statistically significant following a Bonferroni correction for multiple comparisons ($p < 0.017$ for three group comparisons) were considered significant. Mann-Whitney U tests were also applied to look at potential confounding effects of psychotropic medication within each group.

Finally, in order to calculate neurochemical correlations of MFS-, CMAI-, CSDD-, and Behave-AD cluster scores in the total group of AD ($n = 10$) and FTD ($n = 10$) patients, nonparametric Spearman's Rank Order correlation statistics were applied. Again, a Bonferroni correction was performed and only those significant data were taken into account ($p < 0.000022$). All analyses were performed using SPSS 22.0 for Windows (IBM SPSS Software, Armonk, NY, IBM Corp).

RESULTS

Demographics clinical data behavioral assessment scores dementia staging and pH values

Corresponding data are summarized in Table 1 and the electronic Supplementary Material. The AD, FTD and control groups were age- and gender-matched. Moreover, the number of patients taking/not taking psychotropic medication was comparable between all groups ($p = 0.5$). In the AD group, administered subtypes of psychotropic medication were antidepressants ($n = 1$), antipsychotics ($n = 2$), and cholinesterase inhibitors ($n = 1$). In the FTD group, patients were on antidepressants ($n = 4$), antipsychotics ($n = 2$), cholinesterase inhibitors ($n = 1$), and benzodiazepines ($n = 2$). Lastly, some control subjects were on antidepressants ($n = 1$) and benzodiazepines ($n = 1$). There were statistically significant differences regarding storage times of the frozen brain material between the AD and control group, and, the FTD and control group ($p = 0.0005$ and $p = 0.004$, respectively). The average interval between the last behavioral rating and time of death was 0 and 2.9 days for the AD and FTD groups, respectively. Postmortem delay, GDS scores, and pH-values were comparable between groups. Additionally, all FTD and control subjects had cerebellar pH values > 6.1. In contrast, two AD patients had low cerebellar pH-values (<6.1), for which supplementary pH analyses on the remaining 11 brain

Table 1
Demographics, clinical data, dementia staging, and pH values

Parameter	AD ($n=10$)	FTD ($n=10$)	Controls ($n=10$)	Kruskal-Wallis/Mann-Whitney U
Demographics and clinical data				
Age at onset dementia (y)	60.0 ± 7.8 (49–71)	56.5 ± 5.7 (47–65)	N/A	$p=0.225$
Age at death (y)	66.4 ± 6.4 (58–75)	63.4 ± 6.3 (51–72)	65.7 ± 5.3 (57–73)	$p=0.610$
Gender, male/female (n)	7/3	7/3	6/4	Fisher's Exact test = 0.418 $p=1.000$
Storage time tissue at –80°C (y)	3.0 ± 1.2^{aa} (0.3–9.9)	4.9 ± 1.4^{b} (0.7–15.0)	9.9 ± 0.7^{aa,b} (4.3–11.6)	$p=$ **0.001**
Postmortem delay* (h)	3.9 ± 0.4 (2.8–7.0)	4.2 ± 0.6 (2.0–7.6)	5.1 ± 0.5 (2.5–7.0)	$p=0.249$
Taking/not taking psychotropic medication (n)	3/7	5/5	2/8	Fisher's Exact test = 2.015 $p=0.500$
Dementia staging and pH data				
GDS score (/7): Dementia staging	6.6 ± 0.7 (5–7)	6.7 ± 0.7 (5–7)	N/A	$p=0.654$
pH values cerebellar brain tissue	6.3 ± 0.4 (5.9–7.1)	6.4 ± 0.3 (6.1–6.8)	6.3 ± 0.2 (6.1–6.6)	$p=0.481$

Mean±SD; minimum-maximum range is displayed in parentheses. The results of Kruskal-Wallis and Mann-Whitney U tests for 3 and 2 group comparisons, respectively, are displayed in the rightmost column. Significant p values (<0.05) are depicted in bold. One and two superscript letters are used to indicate significant group differences after *post hoc* Mann-Whitney U analyses with $p<0.017$ and $p<0.001$, respectively. Letters a and b signify differences between AD and controls and FTD and controls, respectively; *Postmortem delay indicates the number of hours between time of death and storage of the brain at –80°C. AD, Alzheimer's disease; FTD, frontotemporal dementia; GDS, Global Deterioration Scale; N/A, not applicable.

regions were performed. Eventually, brain samples with acidotic pH values were excluded from statistical analysis, i.e., BA8 ($n=1$), BA9 ($n=1$), BA22 ($n=1$), and BA46 ($n=1$).

Brain MRI data (not shown) revealed that 7 out of 10 FTD subjects had no asymmetric brain degeneration on average two years before brain autopsy. There was a maximum of 39 and minimum of 3 months between MRI scans and death. Of the three individuals with asymmetric brain atrophy, one had predominant left atrophy of temporal and frontal lobes, and the two others had a slightly more pronounced right atrophy of the temporal horn (but not frontal lobe). As for the behavioral data, Behave-AD cluster B ($p=0.013$), AB ($p=0.013$), D ($p=0.008$), and total ($p=0.013$) scores, as well as the CMAI cluster 1 ($p=0.006$), 3 ($p=0.023$), and, CSDD total scores ($p=0.034$) were all significantly higher in the AD group as compared to their FTD counterparts (Supplementary Table 1).

Neurochemical results

Monoaminergic data of the intergroup comparisons are summarized in Table 2. Nonsignificant data were omitted. Likewise, the most significant group differences regarding 5-HIAA/5-HT ratios, 5-HT levels, MHPG/NA ratios, and NA levels are represented in Figs. 1–4.

Serotonergic findings

Firstly, 5-HIAA/5-HT ratios, indicative of the catabolic serotonergic turnover, were significantly lower in FTD compared to AD subjects in eight brain regions (BA8, $p=0.002$; BA9, $p=0.010$; BA10, $p=0.009$; BA11, $p=0.004$; BA12, $p=0.004$; BA22, $p=0.00002$; BA46, $p=0.013$; and hippocampus, $p=0.003$). The same applied to five brain regions for the FTD versus control group comparison (BA8, $p=0.011$; BA9, $p=0.007$; BA11, $p=0.015$; BA12, $p=0.015$; and BA22, $p=0.004$). Remarkably, only in BA22, a significant difference in 5-HIAA/5-HT ratios could be detected between AD and control subjects, with higher values in the AD group ($p=0.004$; Fig. 1).

Furthermore, 5-HT concentrations were higher in FTD than in AD patients in nine out of 11 brain regions (BA6, $p=0.009$; BA8, $p=0.002$; BA9, $p=0.008$; BA11, $p=0.007$; BA12, $p=0.001$; BA22, $p=0.001$; BA46, $p=0.003$; amygdala, $p=0.002$; and hippocampus, $p=0.003$). In the amygdala and hippocampus, 5-HT levels were significantly higher in control subjects compared to AD patients as

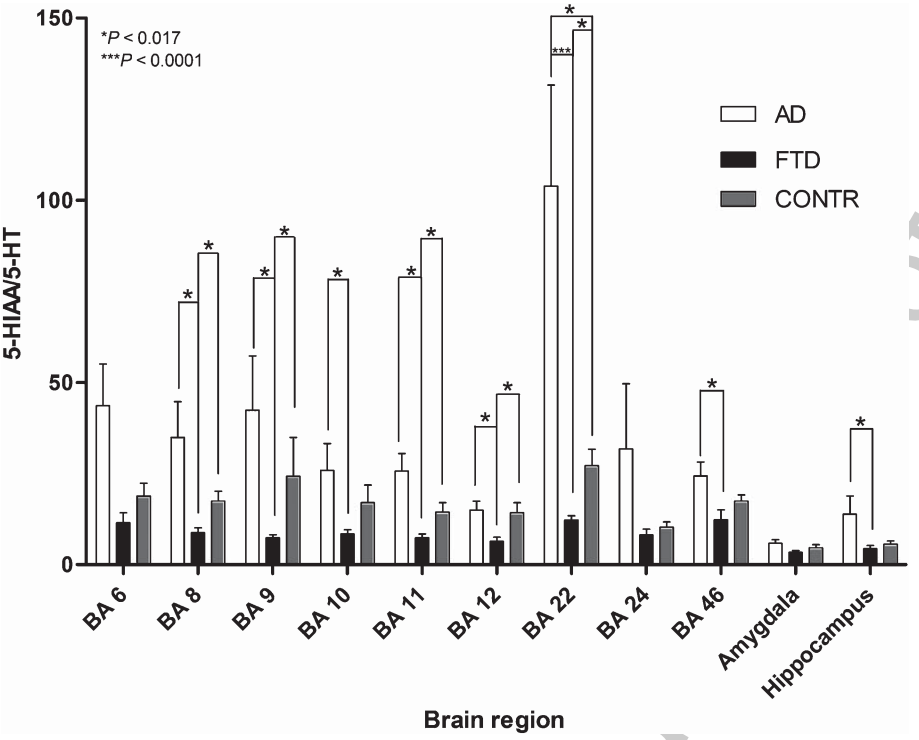

Fig. 1. 5-HIAA/5-HT ratios, indicative of the catabolic serotonergic turnover, across eleven brain regions in AD compared to FTD and control subjects. Data are presented as mean with SD. Only differences (Kruskal Wallis, $p < 0.05$) remaining statistically significant after *post hoc* Mann-Whitney U tests with Bonferroni correction are indicated by asterisks ($^{*}p < 0.017$; $^{***}p < 0.0001$). 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; AD, Alzheimer's disease; BA, Brodmann area; CONTR, controls; FTD, frontotemporal dementia.

well ($p = 0.00009$ and $p = 0.001$, respectively). No significant differences, however, were identified between the FTD and control group. Notably, in BA10 and BA24, no significant group differences were found (Fig. 2).

Lastly, in BA22, 5-HIAA concentrations were significantly higher in AD compared to FTD patients ($p = 0.013$), whereas in the amygdala, 5-HIAA levels were significantly lower in AD compared to control subjects ($p = 0.001$) (Table 2).

Noradrenergic findings

For all brain regions analyzed, FTD patients had the lowest MHPG/NA ratios, indicative of the catabolic noradrenergic turnover, compared to their AD counterparts, with the most pronounced differences in BA6, BA9, BA10, BA11, BA24, amygdala and hippocampus (all $p < 0.0001$; Fig. 3). Additionally, in BA9 ($p = 0.007$), BA10 ($p = 0.007$), BA11 ($p = 0.003$), BA22 ($p = 0.005$), BA24 ($p = 0.009$), and BA46 ($p = 0.007$), MHPG/NA ratios were significantly lower in FTD as opposed to control subjects as well. Moreover, in the prefrontal cortex, ratios were higher in the AD compared to the control group (BA6, $p = 0.004$; BA8, $p = 0.006$; BA9, $p = 0.001$; and BA10, $p = 0.005$).

In contrast, NA levels were significantly higher in the FTD than in the AD group for all 11 brain regions, while in BA6 ($p = 0.0003$), BA8 ($p = 0.001$), BA9 ($p = 0.0002$), BA10 ($p = 0.004$), BA11 ($p = 0.01$), BA24 ($p = 0.004$), and amygdala ($p = 0.006$) NA levels were significantly lower in AD as opposed to controls. Furthermore, in BA8 ($p = 0.015$) and BA46 ($p = 0.003$), NA levels were significantly higher in FTD patients in comparison with control subjects (Fig. 4).

Finally, MHPG concentrations were significantly lower in FTD than in AD patients in eight regions (BA9, $p = 0.001$; BA10, $p = 0.0005$; BA11, $p = 0.0002$; BA22, $p = 0.0003$; BA24, $p = 0.002$; BA46, $p = 0.003$; amygdala, $p = 0.0003$; and hippocampus, $p = 0.001$). There were no significant differences in MHPG levels between the AD and control group. Contrarily, MHPG concentrations were higher in control subjects than in FTD patients in BA9

Table 2
Brain region-specific monoaminergic data of AD, FTD, and control subjects

Brain region	MA, MT, or ratio	AD ($n=10$)	FTD ($n=10$)	Controls ($n=10$)	Kruskal Wallis
BA6	NA (ng/g)	13.7 (8.7–18.7) $n=10^{aa,ccc}$	83.9 (65.9–101.5) $n=10^{ccc}$	54.9 (30.8–87.7) $n=10^{aa}$	$p=0.0002$
	DA (ng/g)	6.5 (4.0–10.6) $n=10$	10.0 (6.2–15.7) $n=10^{b}$	4.5 (2.7–7.0) $n=10^{b}$	$p=0.039$
	5-HT (ng/g)	3.9 (1.4–10.7) $n=10^{c}$	16.8 (11.0–22.1) $n=10^{c}$	9.4 (6.5–15.4) $n=10$	$p=0.017$
	HVA/DA	20.2 (16.5–38.2) $n=10$	15.3 (8.5–29.1) $n=10^{b}$	41.3 (17.6–68.0) $n=10^{b}$	$p=0.022$
	MHPG/NA	23.5 (13.7–40.7) $n=10^{a,ccc}$	1.9 (0.9–2.6) $n=10^{ccc}$	3.1 (1.2–8.8) $n=10^{a}$	$p=0.0004$
BA8	NA (ng/g)	7.9 (6.1–15.1) $n=9^{a,cc}$	63.8 (46.2–82.6) $n=10^{b,cc}$	33.8 (19.5–54.4) $n=10^{a,b}$	$p=0.0002$
	5-HT (ng/g)	2.8 (1.8–9.1) $n=9^{c}$	19.1 (9.5–23.4) $n=10^{c}$	9.4 (5.3–12.6) $n=10$	$p=0.006$
	5-HIAA/5-HT	24.6 (12.8–45.1) $n=9^{c}$	7.2 (5.9–10.8) $n=10^{b,c}$	15.2 (11.7–23.5) $n=10^{b}$	$p=0.003$
	MHPG/NA	48.9 (16.6–54.6) $n=9^{a,cc}$	2.1 (1.4–3.5) $n=10^{cc}$	4.6 (1.5–16.2) $n=10^{a}$	$p=0.001$
BA9	MHPG (ng/g)	465.7 (311.8–539.4) $n=9^{c}$	90.2 (57.9–175.4) $n=10^{b,c}$	291.4 (156.9–553.2) $n=10^{b}$	$p=0.002$
	NA (ng/g)	4.0 (2.7–16.0) $n=9^{aa,ccc}$	55.0 (34.1–59.5) $n=10^{ccc}$	33.5 (26.0–46.3) $n=10^{aa}$	$p=0.00008$
	5-HT (ng/g)	6.0 (2.9–11.9) $n=9^{c}$	19.0 (10.1–25.8) $n=10^{c}$	11.6 (6.1–15.4) $n=10$	$p=0.019$
	5-HIAA/5-HT	28.0 (10.4–72.5) $n=9^{c}$	6.4 (5.5–9.9) $n=10^{b,c}$	15.2 (8.9–20.3) $n=10^{b}$	$p=0.008$
	MHPG/NA	59.3 (23.5–237.3) $n=9^{a,ccc}$	2.3 (1.2–4.4) $n=10^{b,ccc}$	6.5 (4.7–23.4) $n=10^{a,b}$	$p=0.00007$
BA10	MHPG (ng/g)	508.9 (246.1–1080.1) $n=10^{cc}$	113.7 (65.3–174.1) $n=10^{cc}$	245.9 (146.6–567.7) $n=10$	$p=0.002$
	NA (ng/g)	5.1 (3.8–16.1) $n=10^{a,ccc}$	36.5 (31.1–40.7) $n=10^{ccc}$	24.4 (16.1–36.11) $n=10^{a}$	$p=0.0003$
	5-HIAA/5-HT	19.7 (10.1–33.6) $n=10^{c}$	7.9 (5.9–10.2) $n=10^{c}$	13.0 (7.4–19.0) $n=10$	$p=0.021$
	MHPG/NA	97.4 (21.1–236.0) $n=10^{a,ccc}$	3.0 (2.2–4.7) $n=10^{b,ccc}$	9.7 (4.6–31.2) $n=10^{a,b}$	$p=0.0001$
BA11	MHPG (ng/g)	473.6 (257.7–728.0) $n=10^{cc}$	113.3 (50.7–182.5) $n=10^{b,cc}$	432.8 (287.7–671.9) $n=10^{b}$	$p=0.001$
	NA (ng/g)	8.0 (6.2–17.3) $n=9^{a,cc}$	29.8 (26.3–31.7) $n=10^{cc}$	19.7 (13.2–25.9) $n=10^{a}$	$p=0.001$
	5-HT (ng/g)	11.2 (6.5–13.8) $n=10^{c}$	21.8 (14.0–27.5) $n=10^{c}$	18.0 (11.0–21.7) $n=10$	$p=0.017$
	5-HIAA/5-HT	27.9 (12.2–37.4) $n=10^{c}$	6.4 (4.2–10.2) $n=10^{b,c}$	11.7 (9.0–18.8) $n=10^{b}$	$p=0.005$
	MHPG/NA	56.0 (29.5–99.7) $n=9^{ccc}$	3.8 (2.5–6.7) $n=10^{b,ccc}$	21.6 (9.0–39.9) $n=10^{b}$	$p=0.0003$
BA12	NA (ng/g)	8.1 (5.7–19.3) $n=10^{c}$	30.2 (19.4–42.8) $n=10^{c}$	20.7 (13.6–41.7) $n=10$	$p=0.004$
	A (ng/g)	4.2 (2.4–6.0) $n=5^{c}$	12.4 (9.1–12.9) $n=7^{c}$	8.0 (4.9–14.5) $n=4$	$p=0.017$
	DA (ng/g)	2.9 (2.5–5.7) $n=10^{c}$	8.9 (5.0–17.2) $n=10^{c}$	4.3 (2.7–6.6) $n=10$	$p=0.017$
	5-HT (ng/g)	16.1 (9.3–22.8) $n=10^{c}$	31.1 (23.9–85.5) $n=10^{c}$	23.5 (15.1–33.0) $n=10$	$p=0.006$
	5-HIAA/5-HT	13.6 (9.2–20.7) $n=10^{c}$	5.3 (3.7–9.0) $n=10^{b,c}$	12.5 (6.5–20.3) $n=10^{b}$	$p=0.010$

(*Continued*)

Table 2
(*Continued*)

	HVA/DA	54.4 (31.0–73.5) $n = 10^{c}$	17.2 (13.4–22.6) $n = 10^{c}$	51.4 (21.0–71.1) $n = 10$	$p = 0.018$
	MHPG/NA	40.8 (16.5–69.5) $n = 10^{c}$	3.2 (2.7–5.1) $n = 10^{c}$	11.3 (1.7–39.1) $n = 10$	$p = 0.012$
BA22	MHPG (ng/g)	554.9 (471.5–779.9) $n = 9^{cc}$	103.1 (60.3–198.0) $n = 10^{b,cc}$	360.5 (260.4–360.7) $n = 10^{b}$	$p = 0.001$
	NA (ng/g)	10.0 (6.23–19.9) $n = 7^{c}$	33.6 (21.8–50.6) $n = 10^{c}$	18.7 (13.6–28.5) $n = 10$	$p = 0.006$
	5-HIAA (ng/g)	317.7 (144.8–485.8) $n = 9^{c}$	117.7 (97.4–197.0) $n = 10^{c}$	171.5 (121.7–320.6) $n = 10$	$p = 0.026$
	5-HT (ng/g)	4.2 (1.9–7.3) $n = 9^{c}$	12.8 (8.3–13.5) $n = 10^{c}$	7.4 (3.8–11.9) $n = 10$	$p = 0.009$
	5-HIAA/5–HT	67.8 (33.8–175.4) $n = 9^{a,ccc}$	11.9 (9.0–14.9) $n = 10^{b,ccc}$	23.5 (18.0–36.7) $n = 10^{a,b}$	$p = 0.00008$
	DOPAC/DA	2.4 (1.5–4.7) $n = 8^{a}$	1.1 (0.8–2.6) $n = 10$	1.0 (0.5–1.5) $n = 10^{a}$	$p = 0.044$
	MHPG/NA	83.5 (27.1–91.4) $n = 7^{cc}$	3.6 (2.0–5.7) $n = 10^{b,cc}$	20.0 (7.8–46.9) $n = 10^{b}$	$p = 0.001$
BA24	MHPG (ng/g)	552.0 (252.0–856.3) $n = 10^{cc}$	101.4 (65.7–164.6) $n = 10^{b,cc}$	336.9 (158.1–587.5) $n = 10^{b}$	$p = 0.001$
	NA (ng/g)	17.6 (10.5–22.7) $n = 9^{a,cc}$	45.3 (36.8–53.0) $n = 10^{cc}$	33.5 (26.4–43.4) $n = 10^{a}$	$p = 0.001$
	MHPG/NA	36.9 (14.9–67.0) $n = 9^{ccc}$	2.3 (1.8–3.5) $n = 10^{b,ccc}$	9.3 (3.4–21.9) $n = 10^{b}$	$p = 0.0004$
BA46	MHPG (ng/g)	205.6 (145.4–514.3) $n = 9^{c}$	82.6 (47.4–156.1) $n = 10^{c}$	105.8 (58.6–462.4) $n = 10$	$p = 0.035$
	NA (ng/g)	5.5 (4.4–14.8) $n = 9^{cc}$	41.7 (30.8–52.1) $n = 10^{b,cc}$	12.7 (6.8–22.1) $n = 10^{b}$	$p = 0.001$
	DA (ng/g)	3.0 (2.1–8.4) $n = 9^{c}$	12.2 (6.9–17.7) $n = 10^{b,c}$	4.2 (3.4–5.5) $n = 10^{b}$	$p = 0.005$
	5-HT (ng/g)	6.0 (3.0–7.6) $n = 9^{c}$	18.1 (9.4–22.4) $n = 10^{c}$	9.9 (6.6–14.6) $n = 10$	$p = 0.006$
	5-HIAA/5-HT	27.8 (11.6–33.4) $n = 9^{c}$	9.4 (7.1–14.2) $n = 10^{c}$	17.8 (15.3–20.0) $n = 10$	$p = 0.024$
	HVA/DA	29.1 (15.1–52.4) $n = 9$	11.1 (7.8–18.5) $n = 10^{b}$	32.8 (16.7–48.0) $n = 10^{b}$	$p = 0.022$
	MHPG/NA	57.8 (20.5–75.8) $n = 9^{c}$	2.7 (1.1–3.0) $n = 10^{b,c}$	11.4 (4.2–37.9) $n = 10^{b}$	$p = 0.001$
Amygdala	MHPG (ng/g)	651.3 (327.3–1009.7) $n = 9^{cc}$	124.9 (70.8–205.1) $n = 9^{b,cc}$	304.8 (193.5–759.3) $n = 10^{b}$	$p = 0.002$
	NA (ng/g)	59.8 (40.3–78.7) $n = 9^{a,c}$	104.3 (66.2–114.5) $n = 9^{c}$	84.5 (77.5–121.8) $n = 10^{a}$	$p = 0.010$
	5-HIAA (ng/g)	481.2 (317.4–668.1) $n = 9^{a}$	765.6 (471.9–878.4) $n = 9$	999.8 (754.5–1270.4) $n = 10^{a}$	$p = 0.004$
	HVA (ng/g)	558.6 (378.5–706.9) $n = 9^{a}$	562.5 (395.3–779.1) $n = 9^{b}$	1132.5 (751.0–1421.4) $n = 10^{a,b}$	$p = 0.006$
	5-HT (ng/g)	109.6 (49.4–139.9) $n = 9^{aaa,c}$	225.2 (173.3–274.5) $n = 9^{c}$	244.9 (221.7–297.2) $n = 10^{aaa}$	$p = 0.001$
	MHPG/NA	7.9 (4.8–26.5) $n = 9^{ccc}$	1.7 (0.8–2.1) $n = 9^{ccc}$	3.0 (1.4–9.9) $n = 10$	$p = 0.001$
Hippocampus	MHPG (ng/g)	716.8 (290.0–1099.3) $n = 10^{c}$	140.0 (71.9–193.7) $n = 9^{b,c}$	416.3 (232.9–713.5) $n = 10^{b}$	$p = 0.002$
	NA (ng/g)	23.0 (17.2–36.7) $n = 9^{c}$	50.4 (40.6–82.3) $n = 9^{c}$	43.0 (25.8–81.9) $n = 10$	$p = 0.014$
	5-HT (ng/g)	41.4 (20.2–61.1) $n = 10^{a,c}$	85.9 (59.0–144.6) $n = 9^{c}$	87.8 (69.3–111.2) $n = 10^{a}$	$p = 0.002$

(*Continued*)

Table 2
(*Continued*)

Brain region	MA, MT, or ratio	AD ($n=10$)	FTD ($n=10$)	Controls ($n=10$)	Kruskal Wallis
	5-HIAA/5-HT	9.3 (6.5–12.7) $n=10^{c}$	3.3 (2.6–5.9) $n=9^{c}$	5.3 (3.4–7.3) $n=10$	$p=0.008$
	MHPG/NA	20.1 (9.2–70.7) $n=9^{ccc}$	2.8 (1.1–3.5) $n=9^{ccc}$	8.2 (2.3–31.4) $n=10$	$p=0.001$

Median with IQR denoted between parentheses and *p* values after Kruskal Wallis analysis in the rightmost column ($p<0.05$). Only data that remained statistically significant after *post hoc* Mann-Whitney U tests with Bonferroni correction ($p<0.017$ for three group comparisons, one superscript letter) are displayed. Two and three superscript letters are used to indicate significant differences with $p<0.001$ and $p<0.0001$, respectively. Superscript letters a, b and c signify differences between AD and controls, FTD and controls, and, AD and FTD, respectively. 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine (serotonin); A, adrenaline; AD, Alzheimer's disease; BA, Brodmann area; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; FTD, frontotemporal dementia; HVA, homovanillic acid; MA/MT, monoamine and metabolite; MHPG, 3-methoxy-4-hydroxyphenylglycol; NA, noradrenaline.

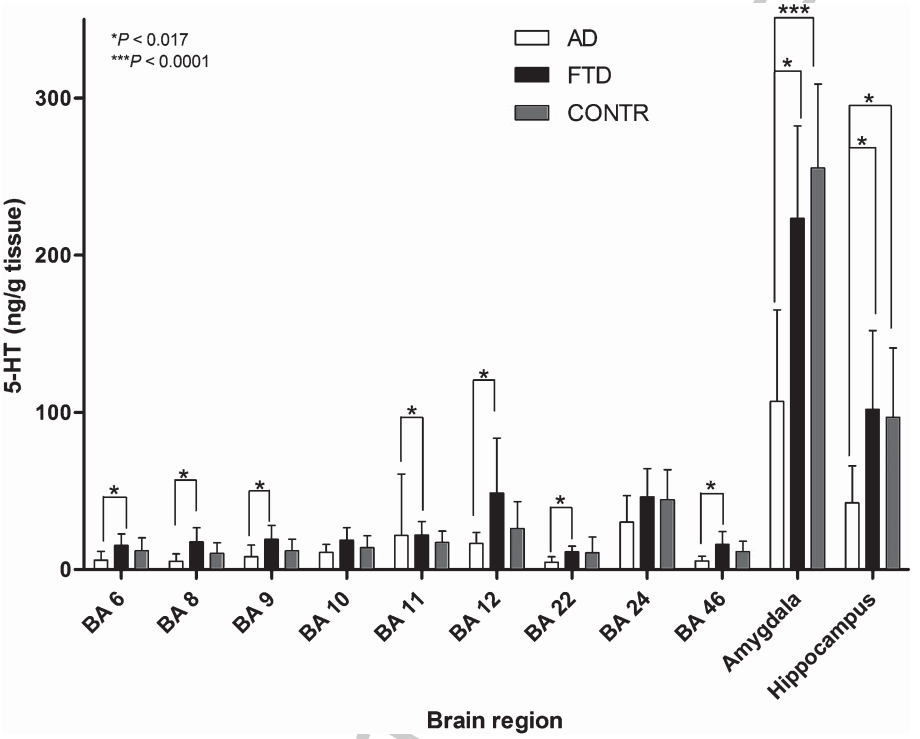

Fig. 2. 5-HT levels across eleven brain regions in AD compared to FTD and control subjects. Data are presented as mean with SD. Only differences (Kruskal Wallis, $p<0.05$) remaining statistically significant after *post hoc* Mann-Whitney U tests with Bonferroni correction are indicated by asterisks ($^{*}p<0.017$; $^{***}p<0.0001$). 5-HT, 5-hydroxytryptamine; AD, Alzheimer's disease; BA, Brodmann area; CONTR, controls; FTD, frontotemporal dementia.

($p=0.007$), BA11 ($p=0.002$), BA22 ($p=0.002$), BA24 ($p=0.009$), amygdala ($p=0.013$), and hippocampus ($p=0.002$).

Dopaminergic findings

Overall, significant dopaminergic group differences were scarce, with only higher DA levels and lower HVA/DA ratios, indicative of the catabolic dopaminergic turnover, in BA12 ($p=0.007$ and $p=0.002$, respectively), and higher DA levels in BA46 ($p=0.008$) in FTD compared to AD patients. In BA6 and BA46, higher DA levels ($p=0.01$ and $p=0.003$) and lower HVA/DA ratios ($p=0.005$ and $p=0.01$) in FTD compared to control subjects were observed accordingly. No significant differences concerning HVA/DA ratios were found between AD and control groups, apart from higher DOPAC/DA ratios in BA22 of AD subjects ($p=0.016$; Table 2).

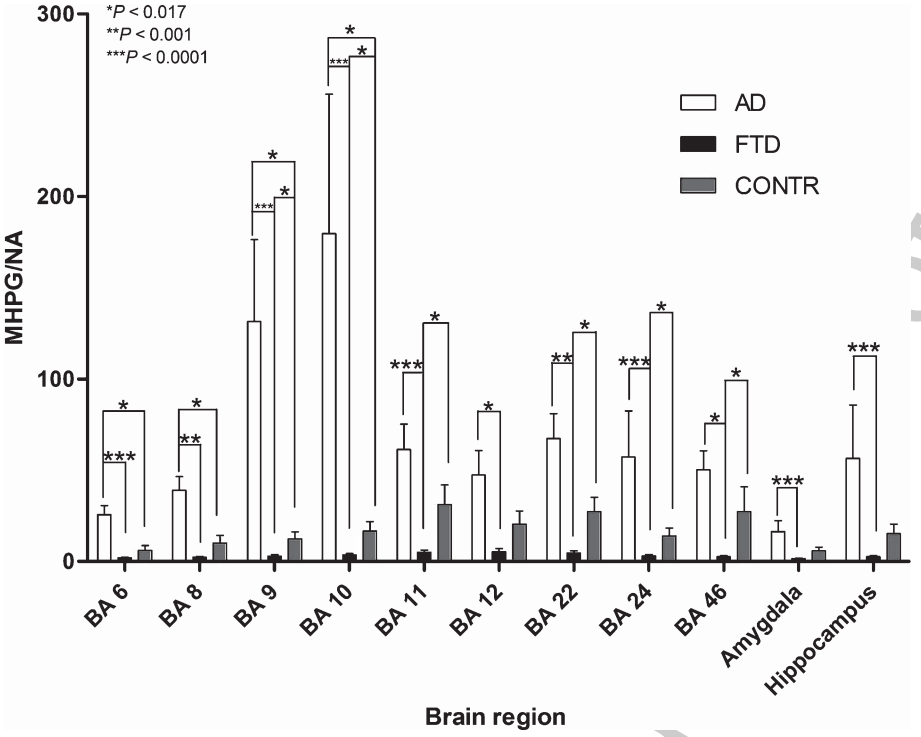

Fig. 3. MHPG/NA ratios, indicative of the catabolic noradrenergic turnover, across eleven brain regions in AD compared to FTD and control subjects. Data are presented as mean with SD. Only differences (Kruskal Wallis, $p < 0.05$) remaining statistically significant after *post hoc* Mann-Whitney U tests with Bonferroni correction are indicated by asterisks ($^{*}p < 0.017$; $^{**}p < 0.001$; $^{***}p < 0.0001$). AD, Alzheimer's disease; BA, Brodmann area; CONTR, controls; FTD, frontotemporal dementia; MHPG, 3-methoxy-4-hydroxyphenylglycol; NA, noradrenaline.

Neurochemical correlates of NPS in AD and FTD

Neurochemical correlates of NPS in the AD and FTD groups are presented in Supplementary Table 2. Due to the extensive amount of data with $p < 0.05$, only the most significant correlations were finally retained ($p < 0.005$). None of these correlations, however, remained statistically significant following a total Bonferroni correction for multiple comparisons (i.e., $p < 0.00002$).

As for the AD group, NA levels in BA12 correlated with CMAI cluster 1 scores (aggressive behavior) ($p = 0.0002$, $R = +0.914$, $n = 10$), whereas in BA22, HVA/5-HIAA ratios correlated with Behave-AD cluster G scores (anxieties and phobias) ($p = 0.001$, $R = +0.850$, $n = 9$). Furthermore, hippocampal 5-HIAA concentrations inversely correlated with Behave-AD total scores, CMAI cluster 3 scores (verbally agitated behavior) and CMAI total scores ($p = 0.0001$, $R = –0.927$, $n = 10$; $p = 0.00006$, $R = –0.939$, $n = 10$; and $p = 0.0003$, $R = –0.903$, $n = 10$). Hippocampal HVA/5-HIAA ratios also correlated with Behave-AD cluster G scores, CMAI cluster 2 (physically nonaggressive behavior) and cluster 3 scores ($p = 0.004$, $R = +0.816$, $n = 10$; $p = 0.001$, $R = +0.872$, $n = 10$; and $p = 0.001$, $R = +0.865$, $n = 10$), as well as Behave-AD and CMAI total scores ($p = 0.0004$, $R = +0.902$, $n = 10$; and $p = 0.0003$, $R = +0.903$, $n = 10$).

In the FTD group, NA levels in BA9 inversely correlated with Behave-AD cluster D scores (aggressiveness) ($p = 0.004$, $R = –0.813$, $n = 10$). Additionally, 5-HT concentrations in BA12 were inversely associated with Behave-AD global scores ($p = 0.003$, $R = –0.834$, $n = 10$). Lastly, in BA46, HVA/DA ratios correlated with CMAI total scores ($p = 0.004$, $R = +0.812$, $n = 10$).

Possible confounding effects of psychotropic medication

In the AD group, 5-HT levels in BA10 ($p = 0.017$), DOPAC/DA ratios in BA22 ($p = 0.036$), and MHPG/NA ratios in the hippocampus ($p = 0.024$) were significantly higher in patients who were on psychotropic medication before death ($n = 3$) compared to patients free of such medication ($n = 7$).

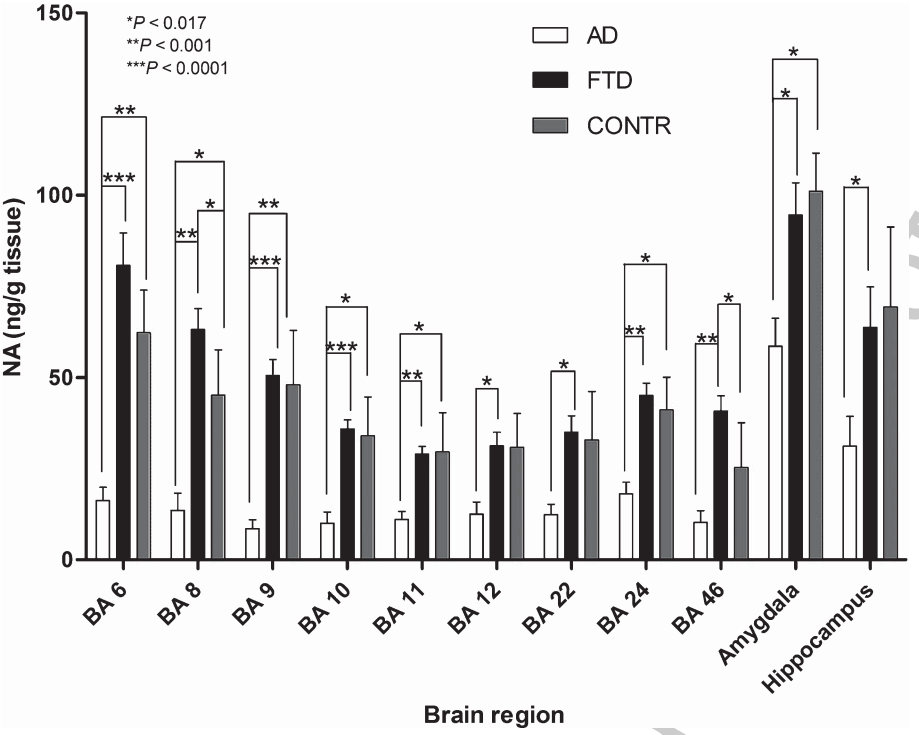

Fig. 4. NA levels across eleven brain regions in AD compared to FTD and control subjects. Data are presented as mean with SD. Only differences (Kruskal Wallis, $p < 0.05$) remaining statistically significant after *post hoc* Mann-Whitney U tests with Bonferroni correction are indicated by asterisks ($^{*}p < 0.017$; $^{**}p < 0.001$; $^{***}p < 0.0001$). AD, Alzheimer's disease; BA, Brodmann area; CONTR, controls; FTD, frontotemporal dementia; NA, noradrenaline.

Concerning the FTD group, MHPG/NA ratios in both BA6 ($p = 0.008$) and BA8 ($p = 0.032$) were significantly lower in pharmacologically-treated patients ($n = 5$) compared to those who were free of psychotropic medication ($n = 5$). The same applies to MHPG levels in BA8 ($p = 0.032$), amygdala ($p = 0.016$), and hippocampus ($p = 0.016$), and, finally, DA levels in BA9 ($p = 0.016$), DOPAC ($p = 0.032$), and 5-HT ($p = 0.032$) levels in the amygdala.

As for the control subjects, 5-HIAA levels in BA6, BA8, BA12, BA22, BA46 and amygdala, as well as NA levels in BA10 and BA22 were significantly lower in patients on psychotropic agents ($n = 2$) compared to their medication-free counterparts ($n = 8$; for all, $p = 0.044$).

Because four times as many FTD patients where on antidepressants ($n = 4$; i.e., trazodone, $n = 2$; amitriptyline $n = 1$; and serlain $n = 1$) compared to AD and control subjects ($n = 1$ for both groups), these drugs may certainly have influenced our serotonergic and/or noradrenergic results. However, only 5-HIAA/5-HT ratios in BA6 and BA9 and MHPG/NA ratios in BA6, BA8, and hippocampus were significantly lower in the subgroup of FTD subjects on antidepressants ($n = 4$) compared to their antidepressant-free counterparts ($n = 6$). There were no significant alterations regarding 5-HT or NA levels.

DISCUSSION

Monoaminergic findings

The key findings of the present study are that FTD patients have a remarkably distinct monoaminergic profile compared to AD patients, with predominantly imbalanced levels of brain serotonergic and noradrenergic compounds and an apparently unaltered dopaminergic neurotransmitter system. The latter is in sharp contrast with some of the aforementioned studies [2, 8, 15] and our initial expectations.

Increased 5-HT levels, corresponding with the findings of Bowen et al. [4], combined with decreased 5-HIAA/5-HT ratios in practically every analyzed brain region of FTD compared to AD and/or control subjects, could possibly be indicative of a serotonergic neurotransmitter imbalance in patients suffering

from FTD. In general, this presenile dementia subtype is characterized by a more extensive loss of pyramidal neurons in the supragranular layers of the frontotemporal cortex than AD [44, 45]. In addition, these layers are enriched with 5-HT_{1A} receptors that have been reported to index the soma of corticocortical glutamatergic pyramidal neurons in the neocortex [11, 46], and 5-HT concentrations have shown to inhibit glutamate release via these receptors [47]. Our observed serotonergic alterations could therefore reflect a relative excess of extraneuronal 5-HT in relation to the number of surviving glutamatergic pyramidal neurons. As a consequence, cognitive and behavioral symptoms in FTD might arise as a result of deficient glutamatergic neurotransmission. This excess of 5-HT may be due to preservation of 5-HT afferents, as was claimed by Bowen et al. [4], based on increased 5-HT reuptake site measures in FTD compared to AD. The mean reuptake value in temporal, parietal and frontal cortex for their FTD group was no less than 153% and 120% of AD and control values. Such preservation of serotonergic indices could be due to collateral sprouting, as has been demonstrated in animal models [48]. The authors further suggest that a 5-HT_{1A} receptor antagonist may be indicated to counteract the overstimulation of 5-HT on these receptors [4]. Interestingly, 5-HT_{1A} receptors or even 5-HT itself could represent neurochemical markers for glutamatergic neurotransmission in FTD accordingly [49]. Clinical trials with SSRIs (e.g., paroxetine, fluvoxamine, sertraline, citalopram) or other serotonergic antidepressants (e.g., trazodone) also led to conflicting results, with mixed treatment effects on both cognition and behavior, and, even a worsening of cognitive functioning in one trial (paroxetine; for Review, see [2] and [3]), which fits our aforementioned postulation.

As for the serotonergic metabolite 5-HIAA, levels were only significantly decreased in the temporal cortex (BA22) of FTD compared to AD subjects. Such preservation of 5-HIAA is largely consistent with previous comparative studies that not only examined frontal, temporal and parietal brain tissue [4], but CSF as well [6–8].

Apart from neocortical brain areas, the present study also analyzed amygdala and hippocampus, which yielded similar results, and therefore our findings point in the direction of a more diffuse serotonergic brain deficiency in FTD. In this context, a 40% reduction in the number of serotonergic raphe nuclei (RN) neurons has previously been observed [50], even though the accompanying postsynaptic serotonergic dysfunction via their ascending projections to the forebrain, including the amygdala, hippocampus and other subcortical nuclei, certainly necessitates further neurochemical investigation, given that a neuronal loss in RN is rather suggestive for an overall decrease (and not increase) in 5-HT levels. Unfortunately, glutamate levels, 5-HT reuptake values or RN atrophy measures are lacking in our study.

Similar to the serotonergic data, we found even more significantly increased NA levels and decreased MHPG/NA ratios in all 11 regions in FTD brain. Additionally, MHPG levels were strongly decreased in eight out of 11 brain regions. These noradrenergic abnormalities suggest that the LC, the principal site for brain synthesis of NA, may be severely damaged in FTD. Yang and Schmitt [50], however, found no neuronal loss in the LC of 12 FTD compared to 30 AD patients, whereas in latter AD group, both the RN and LC were severely affected. Nevertheless, the LC also receives serotonergic innervation from the upper RN [51] with predominantly inhibitory effects on the firing of these LC neurons, which is mediated by receptors of the 5-HT_1 family [52, 53]. Hence, even though the LC appears to be structurally intact, it cannot be excluded that serotonergic deafferentation might eventually lead to secondary, postsynaptic noradrenergic changes in the frontotemporal cortex, hippocampus, and amygdala among others. This remains purely hypothetical. Contrarily, previous studies that examined NA levels and its main metabolite, MHPG in CSF, did not find such noradrenergic alterations [7, 8, 14], except for Sjögren et al. [6], who found higher CSF MHPG levels in FTD compared to both early- and late-onset AD. Lastly, it is of interest to mention that Sparks et al. [54] observed a decreased monoamine oxidase A (MAO-A) enzyme activity in the temporal lobe of patients with Pick's disease, which might – at least partly – explain the increased NA levels and decreased MHPG/NA ratios in our FTD group. This particular enzyme plays a strategic role in inactivating catecholamines that are free within the nerve terminal endings, of which MAO-A preferentially deaminates NA and 5-HT [55]. On the other hand, the authors found increased MAO-A activity in the frontal lobe of AD subjects, which corresponds to the decreased NA levels and increased ratios of our AD group as well.

Similarly, based on our results, it can be concluded that the dopaminergic neurotransmitter system remained largely unaffected in FTD compared

to AD patients and healthy controls. In general, the handful of significant differences that were found, may partly be attributed to psychotropic drug therapy shortly before death (see Results). The latter is in stark contrast to previous findings of Engelborghs et al. [8], who reported an increased activity of dopaminergic neurotransmission due to a potentially altered inhibitory control of the serotonergic system, represented by increased HVA/5-HIAA ratios, in CSF of 25 FTD compared to 181 AD patients. No such differences in HVA/5-HIAA ratios were found in any of the analyzed brain regions in our study, suggesting that the serotonergic inhibitory control on dopaminergic neurotransmitter release was supposedly not affected in AD nor FTD. We have to bear in mind, however, that the hypothesis that monoamine levels in peripheral body fluids reflect central monoaminergic metabolism is based on assumptions (e.g., the assumption that a change in monoaminergic metabolism in a discrete brain area is measurable in large compartments such as CSF) [56]. Conversely, there are other CSF reports that agree with the theory of a relatively spared dopaminergic system in FTD [6, 7]. With regard to studies that analyzed brain tissue samples, one case study found unchanged DA levels in different regions of the neo- and allocortex [57], whereas another case study found dramatically reduced levels of DA and HVA in several neocortical brain areas, as well as thalamus, SN, and striatum [58], both in comparison with identical regions of seven control brains. Lastly, in a small study of only three FTD patients, CSF and brain tissue measurements also suggested that DA release remained relatively intact [11]. Overall, these contradicting results certainly necessitate further investigation since the impairment of the serotonergic system may have a profound impact on the dopaminergic system [50].

Finally, it is of note that serotonergic pathways – apart from strong interactions with dopaminergic, noradrenergic, and glutamatergic systems – are also known to intensively interact with the cholinergic system, and that the 5-HT_{1A} receptor can facilitate various types of memory by enhancing cholinergic as well as glutamatergic neurotransmission if antagonized [59], making it a valuable and strategic therapeutic target.

Neurochemical correlates of NPS

Strikingly, most of the correlations pertained to agitated and/or aggressive behavior in AD as well as FTD patients. In the AD group, considering the ten patients were part of a larger group of 40 in total on which similar research was conducted (See Study population and protocol, above), results therefore correspond well with those of our previous studies [24, 25].

As for FTD patients, an increased enzymatic turnover of DA to DOPAC and then HVA, as represented by HVA/DA ratios, in the middle frontal gyrus (BA46) might be related to agitated behavior (CMAI total score). To some degree, this finding is consistent with Engelborghs et al. [8] who mentioned that CSF DOPAC levels of FTD patients correlated with physically nonaggressive behavior (CMAI cluster 2 score) and agitation in general (CMAI cluster 3 score). Moreover, CSF DOPAC levels were able to predict future aggressive and agitated behavior in FTD patients.

In both study groups, NA levels of the prefrontal cortex (BA12 and BA9) correlated with aggressive behavior, albeit inversely in FTD patients. The correlation in AD partially corresponds with the results of Matthews et al. [60], who observed a similar correlation between aggressive behavior and severity of noradrenergic cell loss in the LC of AD patients before. The same monoamine was thus associated with more and less apparent aggressive behavior in comparable prefrontal regions in AD and FTD brain, respectively.

Results need to be interpreted with caution, however, since none of these correlations described above remained statistically significant following Bonferroni correction for multiple comparisons.

Study strengths and weaknesses

All AD and FTD patients were clinically and behaviorally well-characterized by combining clinical, neuropsychological, and neuroimaging data, as well as behavioral assessment scores obtained during baseline and follow-up investigations. These data gave rise to the clinical diagnosis of *probable* AD or FTD. In addition, postmortem neuropathological examination of the paraformaldehyde-fixated right hemisphere established the diagnosis of *definite* AD or FTD, and all groups were age- and gender-matched. Moreover, all brain dissections of the frozen left hemispheres, as well as the neuropathological assessments of the right hemispheres were always performed by the same neuropathologist(s)/scientist, thus minimizing variability. Postmortem degradation of the neurochemical compounds was minimized by

the inclusion of two quality control measures. Firstly, only nonacidotic brain tissue samples were included. Secondly, the average postmortem delay in all three groups remained more than sufficient within the 6–8 h range (4-5 h) (e.g., if compared to the study of Bowen et al. [4] (30–48 h)).

As opposed to aforementioned strengths, this study also comprised a number of limitations. As such, behavioral assessment scores of the control group were not available and those of the FTD group lacked sufficient variation (Supplementary Table 1) so that, eventually, very little significant neurochemical correlates of NPS were found in this group. Nonparametric tests were also applied due to the limited number of subjects in each study group ($n = 10$) and ordinal variables (behavioral scores), and, because our data did not comply with those of a normally distributed population (data not shown), statistical power might potentially have been lost. Unfortunately, MMSE scores were unsuitable or absent for data analysis owing to the severe disease progression in some patients (e.g., mutism) or because obtained MMSE scores were not recent enough. Furthermore, the existing information on monoamine deficits in particular with reference to distinct histological FTLD subgroups, such as FTLD with (Pick's disease) and without (FTLD-U) tau pathology [5], is sparse [4], and might have introduced some bias in our neurochemical dataset not only due to heterogeneity in spreading of TDP-43 positive inclusions across the different cortical layers depending on the FTLD-TDP-43 subtype [61], but also due to interindividual variation in topographic distribution of pathology. We also have to acknowledge that most of the included AD and FTD subjects had advanced disease stages, which may lead to dissimilar findings in patients with early disease stages, still the main target group for pharmaceutical intervention and biomarker discovery and verification. The variable degree of cortical atrophy between and within patient groups may also have introduced a neurochemical bias, given that there were three out of ten FTD subjects with asymmetric brain degeneration (visualized on MRI). The latter is in reference with Whitwell et al. [62], who found a minority of 35% of behavioral variant FTD patients with asymmetric frontal lobes (15% asymmetric right and 20% asymmetric left).

With regard to the use of antidepressants, our serotonergic and noradrenergic results may have been influenced by confounding medication effects to some degree, particularly in BA6, BA8, BA9 and hippocampus (See Results). On the other hand, possible confounding psychotropic drug effects in general may have introduced type I errors since several noradrenergic alterations were observed as well, although restricted to MHPG/NA ratios and/or MHPG levels of BA6, BA8, amygdala, and hippocampus (See Results). However, neurochemical effects of psychotropic drugs may last for up to several weeks after cessation of treatment. Therefore, neurochemical data of patients who did not take psychotropic medication at the date of death may also be influenced by these effects.

CONCLUSIONS

By and large, our findings support the premise that FTD and AD are distinguishable by their monoaminergic profiles not only in frontotemporal brain regions, but also in amygdala and hippocampus. More specifically, FTD seems to be predominantly characterized by imbalanced levels of brain serotonergic and noradrenergic compounds and by an apparently unaltered dopaminergic neurotransmitter system. We speculate that the observed serotonergic alterations might be caused by preservation of 5-HT afferents (and thus 5-HT levels), consequently leading to an underactivity of prefrontal glutamatergic neurotransmission, as was previously concluded by Bowen et al. [4]. On the other hand, we are the first, to our knowledge, to report on severe brain noradrenergic neurotransmitter deficiencies in FTD compared to AD, hypothetically resulting from an impaired connection between the RN and LC. Tackling both of these monoaminergic disturbances might, therefore, improve cognitive and/or behavioral deficits in patients suffering from this presenile disorder. Additionally, clinical trials that investigate the effects of 5-HT_{1A} receptor antagonists and/or NA-modulating agents, such as $\alpha_{1/2}$- or β_1-blockers [63, 64], may be considered. For instance, the efficacy of α_2-adrenoreceptor antagonists has been demonstrated before in three FTD subjects [65], and a novel generation of promising α_{2C}-antagonists, such as ORM-10921/ORM-12741, is currently being tested (phase 2 clinical trial NCT02471196), albeit in AD [64, 66].

Our study would of course have been more informative if some of the alternative indicators of neurotransmission, such as binding potential measures of 5-$HT_{1A/2A}$-, glutamate-, α- or β-noradrenergic receptors, and MAO and cholinergic enzyme activities

had been determined simultaneously. Moreover, given the discriminative features of both serotonergic (5-HT levels and 5-HIAA/5-HT ratios) and noradrenergic (MHPG, NA levels, and MHPG/NA ratios) compounds, future studies should examine their added potential in CSF in combination with the traditional set of AD biomarkers, as was previously attempted by Herbert et al. [14], and of which a good first indication came from CSF MHPG levels.

Finally, the strong and reciprocal connections between the RN and LC, as well as their postsynaptic efferents to the neo- and allocortex, certainly necessitate further investigation, accompanied with a complete topographic mapping of monoaminergic alterations in FTD and AD brain, including not only the RN and LC, but also the SN and striatum.

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SUPPLEMENTARY MATERIAL

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