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5-HT$_7$ receptors in Alzheimer's disease

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ABSTRACT

Even though the involvement of serotonin (5-hydroxytryptamine; 5-HT) and its receptors in Alzheimer's disease (AD) is widely accepted, data on the expression and the role of 5-HT$_7$ receptors in AD is relatively limited. Therefore, the objective of the present work was to study the expression of serotonergic 5-HT$_7$ receptors in postmortem samples of AD brains and correlate it with neurotransmitter levels, cognition and behavior. The study population consisted of clinically well-characterized and neuropathologically confirmed AD patients (n = 42) and age-matched control subjects (n = 18). Reverse-transcription quantitative polymerase chain reaction (RT-qPCR) and high-performance liquid chromatography were performed on Brodmann area (BA) 7, BA10, BA22, BA24, hippocampus, amygdala, thalamus and cerebellum to measure mRNA levels of 5-HT$_7$ receptors (*HTR7*), as well as the concentrations of various monoamine neurotransmitters and their metabolites.

Decreased levels of *HTR7* mRNA were observed in BA10. A significant association was observed between *HTR7* levels in BA10 and BEHAVE-AD cluster B (hallucinations) (rs(28) = 0.444, $P < 0.05$). In addition, a negative correlation was observed between *HTR7* levels in BA10 and both MHPG concentrations in this brain region (rs(45) = -0.311; $P < 0.05$), and DOPAC levels in the amygdala (rs(42) = -0.311; $P < 0.05$). Quite surprisingly, no association was found between *HTR7* levels and cognitive status. Altogether, this study supports the notion of the involvement of 5-HT$_7$ receptors in psychotic symptoms in AD, suggesting the interest of testing antagonist acting at this receptor to specifically treat psychotic symptoms in this illness.

1. Introduction

As the prototype of cortical dementias, Alzheimer's disease (AD) accounts for 60–80% of all dementia cases worldwide (Mayeux and Stern, 2012). Patients display progressive cognitive decline affecting various cognitive domains combined with behavioral dysfunction and functional dependence (McKhann et al., 2011). Microscopically, AD is characterized by the deposition of misfolded proteins, namely amyloid-β (Aβ) and hyperphosphorylated tau, into extracellular amyloid plaques and intracellular neurofibrillary tangles, respectively. These protein deposits are associated with local neuroinflammation, decreased synaptic density and finally widespread neurodegeneration, loss of synapses and failure of various neurotransmitter pathways (Van Dam et al., 2016).

Although acetylcholine and glutamate are perhaps the most widely reported neurotransmitters to be disturbed in AD, serotonergic neurotransmission also appears to be affected. Serotonergic raphe nuclei indeed project towards a large range of cortical and limbic structures, and as such, serotonin (5-hydroxytryptamine, 5-HT) plays an important role in various aspects of behavior and cognition, either directly or via modulation of cholinergic, glutamatergic, dopaminergic and GABA (γ-aminobutyric acid)-ergic neurotransmission (Buhot et al., 2000; Rodriguez et al., 2012).

Post-mortem studies have reported decreased 5-HT levels in AD brain (Vermeiren et al., 2014a, 2014b, 2016) correlating with cognitive performance (Lai et al., 2002; Vermeiren et al., 2014b). Further (pre) clinical evidence also highlights the relation between reduced serotonergic neurotransmission and cognitive deficits in ageing, AD and also other neurological disorders, including schizophrenia and depression (reviewed by Rodriguez et al., 2012).

In addition to the declining cognitive function, dementia patients are equally affected by a high prevalence (~90%) of behavioral and

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psychiatric disturbances (Gauthier et al., 2010), that are commonly referred to as behavioral and psychological signs and symptoms of dementia (BPSD) (Finkel et al., 1996) or neuropsychiatric symptoms (NPS) (Lyketsos et al., 2011). Without treatment, BPSD can have a tremendous impact on patients, their carers, and society. These symptoms may result in earlier institutionalisation (Colerick and George, 1986; Morris et al., 1990; Steele et al., 1990; O'Donnell et al., 1992; DeVugt et al., 2005), higher economic costs (Cohen-Mansfield, 1995; Herrmann et al., 2006), poorer quality of life of both patients and carers (Deimling and Bass, 1986; Burgio, 1966; Rabins et al., 1982), increased caregiver and nursing staff burden (Rodney 2000; Draper et al., 2000), and increased disability (Brody 1982; Hinton et al., 2008). Patients with BPSD have higher levels of disability; however, when these symptoms improve or resolve, their functional capacity also increases, improving their quality of life.

These BPSD/NPS can – at least partially – be explained by alterations in multiple neurotransmitter systems. Interestingly, 5-HT has been linked to both cognitive decline and multiple BPSD/NPS domains. Although overall serotonergic dysfunction occurs in AD, circuit-specific alterations in distinct 5-HT receptors are likely associated with the heterogeneous presentation of behavioral and psychiatric symptoms in AD (Chakraborty et al., 2019).

5-HT$_7$ receptors were one of the latest discovered receptors of the serotonergic receptor family (Bard et al., 1993). They are densely expressed in the CNS, particularly in the hypothalamus (in the suprachiasmatic nucleus), the thalamus, the hippocampus and the cerebral cortex (Sarkisyan and Hedlund, 2009; Meneses et al., 2015). Their presence in the dorsal raphe nucleus, has nourished the idea of a potential role in regulating 5-HT levels (Martín-Cora and Pazos, 2004). At the neuronal level, 5-HT$_7$ receptors are expressed in pyramidal neurons of the hippocampus, with a higher density in the CA3 area compared to CA1 (Bonaventure and Nepomuceno, 2004) and a differential expression, with selective localization in cell bodies of CA1 pyramidal neurons (Bickmeyer, 2002).

5-HT$_7$ signals postsynaptically via Gαs or Gα12 (Baker et al., 1998; Kvachnina et al., 2005; Costa et al., 2012). Mainly preclinical evidence has suggested that this G protein-coupled receptor (GPCR) is involved in a myriad of processes, ranging from thermoregulation, through cognition and various behaviors including stress, sleep and circadian rhythmicity, to depression, schizophrenia and pain modulation, while the results of anxiety studies are contradictory (reviewed in Nikiforuk, 2015; Roberts and Hedlund, 2012).

Data on the function of 5-HT$_7$ receptors is relatively limited, mainly due to the lack of specific selective agonists (Leopoldo et al., 2011; Nichols and Nichols, 2008). In addition, possibly owing to the differential transduction mechanisms of 5-HT$_7$, agonism and antagonism of this receptor can produce both deficits or improvement in cognition (Nikiforuk, 2015; Meneses, 2014). Matters are even further complicated by the reported heterodimerization with 5-HT$_{1A}$ (Renner et al., 2012), which may considerably alter signaling mechanisms under certain conditions (Fuxe et al., 2008).

To the best of our knowledge, 5-HT$_7$ has not been studied in the context of AD. The main objective of this work was to map the expression of serotonergic 5-HT$_7$ receptors in postmortem brain samples of AD patients in comparison to age-matched control individuals. 5-HT$_7$ receptor density was evaluated in frontal, temporal, parietal and limbic structures, as well as thalamus and cerebellum. Their correlation with monoaminergic neurotransmitter levels, cognition and behavioral alterations in AD individuals could improve insights into the (patho) physiological occurrence and role of these receptors.

2. Materials and methods

2.1. Study population and sample size

The study population for the investigation of mRNA levels of *HTR7* (gene name for 5-HT$_7$ receptor), consisted of neuropathologically confirmed AD (n = 42) and control (CONTR) subjects (n = 18), which were selected from the NeuroBiobank of the Institute Born-Bunge (University of Antwerp, Antwerp, Belgium). Clinical, neuropsychological and neuropathological characterization of study participants was performed as described previously (Vermeiren et al., 2016).

Human brain samples were dissected according to a standard procedure as previously described (Vermeiren et al., 2014a, 2014b, 2016). For this study, frontal (Brodmann area (BA) 10), temporal (BA22) and limbic regions (BA24, hippocampus, amygdala) relevant to cognition and behavior in AD, as well as BA7, thalamus and cerebellum were analyzed using quantitative polymerase chain reaction (qPCR). Receptor density data was correlated with brain-region specific monoamine levels as determined by high-performance liquid chromatopgraphy (HPLC) in previous studies within the same study population (Vermeiren et al., 2014a, 2014b). The monoamine of interest are (nor)adrenaline, dopamine, serotonin and their main metabolites (3-methoxy-4-hydroxyphenylglycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and, 5-hydroxyindoleacetic acid (5-HIAA).

2.2. RNA extraction cDNA synthesis and primer design

Ribonucleic acid extraction from 20 mg of human brain tissue was performed using the Nucleospin RNA kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. Afterwards, RNA concentration and purity were determined with UV-VIS spectrophotometry using the Nanodrop 1000 instrument (Thermo Fisher Scientific, Waltham, MA, USA), and samples were stored at −80 °C until further use. Reverse transcription was conducted on 300 ng RNA, using the SuperScript III First-Strand Synthesis System (Thermo Fisher Scientific, Waltham, MA, USA). All resulting cDNA samples were diluted 1:10 and stored at −80 °C until qPCR analyses.

The qPCR assay employed specific Taqman probes pre-designed by Applied Biosystems (Foster City, CA, USA): Hs-04194798_s1 (*HTR7*) for the target receptor, while 18s was used as housekeeping gene (Hs03003631_g1).

2.3. qPCR

Quantitative PCR analyses were performed on a CFX384 real-time system (Bio-Rad, Hercules, California, USA). Cycling conditions were applied as recommended by Applied Biosystems. Assays were carried out in 10 μL reactions, containing 5.5 μL Taqman Universal PCR master mix, 0.5 μL of TaqMan probe, in addition to 4.5 μL cDNA. CFX Manager version 3.1 (Bio-Rad, Hercules, California, USA) and Microsoft Excel were used to calculate fold change relative to samples belonging to the control group by applying the delta-delta Cq method. A replicate was excluded in case its Cq value was 0.5 units higher or lower compared to the other replicates, with minimum and maximum Cq values set at 15 and 35, respectively. A single reference gene, 18s, was used as normalization factor.

2.4. Statistical analysis

Since the (log-transformed) fold changes of *HTR7*, as well as storage time were characterized by non-normal distributions, nonparametric Mann-Whitney U tests were applied. In contrast, age at death was analyzed using the independent-samples T-test and chi-square statistics were adopted to assess the association of diagnostic group (AD or CONTR), gender and psychotropic medication use. Spearman's rank order correlation was applied to verify the association between fold changes of *HTR7* and storage time, behavioral data, and monoaminergic data in corresponding brain regions.

All statistical analyses were performed using SPSS 25.0 for Windows.

3. Results

3.1. Demographics

Demographic characteristics of the human study population are presented in Table 1. No statistical differences were found in age at onset, age at death, sex distribution or post-mortem delay. Cognitive performance (expressed in terms of Mini-Mental State Examination (MMSE) score) or behavioral evaluation (expressed in terms of Behave-AD score) were not available for controls. As shown in Table 1, marked cognitive deficiencies in the AD population were observed. i.c. an average MMSE score of 11.0 ± 6.5 and a median Global Deterioration Scale (GDetS) score of 6, both indicative of an advanced dementia stage. No associations were observed between storage time, postmortem delay and age of onset, and *HTR7* mRNA between AD and CONTR.

3.2. Expression of HTR7 in human brain

HTR7 mRNA was found to have an approximately five-fold (0.22 ± 0.21) lower expression in AD versus control subjects (U = 109.000; $P \leq 0.001$) in the BA10 region (Fig. 1). This effect was similar in both males and females.

On the other hand, there was an increased expression of this receptor in the thalamus, both in males and females (U = 108.000; $P \leq 0.05$) (Fig. 2).

Table 1
Demographics and clinical data.

Parameter	AD	CONTR	Test statistic
Age at onset (years)	75.4 ± 11.6 (n = 42)	N/A	N/A
Age at death (years)	80.2 ± 10.0 (n = 42)	74.7 ± 9.5 (n = 18)	t(58) = 1.995 $P > 0.05$
Male/female (n)	25/17	10/8	$X^2 = 0.082$ $P > 0.05$
Storage time (years)	2.9 (7.7) (n = 42)	9.3 (3.0) (n = 18)	U = 166.500 $P \leq 0.001$
Postmortem delay (hours)	3.4 (1.3) (n = 42)	5.4 (3.3) (n = 18)	U = 227.500 $P < 0.05$
Taking/not taking psychotropic medication (n)	23/18	7/11	$X^2 = 1.482$ $P > 0.05$
Anti-Alzheimer's	5/36	0/18	Fisher's Exact = 2.398 $P > 0.05$
Anti-psychotics	14/27	0/18	Fisher's Exact = 8.059 $P < 0.05$
Anti-Parkinson's	2/39	0/18	Fisher's Exact = 0.909 $P > 0.05$
Antidepressants	13/28	4/14	$X^2 = 0.549$ $P > 0.05$
Hypnotic, sedative and anxiolytic medication	5/36	4/14	Fisher's Exact = 0.973 $P > 0.05$
Anti-epileptics	0/41	1/17	Fisher's Exact = 2.317 $P > 0.05$
Dementia stage: GDetS (/7)	6 (1) (n = 41)	N/A	N/A
MMSE (/30)	11.0 ± 6.5 (n = 25)	N/A	N/A
BEHAVE-AD (/75)	7.0 (11) (n = 41)	N/A	N/A

Parameters characterized by a normal distribution are depicted by mean ± standard deviation, while non-normally distributed data are depicted by median with interquartile range between brackets. Postmortem delay indicates the number of hours between time of death and storage of the brain at −80 °C. Abbreviations: AD: Alzheimer's disease; BEHAVE-AD: Behavioral Pathology in Alzheimer's disease rating scale; CONTR: control subjects; GDetS: Global Deterioration Scale; MMSE: Mini-Mental State Examination; N/A: not applicable.

No statistically significant differences in the expression of this receptor were found in any of the other studied regions (Suplem Fig. 1).

3.3. Correlation of expression of HTR7 with cognitive and behavioral data

Of note, correlation analysis with behavioral data could only be performed in the AD group, since no such data were available for control subjects. No significant Spearman's rank order correlations could be detected for global dementia staging using the GDetS or cognitive performance based on the MMSE score.

A significant association was observed between *HTR7* mRNA levels in BA10 and BEHAVE-AD cluster B (hallucinations) (rs(28) = 0.444, $P < 0.05$). No other statistically significant correlations between *HTR7* levels and were noted.

3.4. Correlation of expression of HTR7 with monoaminergic concentrations

Spearman's rank order correlation analyses were performed on *HTR7* mRNA levels and monoamines/metabolites (Table 2) showing significant differences between AD and control subjects. Negative relationships were observed when *HTR7* levels in BA10 were correlated with both MHPG concentrations in this brain region (rs(45) = -0.311; $P < 0.05$), and DOPAC levels in the amygdala (rs(42) = -0.311; $P < 0.05$).

3.5. Influence of medication

Differences in the expression of HTR7 in the BA10 region continued to be significative in the medication-free subgroup (U = 31.000; $P \leq 0.01$). However, no differences in the thalamus were found in this medication-free group.

Higher levels of MHPG in BA10 (U = 36.000, $P < 0.05$), could confirm our findings in the total study population. Nevertheless, additional differences were found in subjects who did not take medication. Decreased HVA/5-HIAA ratios in BA10 were noted in AD (U = 34.000; $P < 0.05$), while 5-HIAA/5-HT (U = 45.000; $P < 0.05$), DOPAC/DA (U = 49.000; $P < 0.05$) and MHPG/NA ratios (U = 42.000; $P < 0.05$) were increased in this condition. The cingulate cortex showed increased MHPG concentrations in AD (U = 33.000; $P < 0.05$) and a trend towards increased MHPG/NA ratios (U = 45.000; $P = 0.052$), while in another part of the limbic system, the amygdala, decreased 5-HIAA (U = 29.000; $P < 0.05$), 5-HT (U = 35.000; $P < 0.05$) and HVA (U = 35.000; $P < 0.05$) levels were observed as well. Similarly, decreased 5-HT (U = 25.000; $P \leq 0.001$) and HVA (U = 52.000; $P < 0.05$) ratios were noted in the hippocampus of AD subjects as well. Elevated thalamic MHPG (U = 23.000; $P \leq 0.001$) levels and MHPG/NA ratios (U = 21.000; $P \leq 0.05$), as well as decreased 5-HIAA (U = 48.000; $P < 0.05$) and HVA concentrations (U = 43.000; $P < 0.05$) were noted in AD versus control. Serotonergic turnover was increased in the temporal cortex (U = 55.000; $P < 0.05$), while DOPAC/DA ratios (U = 35.000; $P < 0.05$) were decreased in AD patients. In addition, a trend towards lowered DA levels (U = 52.000; $P = 0.051$) was found in BA22 of AD versus control subjects as well. Finally, cerebellar HVA/5-HIAA ratios were decreased in AD subjects (U = 47.000; $P < 0.05$).

In this medication-free subgroup, a positive association was found between frontal cortex *HTR7* expression and hallucinations (rs(13) = 0.517; $P < 0.05$; Behave-AD cluster AB (rs(13) = 0.563; $P < 0.05$), affective disturbances (rs(13) = 0.598; $P < 0.05$), and Behave-AD Total scores (rs(13) = 0.616; $P < 0.05$), physically nonaggressive (rs(13) = 0.695; $P < 0.05$), verbally agitated behaviour (rs(13) = 0.520; $P < 0.05$), and CMAI Total scores (rs(13) = 0.636; $P < 0.05$). None of these associations remained significant after Benjamini-Hochberg corrections.

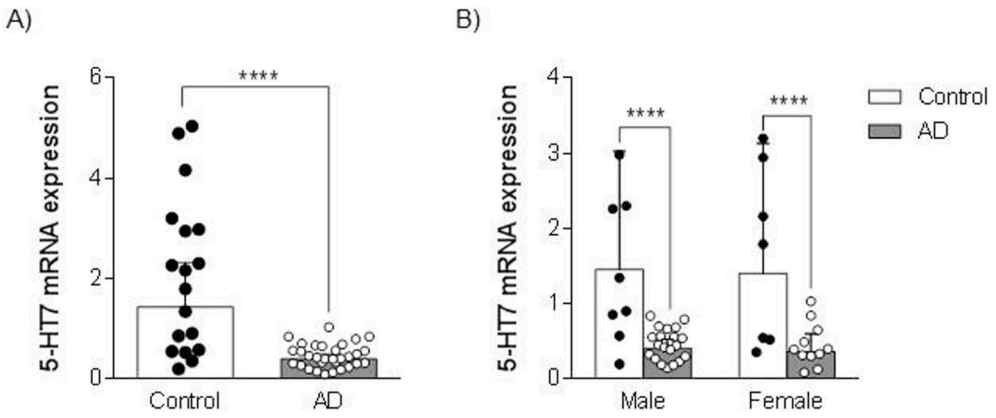

Fig. 1. 5-HT$_7$ mRNA expression in BA10. In A) Control vs Alzheimer's. In B) Control and Alzheimer's distributed by gender. The data are presented according to the ± min and max values (2-ΔΔct). In A, ****p < 0.0001, Student's t-test; In B, ****p < 0.0001 disease main effect, Two-way ANOVA. Ctrl: Control; AD: Alzheimer's disease.

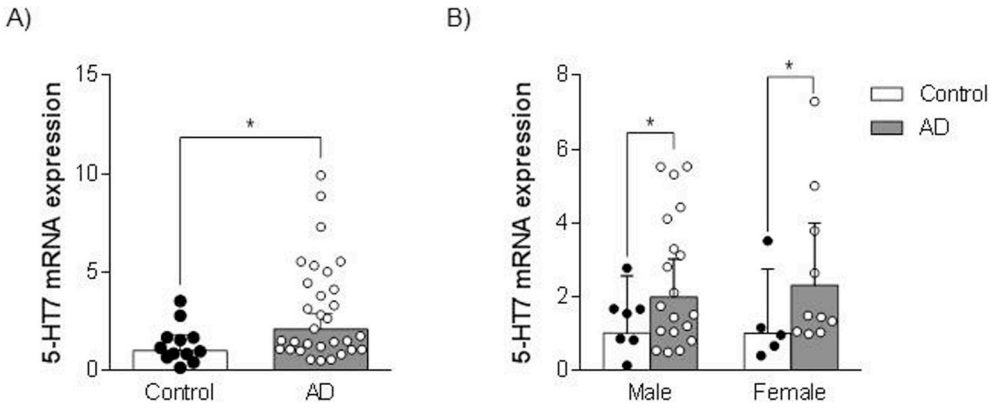

Fig. 2. 5-HT$_7$ mRNA expression in the thalamus. In A) Control vs Alzheimer's disease. In B) Control and Alzheimer's disease distributed by gender. The data are presented according to the ± min and max values (2-ΔΔct). In A, *p < 0.05, Student's t-test; In B, *p < 0.05 disease main effect, Two-way ANOVA. Ctrl: Control; AD: Alzheimer's disease.

Table 2
Monoamine neurotransmitters and metabolites in AD and CONTR.

Parameter	AD	CONTR	Test statistic
Hippocampus			
5-HT (ng/g)	55.93 (48.64) n = 40	83.10 (41.87) n = 17	U = 191.000 $P < 0.05$
Amygdala			
DOPAC (ng/g)	24.28 (24.25) n = 37	18.74 (14.38) n = 17	U = 203.000 $P < 0.05$
HVA (ng/g)	639.67 (298.69) n = 37	931.93 (682.41) n = 17	U = 188.000 $P < 0.05$
5-HT (ng/g)	133.51 (125.53) n = 37	230.81 (116.47) n = 17	U = 162.000 $P < 0.05$
DOPAC/DA	0.49 (0.44) n = 37	0.27 (0.21) n = 17	U = 173.500 $P < 0.05$
BA10			
MHPG (ng/g)	672.60 (608.49) n = 41	245.68 (312.63) n = 18	U = 149.000 $P < 0.001$
MHPG/NA	51.28 (97.01) n = 37	14.93 (23.57) n = 18	U = 200.000 $P < 0.05$

Only statistically significant differences after Mann-Whitney U analyses are presented. Data are depicted as median with interquartile range between brackets. Abbreviations: 5-HT: 5-hydroxytryptamine or serotonin; AD: Alzheimer's disease; BA: Brodmann area; CONTR: control subjects; DOPAC: 3,4-dihydroxyphenylacetic acid; HVA: homovanillic acid; MHPG: 3-methoxy-4-hydroxyphenylglycol; NA: noradrenaline.

4. Discussion

Given the extensive serotonergic denervation in AD brain, as well as the established role of 5-HT in both cognition and behavioural control, and the modulating role of 5-HT on other central neurotransmitter systems, we sought to explore the expression levels of the 5-HT7 receptor, the most recently identified member of the 5-HT receptor family. Brain region-specific mRNA levels were compared between AD and control individuals, and correlated with functional data and monoamine levels.

Our findings show a reduced expression in BA10, that positively correlated with the presence of hallucinations and the altered neurochemical profile in this brain region. On the other hand, unexpectedly, no link between HTR7 expression and cognitive deficits was found.

4.1. Strengths and limitations

The human study population was well characterized, both clinically and neuropathologically. In addition, it was beneficial to generate paired neurotransmitter- and receptor measurements in brain regions of the same subjects. Sample handling, did not appear to have confounded our results, nor did age of AD onset. Nevertheless, medication effects might have influenced our serotonergic results, so caution should be exerted while interpreting these findings. Another limitation of the human study population was the imbalance in AD and control group sizes and the fact that nonparametric statistics needed to be used, which

decreased the power of the analyses. It is to note that the large error bars observed in the figures are due exclusively to the use of geometric means in the analysis.

4.2. Altered expression of 5-HT7 receptors in AD

One of the most interesting areas studied for its involvement in AD is the frontal cortex (BA10), in particular the rostral prefrontal cortex. Serotonergic neurons in the dorsal and median raphe nuclei primarily project to various forebrain regions, but of all cortical regions, the frontal lobe contains the highest density of serotonergic terminals and receptors (Sarkisyan and Hedlund, 2009; Meneses et al., 2015).

Altered expression of the 5-HT_7 receptor has been described in other cognitive disorders, like schizophrenia (East et al., 2002). However, to our knowledge, this work is the first study the expression of the 5-HT_7 receptor in AD.

The functional consequences of preservation in some regions along with the loss of this receptor in BA10 in AD brains is not completely understood. Changes in serotonin function are postulated to contribute to non-cognitive functions including depression, anxiety, fear, irritability, and aggressiveness. These behavioral disturbances typically occur in more advanced stages of AD (Meltzer et al., 1998) and are likely the result of a complex interaction of genetic and environmental factors, as well as the medications to which patients are exposed. The relationship between the onset of these symptoms in AD patients and deficits in serotonin receptors in the different brain regions has not yet been determined.

In animal studies, the use of agonist and antagonist on this receptor has led to suggest that 5-HT_7 receptors in the prelimbic areas are involved in the regulation of anxiety-like behaviors, which is attributable to changes in dopamine, 5-HT and NA levels in the limbic and limbic-related brain regions (Du et al., 2018).

5-HT7 receptors have been linked to a number of psychiatric disorders including anxiety and depression. The localization of 5-HT7 receptors in the thalamus, a key sensory processing center, and the high affinity of many atypical antipsychotic compounds for these receptors have led to the speculation of the utility of 5-HT_7 antagonists in schizophrenia. Studies that have examined the effects of pharmacologic blockade and genetic ablation of 5-HT_7 receptors in animal models have been shown predictive of antipsychotic-like activity. Commonly used second generation antipsychotics such as clozapine, risperidone, olanzapine, sertindole, and ziprasidone bind to 5-HT_7 receptors with high affinity (Meltzer, 2012). In addition, it has been shown that the 5-HT_7 antagonist properties of amisulpride, an antipsychotic with antidepressant properties in schizophrenia, are required for its antidepressant action (Abbas et al., 2009). It is interesting to note that similar results have been found in the medication-free group.

4.3. 5-HT7 receptors and cognition

Although the role of 5-HT_7 receptors modulating cognitive processes under physiological conditions, let alone in memory disorders, is not fully understood, recent studies suggest the efficacy of 5-HT_7 receptor agonists to overcome cognitive damage in disease models (Nikiforuk, 2015). Among the agonists studied, LP-211 or AS-19 produce an improvement in long-term memory (Meneses and Adriani, 2014). Studies using 5-HT_7 receptor knock-out mice revealed a crucial role of this receptor in hippocampal-dependent memory (Sarkisyan and Hedlund, 2009). In fact, the absence of this receptor is associated with a deterioration in the contextual memory test of conditioned fear and a reduced ability to induce long-term potentiation in the hippocampus. Therefore, the 5-HT_7 receptor is considered to be involved in mnemonic processes and in particular, hippocampal functioning (Sarkisyan and Hedlund, 2009).

Therefore, the lack of relationship between the expression of 5-HT_7 receptors and cognition in this study was somehow, unexpected. Not only that, but no differences were found in expression of this receptor in the hippocampus. However, it is to note that, in the literature, some discrepancy is observed in relation to their involvement in cognition, and it seems that both agonist and antagonist could have a beneficial effect on cognition, and that the form of administration or the behavioral test used have also a great influence (Cifariello et al., 2008; Meneses, 1998; Vasefi et al., 2013). Moreover, were results obtained in 5-HT7 receptor knock-out mice also highly dependent on the applied behavioural paradigm with not all hippocampus-dependent setups showing deviant cognitive function (Gasbarri and Pompili, 2014)

To end up, for future studies will be interesting to include younger subjects, since it has been described a decrease in the expression of the 5-HT_7 receptor associated with age (Beaudet et al., 2015).

4.4. Future perspectives and conclusions

Since we only determined expression levels and concentrations of neurotransmitters possibly playing a role in AD, we still have limited information regarding the functionality of the interaction between receptors and their ligands. Radioligand binding and protein expression levels by immunoblotting experiments on postmortem brain material could therefore be initiated (Becker et al., 2014).

Altogether, this study has further supported the notion of the involvement of 5-HT_7 receptors in psychotic symptoms in AD. This is an interesting point, as the treatment of behavioral symptoms in AD is an unmet therapeutic target. This study also supports the notion of the interest to include a larger sample of medication-free subjects in future neurochemical analyses on human postmortem brain material, in order to delimitate which of the neurochemical effects are illness-dependent or medication-dependent.

Author statement

M. Solas, J. Janssens and U. Ocariz performed experiments, D. Van Dam, Y. Vermeiren and P.P. De Deyn collected the samples, M.J. Ramirez designed the experimental work. M. Solas, D. Van Dam, P.P. De Deyn and M.J. Ramirez wrote the manuscript. All authors have checked and approved the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuint.2021.105185.

Declaration of competing interest

All authors declare no conflicts of interest.

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