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CHAPTER 7

GABA AND GLUTAMATE CHANGES IN THE FRONTAL CORTEX: COMPARING ALZHEIMER PATIENTS WITH AND WITHOUT DEPRESSION AND THE APPSWEPS1DE9 MOUSE

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Manuscript under revision

ABSTRACT

Background: We hypothesized that frontal cortex amyloid plaques induce alterations in the GABAergic and glutamatergic pathways, which cause activation of the hypothalamic-pituitary-adrenal (HPA) axis, and may so contribute to the high prevalence of depression in Alzheimer's disease (AD).

Materials and Methods: We determined the expression of genes in these pathways by Q-PCR in the tissue from dorsolateral prefrontal cortex of AD patients with and without depression, we assessed their relation to hyperactivity of the HPA axis, and studied whether which of these changes are reflected in APPswePS1dE9 transgenic (Tg)AD mice.

Results: In the depressed AD patients, GABRA5 mRNA expression was significantly decreased. VGluT1 mRNA levels were positively correlated to the Cornell score for depression severity. In the TgAD mouse there was no GABA or glutamate related gene expression change that was comparable to those observed in depressed AD patients. The HPA axis was not activated in the TgAD mice either, and no difference in sucrose preference was found.

Conclusions: GABA and glutamate related gene expression was affected in depressed AD patients, while the TgAD animal model did not reflect these changes.

INTRODUCTION

Depression affects up to 50% of patients with Alzheimer's disease (AD) (Lyketsos and Lee 2004) which seriously increases patient and caregiver burden and is frequently the reason for hospitalization. Although the etiologies of AD, major depressive disorder (MDD) and depression in AD are different, they share similarities in brain structures and neurotransmitters that are involved. All three disorders are characterized by the hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis in a large part of the patients (Meynen et al., 2007; Bao, et al., 2008; Belmaker and Agam 2008). Previously, we found a positive correlation between the Cornell score for depression severity in dementia and the number of corticotropin-releasing hormone (CRH) expressing neurons, showing that MDD and depression in AD share, at least this characteristic (Meynen, et al., 2007). Furthermore, both MDD and AD are characterized by hypoactivity of the prefrontal cortex (PFC) (Swaab et al., 2000; Drevets et al., 2008). Moreover, the accumulation of plaques in the neocortex was found to be related to the severity of depression in AD (Meynen et al., 2009).

Animal studies have shown that the HPA axis activity is affected by both gamma aminobutyric acid (GABA) and glutamate afferents from the PFC (Gao and Bao, 2011; Herman et al., 2003). Both GABAergic and glutamatergic neurotransmission systems are affected in MDD, AD and depression in AD. Decreases in GABA level (Garcia-Alloza et al., 2006); (Lowe et al., 1988; Hasler et al., 2007) and GABA-A receptors (Lloyd et al., 1991; Merali et al., 2004; Luchetti et al., 2009) have, previously, also been described in the frontal cortex of AD patients and depression. Furthermore, a HPLC study showed a correlation between GABA level and depression factors (Garcia-Alloza et al., 2006). Although no changes in glutamate levels were found in the cerebral cortex of AD patients (Garcia-Alloza et al., 2006), reduced functional glutamate uptake in the frontal cortex was found in AD patients (Francis 2003). Moreover, lower glutamate or glutamine levels (Hasler et al., 2007), and several ionotropic receptors (Feyissa et al., 2009) have been demonstrated in the PFC of MDD patients.

In the present study we aimed to determine: i) whether GABA and glutamate related gene expression changes occur in the PFC of AD patients, ii) whether such changes have a relationship to the presence of depression in AD, and iii) whether depression in AD has, like MDD, a possible relationship to hyperactivity of the HPA axis. When available, PFC gene expression was, therefore, related to HPA

axis activation parameters of the same patients. In addition we determined iv) which GABA and glutamate gene expression changes in the AD patients are also present in the APPswePS1dE9 that is the widely used as a transgenic (Tg) mouse model for AD (Borchelt et al., 1997) and v) whether characteristics of depression such as anhedonia and CRH activation in the hypothalamus are also reflected in this TgAD model.

MATERIALS AND METHODS

Depressed AD patients

AD patients were studied at six-month intervals within the framework of a prospective longitudinal study of depression in AD. For a detailed description of the procedures see the previous studies that have reported about this cohort of patients (Hoogendijk et al., 1999; Meynen et al., 2007). Patients with major neuropathological co-morbidity were excluded. 13 patients fulfilled the criteria of the NINCDS-ARDA and of the CERAD for probable AD. The presence of depressive symptoms in AD was established by the Cornell Scale for depression in dementia and MDD was established by DSM-IIIIR criteria (Alexopoulos et al., 1988). On average, the last evaluation of the AD patients for depression was three months before death, except for one patient of whom only the Cornell score of 24 months before death was available. Patients in the depressed AD group suffered from a major depressive episode according to DSM-IIIIR at death and had been severely depressed for at least three months or suffered from a transient major depressive episode or dysthymia during the course of AD, the mean Cornell score being 12.7 ± 2.7 (mean \pm SEM). The non-depressed AD patients, which served as control subjects, had a Cornell score of 6.0 ± 1.6 . All these latter subjects remained free of mood disorders throughout the AD process.

The 9 depressed AD patients and 4 non-depressed AD patients were well matched by age ($P = 0.146$), sex ($P = 1.000$), time of death ($P = 0.687$) and CSF pH ($P = 0.833$) except for their PMD, which was significantly longer in the depressed AD group (266 ± 40) comparing to non-depressed AD (212 ± 33 , $P = 0.040$), but this difference did not influence our results (see below). The 4 non-depressed AD patients and 8 controls did not differ with respect to age ($P = 0.128$), sex ($P = 1.000$), time of death ($P = 0.127$), CSF pH ($P = 0.980$) and PMD ($P = 0.073$).

Human brain material

Frozen brain samples were collected by the Netherlands Brain Bank, following written informed consent from the patient or the next of kin for a brain autopsy and the use of the material and clinical information for research purposes. Tissue from the left dorsolateral PFC (DLPFC) was snap-frozen and 50 μm -thick cryostat sections were cut. Grey matter containing the complete all six layers of the neocortex was isolated (Bossers et al., 2010). Postmortem neuropathological evaluation took place in a systematic way for all patients (van de Nes et al., 1998) with an estimation of the distribution of the AD changes according to the classification of Braak (Braak and Braak 1991; Hoogendijk et al., 1999). Control subjects did not have any primary neurologic or psychiatric disease (for exceptions see Table 1).

Sucrose preference test in mice

All described experiments in mice were conducted under the approval of the Animal Care Committee of the Royal Netherlands Academy of Science. Adult transgenic APP^{swePS1dE9} AD mice (age: 12-14 months; body weight: 34.2 ± 0.4 g) were housed at room temperature with a 12 hr light/dark schedule (lights on at 7:00 a.m.). At 2 days before the behavioural experiments, mice were housed single. During the test, mice were given, for 48 h, a free choice between two bottles, one with 1% sucrose solution and another with tap water. To prevent possible effects of side preference in drinking behaviour, before experiment the bottles were present at the left side for 1 day and then changed to the right side for the second day, repeated for 2 more days. During the experiment the position of the bottles was switched every 24 h. No food or water deprivation was applied before the test. The consumption of water and sucrose solution was estimated simultaneously in control and experimental groups by weighing the bottles. The sucrose intake was calculated as an amount of consumed sucrose in mg per gram body weight. The preference for sucrose was calculated as a percentage of consumed sucrose solution of the total amount of liquid drunk (Strekalova et al., 2004).

Table 1 Clinico-pathological information of patients with AD and control subjects

Group	NBB number	Sex	Age (year)	PMD (h:min)	pH CSF	Brain weight (g)	ApoE	Clock time at death	Braak stage ¹	Cornell score ²	Type and courses depression duration (months)	Medication taken in the past	Medication taken in the last 3 month	Cause of death
AD	92-083	M	82	4:15	6.95	1317	44	13:00	5	26	MDD at onset and before AD, No, MDD (36)	BZD, phenytoin	Hal, promethazine	Pneumonia
AD	93-026	M	76	4:55	6.55	1050	44	1:30	6	25	MDD at onset AD, No, MD (36)	Mo	BZD, Mo, ZUC	Cardiac pulmonary insufficiency
AD	94-101	F	87	5:00	6.66	852	43	11:15	6	14	No, Dys (10), MDD (5)	BZD, Hal	None	Dehydration
AD	95-081	F	87	4:10	6.90	1047	42	5:00	6	12	No, (Admission for MDD long before AD)	None	Mo, BZD	Cachexia
AD	94-110	F	82	5:00	6.45	866	44	15:30	6	11	MDD at onset AD, no, Dyst (12), MDD (5)	None	BZD, pipamperone, Mo	Pneumonia, dehydration, cachexia
AD	94-012	M	64	3:40	6.26	950	43	13:25	6	10	No, Dyst (10)	Aki, levo, tria	Mo, levo	General physical deterioration
AD	94-091	F	83	4:45	6.74	1043	33	20:00	6	9	No, Dys (24)	None	BZD	Cardiac infarction
AD	96-068	F	82	3:10	6.70	1065	44	0:05	6	9	No mood disorder	BZD, hydrocortison	BZD, Mo	Physical deterioration
AD	93-087	M	81	4:10	7.08	1088	43	12:10	5	8	No mood disorder	None	BZD, Hal	Urinary tract infection, dehydration
AD	92-091	F	94	3:50	6.49	882	43	13:55	5	5	No mood disorder	Hal	Hal	Dehydration, cachexia
AD	98-142	F	78	5:00	6.73	843	43	2:00	6	5	No, Dyst (24)	BZD, Hal	Mo	Dehydration, cachexia, hypertension
AD	97-087	F	78	3:10	6.72	959	43	14:45	5	2	Mo, MDD (6), Dys (6), MDD (6), No (28)	None	Mo	Cachexia

Table 1 Continued, Clinico-pathological information of patients with AD and control subjects

Group	NBB number	Sex	Age (year)	PMD (h:min)	pH CSF	Brain weight (g)	ApoE	Clock time at death	Braak stage ¹	Cornell score ²	Type and courses depression duration (months)	Medication taken in the past	Medication taken in the last 3 month	Cause of death
AD	93-030	F	87	3:00	6.50	1037	43	15:15	6	2	No mood disorder	None	Mo	Pneumonia
Mean±SD	-	4M/9F	81.62 ±7.14	4:09 ± 0:44	6.67 ± 0.22	999 ±130	-	-	5.54 ± 0.66	10.62 ± 7.53	-	-	-	-
Contr	93-035	F	89	4:20	6.68	1152	33	6:15	2	-	-	BZD	BZD, Mo	Heart failure
Contr	06-049	F	84	4:45	6.26	1179	33	11:20	1	-	-	Mo	Mo	Heart failure, dehydration
Contr	07-075	F	82	5:10	6.64	1195	32	9:30	2	-	-	Mo	BZD, Mo	Pneumonia
Contr	97-143	M	79	6:00	6.51	1396	33	6:10	1	-	-	BZD, GnRH agonist	None	Adenocarcinoma of prostate, septic shock
Contr	97-156	F	77	2:40	6.37	1235	33	8:30	1	-	-	None	None	Septic shock, pancreas carcinoma
Contr	04-057	F	81	6:40	7.16	1164	33	13:10	1	-	-	None	Thiopental, pancuronium	Legal euthanasia
Contr	06-037	M	66	7:45	6.70	1590	33	17:45	0	-	-	None	None	Ruptured abdominal aneurysm aorta
Contr	97-068	F	61	10:15	7.18	1312	33	10:15	1	-	-	None	None	Cachexia
Mean±SD	-	2M/6F	77.38 ± 9.37	5:56 ± 2:19	6.69 ± 0.33	1277 ±151	-	-	1.13 ± 0.64	-	-	-	-	-
P-value	-	-	0.254	0.068	0.897	0.001	-	-	-	-	-	-	-	-

Abbreviations: AD, Alzheimer's Disease; Aki, Akimeton; BZD, benzodiazepine; Car, carbamazepine; CSF, cerebrospinal fluid; DL, dorsolateral; Dyst, dysthymia; F, female; Hal, haloperidol; L, left; Lev, levodopa; MDD, major disorder depression; Mo, morphine; M, male; NBB, Netherlands Brain Bank; ND, no data; None, no medication; PFC, prefrontal cortex; PMD, postmortem delay; tria, triamterene; ZUC, zuclopenthixol.

¹ Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol* 1991;82(4):239-259.

² Alexopoulos GS, Abrams RC, Young RC, Shamoian CA. Cornell Scale for Depression in Dementia. *Biol Psychiatry* 1988; 23: 271-84.

Mouse brain material

The animals were anaesthetized using CO₂/O₂ mixture, then decapitated. The left frontal cortex and whole hypothalamus were rapidly dissected and frozen in liquid nitrogen. All mice were sacrificed between 10:00 a.m. and 13:00 p.m.

Gene selection and Primer design

For information on the 65 genes studied see Table 2. The mRNA sequences for the genes were downloaded from the NCBI at www.ncbi.nlm.nih.gov. Sequences and size of the primer pairs are shown in Table 2. Procedures for RNA isolation, cDNA synthesis and real-time quantitative PCR (Q-PCR) have been described before (Wang et al., 2008).

Normalization strategy

To remove sampling-related differences (RNA quality and quantity), a normalization strategy based upon the geNorm approach was used (Vandesompele et al., 2002). The relative absolute amount of target genes were calculated by $10^{10} \times E^{-CT}$ ($E = 10^{-(1/\text{slope})}$) (Kamphuis et al., 2001). The geNorm analysis revealed that the transcript level of all reference genes that were determined both in patients and mice could be included in the calculation of the normalization factor. For information of reference genes see Table 2.

Correlation with hypothalamic paraventricular nucleus (PVN) and Cornell score

For 11 AD patients for whom both a Cornell score and the number of CRH expressing neuron the PVN were available (Meynen et al., 2007), a correlation with our data was performed.

Statistic analysis

For the human postmortem data, analysis of variance (ANOVA) was performed for each molecule to identify statistically significant changes. To identify difference between the depressed AD, non-depressed AD, and control, statistically significant genes were subjected to a *post hoc* Least Significant Difference (LSD) test for the contrasts depressed AD versus non-depressed AD, depressed AD versus control, and non-depressed AD versus control. The correlations between the expression levels of different brain areas were tested by Pearson's correlation. For the mouse experiment, the difference between gene expression levels was determined by

Table 2 Information of Gene Selection, sequence of the primers, gene bank accession numbers, the size of amplified product for the target genes and reference genes

Gene	Official full name	Primer sequences (Forward)	Primer sequences (Reverse)	Accession numbers	Amplicon length (bp)
GABA pathway related molecules (Homo)					
GABRA1	gamma-aminobutyric acid (GABA) A receptor, alpha 1	ATGCCCAACAAACTCCTCGC	ATAGGGAAGCTCCTCCAAATGC	NM_000806 NM_001127643	103
GABRA2	gamma-aminobutyric acid (GABA) A receptor, alpha 2	CTTGGGATGGGAAGAGTGTAGT	GTTCTGTATCATACGGAAAGCCT	NM_000807 NM_001114175	64
GABRA3	gamma-aminobutyric acid (GABA) A receptor, alpha 3	GCCCCACTGAGACCAAGACC	ATGGCAAAGAGCACAGGAAAAGA	NM_000808	81
GABRA4	gamma-aminobutyric acid (GABA) A receptor, alpha 4	TGGGCAACCCGTATCAAGTGTG	GAGGTGGAAGTAAACCCGTCATAA	NM_000809	74
GABRA5	gamma-aminobutyric acid (GABA) A receptor, alpha 5	CAGTCTTGTTCGGCACTTTCA	GAGAGGGGGCTCCTTTTAT	NM_000810	80
GABRB1	gamma-aminobutyric acid (GABA) A receptor, beta 1	AGCCAGAGTCGCACACTAGGAAT	CCAGCAGAGCCAGGAACAC	NM_000812	148
GABRB2	gamma-aminobutyric acid (GABA) A receptor, beta 2	CGGTGGATAGACTCCCTGAA	TGGCAATGTCAATGTTTCATC	NM_000813	91
GABRB3	gamma-aminobutyric acid (GABA) A receptor, beta 3	TGACAACCATCAACACCCACC	CAAAAGACGAAAGCAGCCCATAA	NM_021912 NM_000814	93
GABRD	gamma-aminobutyric acid (GABA) A receptor, delta	ATCGTGAACGCCAAGTCG	TGGAGGTGATTCGGATGCT	NM_000815	104
GABRE	gamma-aminobutyric acid (GABA) A receptor, epsilon	CATCCTCGTATCAATAGCCGTG	GCTCCTCTCCATCACTTCCCT	NM_004961	123
GABRG2	gamma-aminobutyric acid (GABA) A receptor, gamma 2	CGTCTATCCTGGGTGCTTTTC	CAATGGTCTGAGGGTGGTTC	NM_000816 NM_198903	102
GABRQ	gamma-aminobutyric acid (GABA) receptor, theta	CCTGGGAAGGACGATTACT	CCCTCTGAACCTGGAACCT	NM_018558	85
GABBR1	gamma-aminobutyric acid (GABA) B receptor, 1	ATTACCGACCAAATCTACCG	CGCTGGCAATCAAAACACC	NM_021904 NM_021903	78
GABBR2	gamma-aminobutyric acid (GABA) B receptor, 2	TCAGGGTCAAGTGTGATTC	CCTTACCTCCCTGCTGTCT	NM_005458	83
GAD1	glutamate decarboxylase 1 (brain, 67kDa)	CGGCTAAGAACGGTGAGGA	CTTGCGGACATAGTTGAGGAGT	NM_000817 NM_013445	76
GAD2	glutamate decarboxylase 2 (pancreatic islets and brain, 65kDa)	CCACACAAGATGATGGGAGTC	TGCTGAAAGAGGTAGGAGGC	NM_000818 NM_001134366	106

Table 2 Continued, Information of Gene Selection, sequence of the primers, gene bank accession numbers, the size of amplified product for the target genes and reference genes

Gene	Official full name	Primer sequences (Forward)	Primer sequences (Reverse)	Accession numbers	Amplicon length (bp)
Glutamate pathway related molecules (Homo)					
GLS	glutaminase	GCTTTCATGTTGGTCTTCC	ACACTGTTGCCCATCTTATCC	NM_014905	118
VGLUT1 (SLC17A7)	solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 7	TCTTCTACGGGGTCTTTGCTT	CACACTTCTCCTCGCTCATCT	NM_020309	74
VGLUT2 (SLC17A6)	solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6	TAAAAAAGCTAACATCAGACCCC	ATCCAGAAATAGACAAAAATACAC-CAA	NM_020346	102
GluR1 (GRIA1)	glutamate receptor, ionotropic, AMPA 1	ATCCACAGCAATCCATCAA	ACCCGACCAITTCCTCCAC	NM_000827 NM_001114183	100
GluR2 (GRIA2)	glutamate receptor, ionotropic, AMPA 2	ATTGTTGGAGGTGTGGTGG	TAAAGTTAGCCGTGTAGGAGGA	NM_000826 NM_001083619 NM_001083620	62
GluR3 (GRIA3)	glutamate receptor, ionotropic, AMPA 3	TACTTGTGGGAGGTCTGGG	GTGAGITTCATGCGTTTGG	NM_000828 NM_007325	90
GluR4 (GRIA4)	glutamate receptor, ionotropic, AMPA 4	TCACTACGAATGGAACGTGTTT	AGTATCGAGTATCCCCTGTCTG	NM_001077244 NM_000829	59
GluR5 (GRIK1)	glutamate receptor, ionotropic, kainate 1	CCCGAGGAAGACAACAAAGAA	GGCAGCCAGAACAAATGAAGAT	NM_001112812 NM_001077243	74
NR1 (GRIN1)	glutamate receptor, ionotropic, N-methyl D-aspartate 1	ATCCAGATGGCTCTGTGCGGT	GGTGGGAGTGAAGTGGTCCGT	NM_007327 NM_021569	101
NR2A (GRIN2A)	glutamate receptor, ionotropic, N-methyl D-aspartate 2A	TCAAGAAGTAATGGCACCG	CATCATCACCCAGACAGAGG	NM_000832 NM_000833	71
NR2B (GRIN2B)	glutamate receptor, ionotropic, N-methyl D-aspartate 2B	CTGAGAAATAAAACAGACGAGG	AAGGATGTCAATACAGAACCC	NM_001134407 NM_001134408	70
mGluR1 (GRM1)	glutamate receptor, metabotropic 1	CCTACGCCCTCTGTCATTCTGC	CTCTGGCTTGCTTGCCTTTTCT	NM_000838 NM_001114329	91

Table 2 Continued, Information of Gene Selection, sequence of the primers, gene bank accession numbers, the size of amplified product for the target genes and reference genes

Gene	Official full name	Primer sequences (Forward)	Primer sequences (Reverse)	Accession numbers	Amplicon length (bp)
mGluR2 (GRM2)	glutamate receptor; metabotropic 2	GCAAGTATGTTGGGCTCGC	CTCGTTGAAGTTTTTCGGGG	NM_000839 NM_001130063	98
mGluR3 (GRM3)	glutamate receptor; metabotropic 3	ATTGCCCTGCTGGGTTTTTA	AAGGGTGTGTTGTTGTGCT	NM_000840	70
PSD-93 (DLG2)	discs, large homolog 2	TAATCCCTGTTGAGTGTGA	ACGAGTTGCGGTGCTAATGT	NM_001364 NM_001142699	119
PSD-95 (DLG4)	discs, large homolog 4	GGGTAAC TCAAGGCTCTGGGC	CTTGGTGATGAAAATGGATGG	NM_001365 NM_001128827	84
Reference genes (Homo)					
RN18S	18S ribosomal RNA	TTCGTATTGGCGCGCTAGA	TGGCAAATGCTTTCGGCTCT	NR_003286	70
ACTβ	actin, beta	CCCAGCGATGTACGTTGCTA	TCACCGGAGTCCCATCACGAT	NM_001101	65
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	CAATTCCATGGCACCCGTC	TCTCGCTCCTGGAAGATGTT	NM_002046	62
HMBS	hydroxymethylbilane synthase	GATCCCGAGACTCTGTCTCG	ACACTGCAGCCTCCTTCCAG	NM_001024382	70
TUBα	tubulin, alpha 1b	CTTTGAGCCAGCCAACCAGA	GTACAACAGGCAGCAAGCCAT	NM_006082	72
TUBβ4	tubulin, beta 4	GGGCCAAGTTTTTGGGAGGT	CACTGTCCCCATGGTATGTGC	NM_006087	71
Stress related molecules (Mus)					
CRH	Corticotropin releasing hormone	GCCCATGCTTAATTTCTATGIG	CTGGATGACTCCCATCTGCT	NM_205769.2	107
CRHR1	Corticotropin releasing hormone receptor 1	CTTGGGATGCTACCGAGGAC	TTGTAGGGAGGTGGAGTGCC	NM_007762.4	100
CRHR2	Corticotropin releasing hormone receptor 2	TCCATCCTTTACAGGCCTCC	GTGTTCTGTTCTCCTCCCTTC	NM_009953.3	87
OXT	Oxytocin	CTCGGCCTGTACATCCAGA	AGGTCCAGCACAGCCCTCTT	NM_011025.3	57
GR	Nuclear receptor subfamily 3, group C, member 1	GTGGTGGATAGCAACA AAG	TACTCCGGAAGGTCTTGAAA	NM_008173.3	55
MR	Nuclear receptor subfamily 3, group C, member 2	TCTAGGAGAAGTGATGGGTA	CTTGGAAAGGTC TTGAGGAT	NM_001083906.1	102
AR	Androgen receptor	TACAAC TTTCCGGCTGGCTCT	CGTAGTCCAATGGGTTCTCC	NM_013476.3	94
ESR1	Estrogen receptor 1 (alpha)	GGAACAGGCCAAAAGGGATT	AGCAAAGGGAGAAAAGAGAGC	NM_007956.4	61

Table 2 Continued, Information of Gene Selection, sequence of the primers, gene bank accession numbers, the size of amplified product for the target genes and reference genes

Gene	Official full name	Primer sequences (Forward)	Primer sequences (Reverse)	Accession numbers	Amplicon length (bp)
ESR2	Estrogen receptor 2 (beta)	TACGGTGCTGGTCCCTGTG	GCCGGTTCTTGTCTATGGT	NM_207707.1 NM_010157.3	85
AVP	Arginine vasopressin	AAGCAACGCCACACAGC	GCAGAATCCACGGACTCC	NM_009732.1	90
AVP1a	Arginine vasopressin receptor 1A	GGTAGAGAGGAGTTGGGTTG	TTCAGTGTAAATAGAGTGTCAAG	NM_016847.2	91
GABA pathway related molecules (Mus)					
GABRA1	gamma-aminobutyric acid (GABA) A receptor, alpha 1	AGTGGCACCATAGAACCAGAAA	TCCAAATAGCAGCGGAAAAGG	NM_010250.4	123
GABRA2	gamma-aminobutyric acid (GABA) A receptor, alpha 2	GCTTGGGACGGGAAAGAGTGT	GCTACCCGCATAGGCCGTTGTT	NM_008066.3	83
GABRA3	gamma-aminobutyric acid (GABA) A receptor, alpha 3	GCCCTGGAGATGAAGAAGAAA	ATCGCTGTTGGAGTTGAAAGAA	NM_008067.4	164
GABRA4	gamma-aminobutyric acid (GABA) A receptor, alpha 4	ATCAATACAGATGCCGACCAG	TCCAGGCTCTGAGGAACTATG	NM_010251.2	187
GABRA5	gamma-aminobutyric acid (GABA) A receptor, alpha 5	CCATCTTGTTTGGCACTTTCA	GCAGTTTCATTTCTGTGGGAG	NM_176942.4	124
GABRB1	gamma-aminobutyric acid (GABA) A receptor, beta 1	CTCACCTCAGGGAGACTTTG	TCCAGTAGAGCCAGGAACAC	NM_008069.4	97
GABRB2	gamma-aminobutyric acid (GABA) A receptor, beta 2	GGCTGTATGAGTGAGTGTG	GAAAGGCAGGATAATGACGA	NM_008070.3	100
GABRG2	gamma-aminobutyric acid (GABA) A receptor, gamma 2	GTGTTTGGATGGCAAGGACTG	AAAGCGGTAGGGAAGAAGAT	NM_008073.2 NM_177408.5	139
GABBR1	gamma-aminobutyric acid (GABA) B receptor, 1	GCACCGAACCAATTGAGACTTT	CAGCAGCCCTTTGTAAACCATA	NM_019439.3	139
GABBR2	gamma-aminobutyric acid (GABA) B receptor, 2	CTGGGCAGAATCATCTCCTCAA	CAGCCACAGCGTTGTACTCG	NM_001081141.1	157
GAD1	glutamate decarboxylase 1 (brain, 67kDa)	AAGCACCCGCCACAACACTC	CAGCAGCCCATCATCT	NM_008077.4	78
GAD2	glutamate decarboxylase 2 (pancreatic islets and brain, 65kDa)	GTGCTGGTAGAACTATGGAC	GTCTGGGCTAAGTTGTAAAG	NM_008078.2	164
Glutamate pathway related molecules (Mus)					

Table 2 Continued, Information of Gene Selection, sequence of the primers, gene bank accession numbers, the size of amplified product for the target genes and reference genes

Gene	Official full name	Primer sequences (Forward)	Primer sequences (Reverse)	Accession numbers	Amplicon length (bp)
GluR1 (GRIA1)	glutamate receptor; ionotropic, AMPA 1	TACAAATCCCGTAGCGAGTC	TCCACITGGCAGCTTCCTCC	NM_008165.2	135
GluR2 (GRIA2)	glutamate receptor; ionotropic, AMPA 2	AAAAGAACGGCGTGTAATCC	CAGCAGGCTCCCATCAGTAAAT	NM_013540.2	139
NR1 (GRIN1)	glutamate receptor; ionotropic, N-methyl D-aspartate 1	AAGGAGTGGAAACGGAATGAT	AAGGGCTTGGAGAACTCTATGT	NM_008169.1	113
NR2A (GRIN2A)	glutamate receptor; ionotropic, N-methyl D-aspartate 2A	GTCTGGGTGATGATGTTGCT	CCTTTGGCTAAGTTTCTGTGTG	NM_008170.2	104
NR2B (GRIN2B)	glutamate receptor; ionotropic, N-methyl D-aspartate 2B	GGCATCAGTTCATGGTAT	CTCTAAGAAAGGCAGAAAGGTG	NM_008171.3	60
PSD-93 (DLG2)	discs, large homolog 2	ACCAACCTACCCCTTTACCC	AACTAAGCAATCATCCTCCC	NM_011807.2	155
PSD-95 (DLG4)	discs, large homolog 4	ATCCTGTGCGGTCAAATGGTGT	GAATCGGGCTATAC1CTTCTGGT	NM_001109752.1	126
GLS	glutaminase	GCACAGACATGGTTGGGATA	TTCACCAATAATTTGGGCAGA	NM_001081081.1	125
VGLUT1 (SLC17A7)	solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 7	TGTTCTCATAGCCCTCCCTG	CGTCCTCGATTTCACTTTCGT	NM_182993.2	168
VGLUT2 (SLC17A6)	solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6	AATGGCAGTATGTCTTCCTC	TCTTCGCTTGT1TTCCCTCA	NM_080853.2	118
Reference genes (Mus)					
18S	18S ribosomal RNA	GGACCAGAGCGAAAGCATTT	TCGTCTTCGAACTCCGACTT	NR_003278.1	71
ACT β	actin, beta	GCTCCTCTGAGCGCAAG	CATCTGCTGGAAGTGGACA	NM_007393.3	75
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGA	NM_008084.2	87
HPRT1	hypoxanthine phosphoribosyltransferase 1	ATGGGAGGCCATCACAT1TGT	ATGTAATCCAGCAGGTCAGCAA	NM_013556.2	77

Student's *t*-test. All statistical analyses were performed using SPSS (Version 16.0, SPSS Incorporation) and statistical significance was set at $P \leq 0.05$. All tests were two tailed.

RESULTS

Postmortem human study

There was no difference among depressed AD, non-depressed AD patients, and control subjects for any of the putative confounders other than PMD (Table 1). We observed that the PMD was shorter in the non-depressed AD patients when compared to controls. However, gene expression of none of the single target genes was correlated to the PMD in the non-depressed AD and control subjects (Data not shown).

GABA related gene expression in AD patients

Transcript levels of several GABA-A receptor subunits GABRA1 ($P = 0.002$, Figure 1E), GABRA4 ($P = 0.002$, Figure 1F) and GABRA5 ($P = 0.009$, Figure 1G) were significantly decreased in AD patients compared to controls (Table 3). Several other GABA-A receptor subunits, GABRB2 ($P = 0.011$, Figure 1H), GABRG2 ($P = 0.012$, Figure 1I), GABRE ($P = 0.030$, Figure 1J) and one of the GABA-B receptor subunits GABBR2 ($P = 0.018$, Figure 1K), showed a trend towards a reduction in the AD group ($P \leq 0.05$ was considered as a trend). Transcript levels for glutamic acid decarboxylase 1 (GAD1; $P = 0.008$, Figure 1L) were significant lower while GAD2 ($P = 0.014$, Figure 1M) showed a trend towards a reduction in AD.

Transcription levels of GABA-A receptor subunit: GABRA1 ($P = 0.001$), GABRA4 ($P = 0.008$) and GABRA5 ($P = 0.001$ Figure, 1O), GABRB2 ($P = 0.009$), GABRG2 ($P = 0.002$) were significantly lower in depressed AD patients than control subjects, while GABAE ($P = 0.016$) mRNA level was higher. The expression of GABA_B receptor subunit GABBR2 ($P = 0.001$) and glutamic acid decarboxylase 1 (GAD1, $P = 0.012$) were also decreased in the depressed AD group compared to controls. GABA-A receptor subunit GABRA3 ($P = 0.008$) and GABRA5 ($P = 0.011$), and GABA-B receptor subunit GABBR2 ($P = 0.005$) transcription level was also lower in the depressed AD patients than non-depressed AD patients. Non-depressed AD patients had lower level for GABRA4 ($P = 0.009$) expression compared to controls (Figure 1T).

Table 3 P-value on gene expression changes in postmortem study and animal study

Gene	Postmortem study				Animal study				Postmortem study				Animal study	
	DLPFC		Hypo-thalamus		Frontal cortex		Hypo-thalamus		DLPFC		Frontal cortex		Hypothalamus	
	AD vs. Contr	Depressed AD vs. depressed AD	Non-depressed AD vs. Contr	TgAD mice vs. WT	TgAD mice vs. WT	Non-depressed AD vs. Contr	TgAD mice vs. WT	Gene	AD vs. Contr	Depressed AD vs. depressed AD	Non-depressed AD vs. Contr	TgAD mice vs. WT	Frontal cortex	Hypothalamus
<i>GABA pathway related molecules</i>														
GABRA1	0,002**↓	0,147	0,068	0,148	0,352	GluR1	0,233	0,504	0,723	0,661	0,055			
GABRA2	0,248	0,299	0,651	0,917	0,881	GluR2	0,057	0,326	0,347	0,095	0,430			
GABRA3	0,987	0,033*↓	0,260	0,819	0,526	GluR3	0,056	0,251	0,565	-	-			
GABRA4	0,002**↓	0,604	0,017*↓	0,012*↓	0,359	GluR4	0,108	0,980	0,342	-	-			
GABRA5	0,009**↓	0,001**↓	0,659	0,470	0,409	GluR5	0,006**↓	0,603	0,023*↓	-	-			
GABRB1	0,977	0,106	0,396	0,773	0,179	NR1	0,300	0,286	0,153	0,073	0,970			
GABRB2	0,011*↓	0,195	0,272	0,708	0,950	NR2A	0,042*↓	0,304	0,389	0,211	0,847			
GABRB3	0,146	0,040*↓	0,988	-	-	NR2B	0,045*↓	0,123	0,676	0,349	0,016*↓			
GABRG2	0,012*↓	0,071	0,423	0,754	0,145	mGluR1	0,039*↓	0,522	0,084	-	-			
GABRD	0,923	0,703	0,874	-	-	mGluR2	0,616	0,039*↓	0,292	-	-			
GABRE	0,03*↑	0,328	0,183	-	-	mGluR3	0,844	0,050*↓	0,210	-	-			
GABRQ	0,406	0,774	0,400	-	-	VGluT1	0,131	0,288	0,991	0,668	0,300			
GABRR1	0,187	0,079	0,977	0,601	0,050*↑	VGluT2	0,145	0,316	0,734	0,571	0,039*↑			
GABRR2	0,018*↓	0,014*↓	0,887	0,189	0,160	PSD-93	0,909	0,071	0,300	0,768	0,960			
GAD1	0,008**↓	0,744	0,083	0,421	0,715	PSD-95	0,195	0,079	0,711	0,756	0,852			
GAD2	0,014*↓	0,991	0,059	0,620	0,131	GLS	0,089	0,179	0,839	0,357	0,581			

Abbreviations: AD, Alzheimer's disease; Contr, control; DLPFC, dorsolateral prefrontal cortex; WT, wild type; For abbreviations of gene see Table 2.

The differences among gene expression levels in the human postmortem study were determined by Student's *t*-test. Because of multiple comparisons and the relatively small group, a more strict level of $P \leq 0.01$ was considered to be statistically significant. For the mouse experiment, the difference between gene expression levels were determined by Student's *t*-test and statistical significance was set at $P \leq 0.05$.

All tests were two tailed.

** $P \leq 0.01$, * $0.01 < P \leq 0.05$.

↓ decrease, ↑ increase

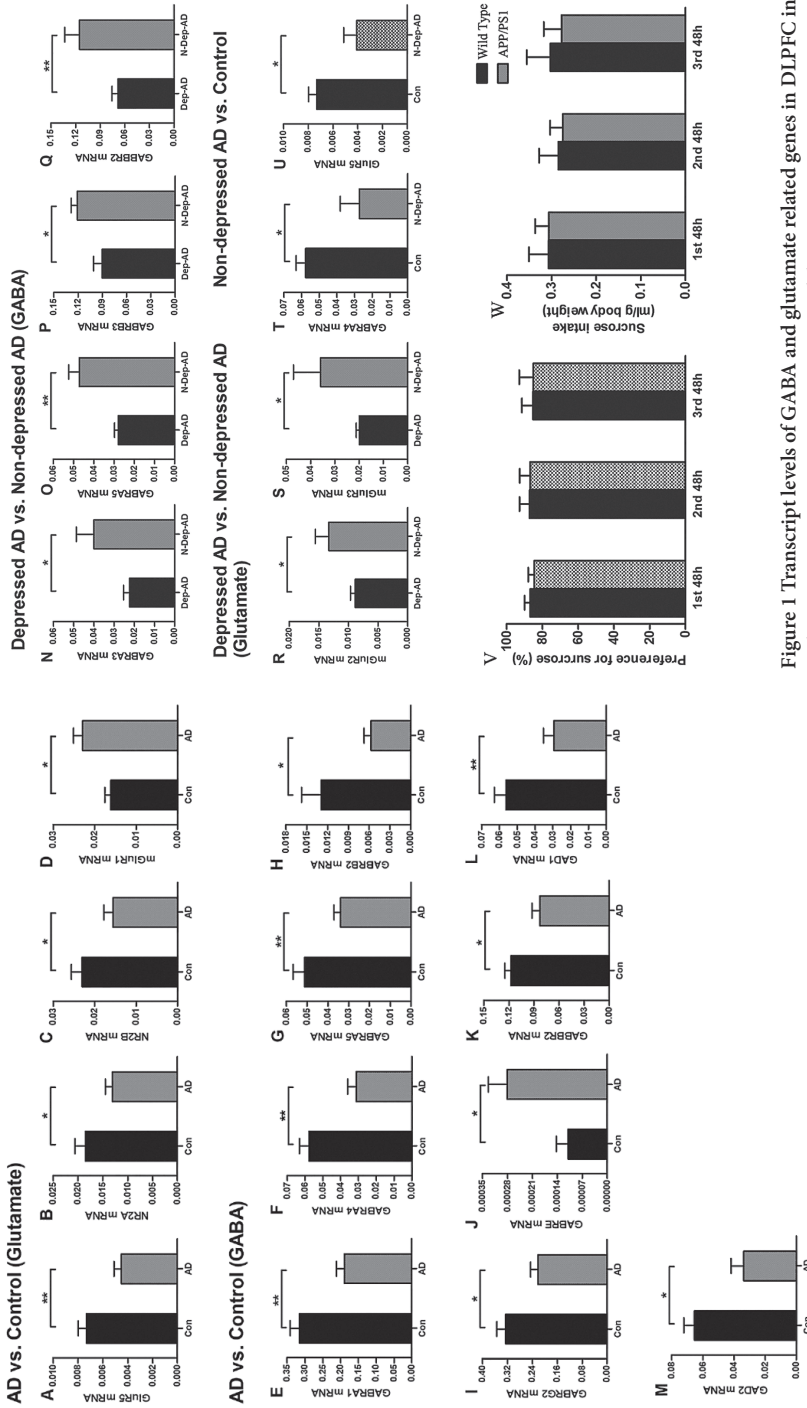


Figure 1 Transcript levels of GABA and glutamate related genes in DLPFC in AD and sucrose consumption test in TgAD model. $**P \leq 0.01$, $*0.01 < P \leq 0.05$

Glutamate related gene expression in AD patients

In the glutamate pathway, the KA receptor subunit GluR5 ($P = 0.006$, Figure 1A) levels were decreased, the NMDA receptor subunits NR2A ($P = 0.042$, Figure 1B) and NR2B ($P = 0.045$, Figure 1C) showed a trend for a decrease while NR1 ($P = 0.300$) was unaltered (Table 3). The expression levels encoding for the AMPA receptor subunits were not changed. Metabotropic glutamate receptor subunits were unchanged except for mGluR1 ($P = 0.039$, Figure 1D) with a trend for an increase in the AD group compared with controls. Glutaminase (GLS), pre-synaptic transporter (VGluT1 and VGluT2) and post-synaptic scaffolding proteins (PSD-93 and PSD-95) were unaltered. KA receptor subunit GluR5 ($P = 0.018$), NMDA receptor subunit NR2B ($P = 0.014$), and post-synaptic scaffolding proteins (PSD-95), GLS ($P = 0.027$) transcription level was lower in the depressed AD patients compared to control subjects. While for the gene expression comparison between depressed AD and non-depressed AD, there was no significant difference other than PSD-95 ($P = 0.038$) in the glutamatergic pathway. For the gene expression comparison between non-depressed AD and control subjects, the transcription level of GluR5 ($P = 0.023$, Figure 1U) was decreased.

Correlations of gene expression levels in relation to the PFC

VGluT1 mRNA was strongly positively correlated to the Cornell score ($\rho = 0.734$, $P = 0.004$) in AD patients. We did not find a correlation between GABA and glutamate related genes and the number of CRH expressing neurons.

Animal study

APPswePS1dE9 and their wild type (WT) controls were studied at the age of 12-14 months. In these TgAD mice plaque deposition starts in the cortex and hippocampus at the age of 5-6 months and plaque load continues to rise thereafter (Jankowsky, Fadale et al. 2004). At 12-14 months plaque load, astrogliosis and microgliosis are extensive in the cortex and hippocampus while in the hypothalamus only few plaques can be found (Unpublished observations WK).

Sucrose preference test

Neither the absolute intake of sucrose solution nor the sucrose intake calculated per body weight in TgAD mice showed a significant difference from that of WT mice (Figure 1V-W).

Stress-related gene expression

In the hypothalamus, only the expression of ESR2 was significantly increased in TgAD mice compared to WT ($P = 0.043$). All other genes (CRH, CRHR1-2, AVP, OXT, MR, GR, AR, ESR1) did not show a difference.

GABA and glutamate related gene expression

In the frontal cortex, for the GABA related genes, only GABRA4 expression was decreased ($P = 0.012$). And for the glutamate related genes, no difference was found between two groups.

DISCUSSION

To our knowledge, this is the first report in which both GABA and glutamate pathway related genes have been studied in the PFC of AD patients that were prospectively followed up for depressive symptoms and depression diagnosis. We observed an alteration in the GABAergic pathway which indicates diminished activity in this pathway. In the AD group VGLUT1 was positively correlated with Cornell score. Since the Cornell score was also correlated to the number of CRH expressing neurons in the PVN (Meynen et al., 2007), the aminoacid neurotransmitters in the PFC may be involved in the activation of the HPA axis in depression in AD as is also the case in mood disorder patients. However, the TgAD mouse showed no depression related behaviour, i.e. anhedonia, and no depression-related changes in the PFC, or the hypothalamus such as a CRH expression increase.

GABA and glutamate related gene expression in AD patients

Post-synaptic GABA-A receptors are ion channels and mediate inhibition in the CNS (Farrant and Nusser 2005). The pentameric receptors are generally comprised of two α , two β , and one γ subunit. The reduced expression of GABRA1, GABRA4-5, GABRB2 and GABRG2 we found in AD suggests a loss of GABAergic inhibition in PFC. Interestingly, a trend for an increase of the transcript level of GABRE was also observed. The ϵ subunit may replace the decreased subunits we mentioned before, resulting in the construction of ϵ subunit-containing receptors (Jones and Henderson 2007; Bollan et al., 2008), that exhibit a reduced deactivation which may compensate for a reduced inhibitory synaptic current (Wagner et al., 2005). The GABA-B receptors are metabotropic and induce the slow, long-lasting component of inhibitory post-synaptic

potentials. The observed trend for a decrease of GABBR2 mRNA also suggests a loss of GABAergic inhibition in the same area.

Decreased mRNA levels of GAD1 and GAD2, which are responsible for synthesis of GABA, also suggests a deficit of GABAergic transmission in PFC in AD. The GABA system is generally considered to be quite robust in AD as GABAergic neuron numbers and GAD enzyme activity in PFC were previously found not to be changed (Rossor et al., 1982; Mountjoy, Rossor et al. 1984; Reinikainen, Paljarvi et al. 1988). However, others reported that the frontal cortex GABA levels were decreased (Lowe, et al., 1988). Moreover, the diminished expression of two GABA-A receptor subunits in the PFC of AD patients (Luchetti et al., 2009) suggests a deficit of the GABA.

Our study indicates the presence of a dysfunction of glutamate system in DLPFC of AD patients. The observed alterations have a different pattern from that previously observed in the hippocampus and entorhinal cortex that strongly suggested that AMPA, NMDA and metabotropic glutamate receptors may play crucial roles in AD pathogenesis while KA receptors were found to be spared (Francis 2003; Parameshwaran et al., 2008). In the present study, the expression of subunits of NMDA and metabotropic glutamate receptors showed a trend in AD patients indicating a dysfunction of these receptors comparable to that in other brain areas. However, we observed a decreased expression of GluR5, suggesting affected KA in the DLPFC. We did not find changes in AMPA receptor subunits in AD.

GABA and glutamate related gene expression in AD patients with or without depression

GABRA5 displayed a decreased mRNA level in the DLPFC in AD patients with depression. The possibility that this alteration is associated with mood disorders is supported by genetic studies. Polymorphisms in this gene were found to be associated to bipolar disorder in different populations (Craddock et al., ; Papadimitriou et al., 1998; Otani et al., 2005). Our results provided additional evidence for a connection between GABRA5 and depression. The decreased mRNA level of GABBR2 we observed in the DLPFC has also been confirmed at the protein level in a depressed patient cohort (Ishikawa et al., 2005). In addition, a recent paper reported increased GABBR2 mRNA in the hippocampus in subjects with major depression (Ghose et al., 2011), which indicates that a reduction of GABBR2 may occur in more brain areas in relation to depression. It is also interesting to note that GABBR2 knockout mice showed depression related

behaviour (Mombereau et al., 2005).

Both the concordant GABA-A receptor and GABA-B receptor subunit changes are of particular interest, since their reduction is likely to be involved in receptor kinetics resulting in a dysfunctional GABAergic transmission. Moreover, the previous finding that changes in the GABA and glutamate ratio correlate with depression in AD (Garcia-Alloza et al., 2006) was confirmed in our finding that the GABAergic pathway is relatively more affected in depressed AD than the glutamatergic pathway.

Relation between PFC gene expression changes and Cornell score

Earlier we reported a positive correlation between the Cornell score and the number of hypothalamic CRH expressing neurons in AD patients (Meynen et al., 2007). Now we observed that, in the AD group, VGluT1 expression was positively correlated with the Cornell score. This supports the idea that alterations in the aminoacid neurotransmitters in the PFC may be involved in the activation of the HPA axis in depression in AD as is also the case in mood disorder patients (Raadsheer et al., 1995; Meynen et al., 2007). Since the amount of neuritic plaques and tangles in the cortex of AD patients is related to the depression severity (Rapp et al., 2008; Meynen et al., 2009), it seems worthwhile to further investigate the possible effect of Alzheimer lesions on cortical aminoacid transmitters and HPA-activity.

Our results strongly suggest that the GABA and glutamate pathways in the PFC are functioning in a concerted action on the activation of the HPA axis in depressed AD patients. However, since observations in human post-mortem material can only reveal correlations and cannot distinguish cause and effect, we studied TgAD mice.

Alterations in TgAD model compared to human postmortem results

Our previous studies showed increased CRH activity in the hypothalamic PVN in both AD and depression (Raadsheer et al., 1995; Wang et al., 2008). In addition there was an imbalance in the receptors that are acting and inhibiting the hypothalamic CRH neurons in depression (Wang et al., 2008). However, in TgAD mice we did not find any change that was observed in the postmortem study, only ESR2 transcript levels were increased in the transgenic mouse, while such an elevation was not observed in the postmortem study (Table 4). These data show that the hypothalamic changes in depressed patients (Wang et al., 2008)

and AD patients (Raadsheer et al., 1995) are not reflected in the hypothalamus of TgAD mice.

Table 4A Comparison between depression postmortem study and TgAD animal study in hypothalamus

Target gene	Depression	TgAD mice
CRF	▲	Unchanged
CRFR1	▲	Unchanged
CRFR2	Unchanged	Unchanged
AVP	▲	Unchanged
AVPR1A	Unchanged	Unchanged
OXT	Unchanged	Unchanged
MR	▲▲	Unchanged
GR	Unchanged	Unchanged
AR	▼	Unchanged
ESR1	▲	Unchanged
ESR2	Unchanged	▲

Table 4B Comparison between AD postmortem study and TgAD animal study in frontal cortex

Target gene	AD vs. Contr	Depressed AD vs. Non-depressed AD	Non-depressed AD vs. Contr	TgAD mice vs. Wild type
GABA related molecules				
GABRA1	▼▼	Unchanged	Unchanged	Unchanged
GABRA3	Unchanged	▼	Unchanged	Unchanged
GABRA4	▼▼	Unchanged	▼	▼
GABRA5	▼▼	▼▼	Unchanged	Unchanged
GABRB2	▼	Unchanged	Unchanged	Unchanged
GABRB3	Unchanged	▼	Unchanged	Unchanged
GABRG2	▼	Unchanged	Unchanged	Unchanged
GABBR2	▼	▼	Unchanged	Unchanged
GAD1	▼▼	Unchanged	Unchanged	Unchanged
GAD2	▼	Unchanged	Unchanged	Unchanged
Glutamate related molecules				
NR1	▼	Unchanged	Unchanged	Unchanged
NR2A	▼	Unchanged	Unchanged	Unchanged
NR2B	▼	Unchanged	Unchanged	Unchanged
mGluR2	Unchanged	▼	Unchanged	Unchanged
mGluR3	Unchanged	▼	Unchanged	Unchanged

▼▼ decrease ($P \leq 0.01$); ▼ decrease ($0.01 < P \leq 0.05$)

In AD patients, we found, 13 molecules that showed a significant decrease or trend, while in TgAD mice only one molecule (GABRA4) was decreased. GABRA4 decrease was present in the non-depressed AD subgroup compared to controls, but no difference was observed between AD patients with and without depression. This suggests that the change of GABRA4 is not related to depression but rather to AD, it also fits with our sucrose preference experiment which indicates there

is no anhedonia in these mice. However, when we consider the entire GABA and glutamate system, the agreement between the changes in AD patients and TgAD mice is neglectable.

For the depressed AD group we found that GABRA5 was significantly decreased, also GABRA3, GABRB3, GABBR2, mGluR2 and mGluR3 showed a decreased trend compared to non-depressed AD patients. The TgAD mice did not show any of the changes that we observed in the human PFC for these six molecules (Table 4B).

Limitations

Several limitations should be mentioned in this study. First, post-mortem human brain studies of psychiatric disorders have their inherent limitations. In our study, the group size is relatively small. However, for Q-PCR, frozen brain material is needed from clinically well-documented patients and well-matched control material with a relatively short post-mortem time and good quality of RNA, which is extremely difficult to obtain. An extra difficulty of the current study is the unique combination of prospectively scoring depression in AD patients over a longer period before death and obtaining permission for a brain autopsy from the same patients after death. Secondly, we could not match PMD between depressed AD and non-depressed AD in our study. However, out of 33 target genes, there were 3 genes (GABRA4, mGluR1 and PSD-95) which were significantly correlated to PMD. Therefore, the significant changes we found in our study are largely unconfounded by the difference in PMD. Third, the brain area available for our study was limited to DLPFC. Many other brain regions have structural and functional disturbances in psychiatric conditions (Drevets et al., 2008), which asks for extensive series of comparisons of these variables in one and the same study in the future. A fourth point that needs to be mentioned is medication as a possible of confounding factor. Anti-depressant treatments appear to eliminate the deficit in cortical GABA levels associated with MDD (Krystal et al., 2002) and benzodiazepine administration appear to reduce cortical GABA levels in healthy subjects (Goddard et al., 2004). And antidepressants influence the SCN which in turn might influence the adrenal cortical sensitivity to ACTH and therefore changes in cortisol. An animal study showed that treatment (with haloperidol and diazepam) was limited to GABA-A receptors that do not contain an $\alpha 5$ subunit (McLeod et al., 2008). Our data showed decreased GABA related gene expression in depressed AD patients, so the differences might even have been

more pronounced without medication. Since postmortem tissue of depressed AD patients who did not receive medication is quite impossible to come by, additional information will hopefully come from in vivo imaging studies of medication-free patients. Another possible confounding factor is the cause of death which might affect brain metabolism for extended periods of time. However, CSF pH, a good indicator of agonal states in brain tissue (Monoranu et al., 2008), was well matched between our groups. This makes it unlikely that the cause of death has influenced our findings.

CONCLUSIONS

The present study shows that GABAergic neurotransmission related gene in the DLPFC is differentially expressed in AD patients with and without depression which may provide possible new targets for therapeutic strategies to treat depression in AD. In AD patients there was a positive correlation between VGlut1 mRNA expression in the DLPFC and depressive symptoms as determined by the Cornell score. The TgAD model did not show these changes in the frontal cortex and hypothalamus, nor did it show a depression-like symptom, i.e. anhedonia, suggesting this model is not suitable to study these aspects of depression in AD.

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