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Prefrontal gene expression changes in mood disorders and suicide

Zhao, J.

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CHAPTER 3

GENE EXPRESSION OF GABA AND GLUTAMATE PATHWAY MARKERS IN THE PREFRONTAL CORTEX OF NON-SUICIDAL ELDERLY DEPRESSED PATIENTS

J. Zhao, A-M. Bao, X-R. Qi, W. Kamphuis, S. Luchetti, J-S Lou and D.F. Swaab

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ABSTRACT

Background: The prefrontal cortex (PFC) is presumed to be involved in the pathogenesis of depression.

Methods: We determined the gene expression of 32 markers of the pathways of the two main neurotransmitters of the PFC, gamma-aminobutyric acid (GABA) and L-glutamic acid (glutamate), by real-time quantitative PCR in human postmortem anterior cingulate cortex (ACC) and dorsolateral PFC (DLPFC) in elderly non-suicidal patients with major depressive disorder (MDD) or bipolar disorder (BD).

Results: We found the transcript levels of GABA-A receptor beta 2 (GABRB2) and post-synaptic density-95 (PSD-95) to be significantly decreased in the ACC in mood disorder. DLPFC mRNA expression of all the detected genes in the mood disorder group did not differ significantly from that of the non-psychiatric controls.

Conclusions: The observed alterations in the GABAergic and glutamatergic pathways indicate a diminished activity. These alterations were only present in the ACC and not in the DLPFC.

INTRODUCTION

Mood disorders are common and have serious consequences. The lifetime prevalence of major depressive disorder (MDD) is estimated to be 16% (Kessler et al., 2003). Neuropsychological, functional neuroimaging and morphological studies indicate that the prefrontal cortex (PFC) is involved in the pathogenesis of depression. A number of studies show that hypofunction of the PFC correlates with disease severity in both MDD and bipolar disorder (BD) (Drevets et al., 2008; Price and Drevets, 2010; Swaab et al., 2000): for example, a strongly altered glucose metabolism was found in the dorsolateral PFC (DLPFC) and anterior cingulate cortex (ACC) (Price and Drevets, 2010). Moreover, BD patients exhibit pronounced impairment of a number of tasks mediated by the PFC (Zihl et al., 1998). In addition, a reduction of grey matter volume was found in the DLPFC in both mood disorders, accompanied by reductions in density and size of neurons and glial cells (Price and Drevets, 2010; Rajkowska et al., 1999; Rajkowska et al., 2001).

Gamma-aminobutyric acid (GABA) and L-glutamic acid (glutamate) are two major neurotransmitters that may affect PFC activity in depression (Hashimoto, 2011; Krystal et al., 2002; Sanacora and Saricicek et al., 2007). In living patients, an increased glutamate content was reported in BD in both ACC and DLPFC (Frye et al., 2007; Lan et al., 2009), whereas prefrontal GABA and glutamate/glutamine concentrations were either reduced or unchanged in MDD patients (Hasler et al., 2007; Walter et al., 2009). A reduced density of GABAergic neurons was found in the postmortem ACC of BD patients and in the DLPFC of MDD patients (Cotter et al., 2002; Rajkowska et al., 2007). Furthermore, the transcript levels of a number of GABA subunits were decreased in the PFC of depressed suicide victims (Merali et al., 2004). For the glutamate pathway, reduced gene expression of NMDA receptor subunits (NR1 and NR2A) were found in the DLPFC of depressed patients (Beneyto and Meador-Woodruff, 2008). Furthermore, a microarray study showed dysregulation of genes related to the GABA and glutamate pathways in the ACC and DLPFC (Choudary et al., 2005).

However, it should be noted that in the postmortem studies data on the major changes in the GABAergic and glutamatergic systems in depression (Gao et al., 2010) were mainly obtained in younger suicidal patients. Therefore, the present study aimed to study GABA and glutamate pathway changes in non-suicidal elderly depressed patients. We used real-time quantitative PCR (Q-PCR) on

isolated grey matter of the ACC and DLPFC in order to compare the expression of components of the GABAergic and glutamatergic pathways in mood disorder patients and matched controls.

METHODS

Subjects

Brain samples were collected by the Netherlands Brain Bank (NBB), after written informed consent from the patient or their next of kin for a brain autopsy and the use of the material and medical records for research purposes. The depressed patients had been diagnosed in psychiatric clinics either as MDD or BD during their lifetime according to the DSM-III, DSM-III-R or DSM-IV criteria, and the diagnosis was confirmed on the basis of the DSM-IV criteria by a board-certified psychiatrist using the extensive medical records of the NBB. Alcohol abuse was an exclusion criterion and other causes of depression, such as Parkinson's and Alzheimer's disease, were excluded by systematic neuropathological investigation (van de Nes et al., 1998). Control patients had no primary neurologic or psychiatric disease (for exceptions see Table 1). The material consisted of ACC (depression N=12, control N=12) and DLPFC (depression N=14, control N=14). The depressed patients and controls were matched for sex, age, postmortem delay (PMD), clock time and month of death, cerebrospinal fluid (CSF) pH, brain weight, ApoE genotype and Braak stage of Alzheimer pathology.

Tissue from the left DLPFC or ACC (for right side exceptions see Table 1) was dissected, then snap-frozen and 50 μ m thick cryostat sections were cut. Grey matter containing all six layers of the neocortex was isolated as described in our previous publication (Bossers et al., 2010).

Table 1 Clinico-pathological information of patients with MDD, BD and control subjects

	NBB number	Sex	Age (y)	PMD (h)	CTD (h)	MTD	CSF pH	BW (g)	RIN (ACC)	RIN (DLPFC)	ApoE	Braak stage	Brain Region	Medication in the past	Medication in the last 3 months	Suicide attempt	Cause of death	
Patients	MDD 01-074	M	45	7:00	02:30	6	6.55	1427	7.0	8.0	43	0	R DLPFC, LACC	SSRI, BZD	SSRI	No	Pontine hemorrhage	
	MDD 02-051	M	81	6:00	15:30	6	6.50	1345	8.0	7.3	43	3	L DLPFC, LACC	BZD, TCA, Hal	Hal	No	Renal insufficiency	
	MDD 06-011	F	60	4:20	16:10	1	ND	1080	8.1	8.9	33	1	L DLPFC, LACC	SSRI, BZD, Hal, Mo, Tam	Hal, Dex	No	Euthanasia due to carcinoma	
	MDD 06-026	M	70	7:15	08:00	3	6.50	1415	7.9	8.4	43	1	L DLPFC, LACC	Met, Hal, ZUC, BZD	BZD, Mo, Clozapine	No	Respiratory insufficiency	
	MDD 07-033	M	88	6:37	21:15	5	6.26	1225	7.0	7.3	43	2	L DLPFC, LACC	Li, BZD, Lev, Mo	BZD, Lev, Mo	No	Multiple epileptic seizures	
	BD 00-074	M	78	7:35	23:00	6	6.27	1227	6.7	7.3	33	0	L DLPFC, LACC	Li, Hal, BZD	BZD	Yes	Colon cancer	
	BD 00-088	M	73	5:15	09:30	7	6.38	1260	7.5	7.2	33	2	R DLPFC, LACC	Li, CBZ, BZD, ECT	Mo	No	Dehydration	
	BD 00-111	M	70	4:50	02:45	10	6.26	1490	-	6.9	33	1	L DLPFC	Li, Hal, BZD, ECT, ZUC	Mo, ZUC	No	Cardiac arrest, ileus	
	BD 02-014	M	68	16:46	00:00	2	6.64	1424	7.2	8.1	33	1	L DLPFC, LACC	Li, ZUC, CBZ, MAOI, Hal	None	No	Subdural hematoma	
	BD 06-021	M	70	6:23	13:07	3	6.53	1488	7.0	7.1	33	3	L DLPFC, LACC	Li	Li, BZD	No	Cerebral Contusion, pneumonia	
	BD 06-075	F	80	9:30	09:30	10	6.33	1190	6.3	6.3	33	1	L DLPFC, LACC	Li	Li, Prednisolone	No	Acute heart death	
	BD 07-076	F	79	7:25	03:10	11	6.26	1231	-	7.2	43	2	L DLPFC	ECT, BZD, CBZ	Mo, CBZ	No	ND	
	BD 97-058	F	90	6:30	10:15	5	ND	1143	7.1	7.4	32	3	L DLPFC, LACC	Li, TCA, Parnate, ECT	Parnate	No	Lung embolism	
	BD 99-118	M	68	5:55	23:15	10	6.82	1204	-	7.7	32	1	L DLPFC	Li	None	No	Cardiac ischemia	
	BD 07-060	M	93	6:00	21:10	9	6.37	1459	7.7	-	33	1	LACC	BZD, SSRI, TCA	BZD	No	Cachexia, renal failure	
	Mean (ACC)		74	7:25	13:27	5	6.43	1307	7.3	-								

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

	NBB number	Sex	Age (y)	PMD (h)	CTD (h)	MTD	CSF pH	BW (g)	RIN (ACC)	RIN (DLPFC)	ApoE	Braak stage	Brain Region	Medication in the past	Medication in the last 3 months	Suicide attempt	Cause of death	
Mean (DLPFC)			72	7:14	12:09	7	5.52	1296	-	7.5								
SEM (ACC)			3.91	0:55	01:56	0.78	0.04	39.36	0.16	-								
SEM (DLPFC)			3.07	0:48	02:02	0.82	0.63	35.53	-	0.18								
Controls	C-1	00-067	M	73	12:45	00:01	6	ND	1267	-	7.8	33	0	L DLPFC	ND	-	-	Massive lung embolism
	C-2	01-033	M	75	6:20	06:10	3	6.18	1180	-	7.3	43	1	L DLPFC	Mo	-	-	Dehydration, adenocarcinoma, pneumonia
	C-3	01-086	M	88	7:00	03:00	7	6.84	1398	-	8.1	32	1	L DLPFC	BZD, Prednisolone	Mo	-	Heart failure
	C-4	04-049	F	77	8:20	07:55	7	6.48	1312	5.9	6.1	32	1	L DLPFC, LACC	BZD	TCA	-	Cachexia and uremia
	C-5	04-057	F	81	6:40	13:10	8	7.16	1164	7.5	8.2	33	1	L DLPFC, LACC	None	Mo	-	Euthanasia
	C-6	05-019	M	74	5:00	02:00	4	6.70	1125	8.1	8.3	43	3	L DLPFC, LACC	Fluticasone, Salbutamol	Mo, Hal, BZD	-	Bronchus carcinoma
	C-7	05-034	M	56	14:00	00:01	5	7.03	1323	-	8.5	33	0	L DLPFC	ND	ND	-	Heart failure
	C-8	05-044	M	80	7:15	07:15	6	5.80	1376	5.2	5.7	33	0	L DLPFC, LACC	None	Mo	-	Cachexia and dehydration
	C-9	05-068	M	56	9:15	04:45	10	6.54	1553	7.9	-	43	0	LACC	None	None	-	Acute myocardial infarction
	C-10	06-037	M	66	7:45	17:45	5	6.70	1590	-	7.8	33	0	L DLPFC	Met	Met	-	Ruptured abdominal aorta aneurysm
	C-11	96-052	M	73	9:10	11:30	5	ND	1500	-	8.5	43	2	L DLPFC	None	None	-	Cardiac arrest
	C-12	97-039	M	87	4:00	15:00	4	7.39	1506	-	8.3	33	3	L DLPFC	None	None	-	Myocardial infarction

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

NBB number	Sex	Age (y)	PMD (h)	CTD (h)	MTD	CSF pH	BW (g)	RIN (ACC)	RIN (DLPFC)	ApoE	Braak stage	Brain Region	Medication in the past	Medication in the last 3 months	Suicide attempt	Cause of death
C-13	F	77	2:40	08:30	11	6.37	1235	-	8.2	33	1	L DLPFC	None	None	-	Septic shock, pancreas carcinoma
C-14	M	50	8:30	11:00	1	6.65	1436	-	7.5	43	0	L DLPFC	None	None	-	Cardiac arrest, septic shock
C-15	F	88	5:40	03:05	9	6.67	1054	-	6.9	33	3	L DLPFC	BZD, Prednisolon	None	-	Cardial decompensation, peritonitis
C-16	M	87	10:20	04:00	3	6.32	1356	7.5	-	33	1	L ACC	ND	Mo	-	Pneumonia, heart infarction, renal insufficiency
C-17	F	89	6:25	21:30	12	6.46	1210	7.1	-	32	2	L ACC	Hal, BZD	None	-	Ruptured abdominal aorta aneurysm
C-18	M	79	6:00	06:10	10	6.51	1392	8.6	-	33	1	L ACC	BZD, Dex	None	-	Metastasized adeno- and lung carcinoma
C-19	M	68	10:10	09:05	4	7.08	1547	8.5	-	33	2	L ACC	None	Adrenaline	-	Heart infarction
C-20	M	80	4:30	14:30	6	6.22	1400	5.4	-	33	2	L ACC	Dex, Mo, Estramustine	None	-	Renal failure
C-21	M	87	6:05	08:05	10	6.96	1568	7.8	-	33	3	L ACC	None	None	-	ND
C-22	M	96	5:23	13:27	2	6.70	1204	7.4	-	33	1	L ACC	None	Mo	-	Pneumonia
		79	7:06	10:32	7	6.58	1351	7.2	-							
Mean (ACC)		74	8:21	07:35	6	5.71	1319	-	7.7							
Mean (DLPFC)		3.91	0:34	01:50	1	0.11	44.39	0.33	-							
SEM (ACC)		2.99	1:26	01:29	1	0.66	42.28	-	0.24							

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

	NBB number	Sex	Age (y)	PMD (h)	CTD (h)	MTD	CSF pH	BW (g)	RIN (ACC)	RIN (DLPFC)	ApoE stage	Brain Region	Medication in the past	Medication in the last 3 months	Suicide attempt	Cause of death
Level of Significance (ACC)	0.34 ¹		0.77 ¹	0.23 ²	0.82 ²	0.24 ¹	0.47 ¹	0.89 ¹	-	0.98 ²	1.00 ²			¹ Student t-Test		
Level of Significance (DLPFC)	0.58 ¹		0.55 ¹	0.81 ²	0.57 ²	0.07 ¹	0.65 ¹	-	0.61 ¹	1.00 ²	0.69 ²			² Mardia-Watson-Test		

Abbreviations: ACC, anterior cingulate cortex; BD, bipolar disorder; BZD, benzodiazepine; BW, brain weight; CBZ, carbamazepine; CSF, cerebrospinal fluid; CTD, clock time of death; Dex, dexamethasone; DLPFC, dorsolateral prefrontal cortex; MTD, month time of death; ECT, electro-convulsive treatment; F, female; Hal, haloperidol; L, left; Lev, Levetiracetam; Li, lithium; MAOI, MAO inhibitor; MDD, major disorder depression; Met, Methylprednisolone; Mo, morphine; M, male; NBB, Netherlands Brain Bank; ND, no data; None, no medication; PMD, postmortem delay; R, right; RIN, RNA integrity number; SSRI, selective serotonin reuptake inhibitor; Tam, Tamoxifen; TCA, tricyclic antidepressant; ZUC, zuclopenthixol.

Q-PCR analysis

The genes related to GABAergic and glutamatergic pathways were selected according to Figure 1, the gene information and their primers are shown in Supplementary Material Table S-1. RNA isolation, cDNA synthesis and Q-PCR were performed by the same protocol as described in our published paper (Wang et al., 2008).

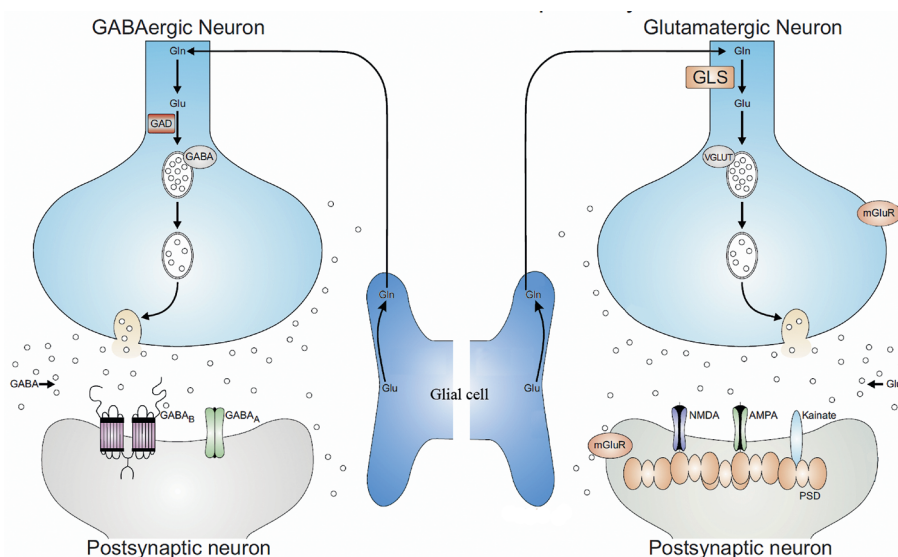


Figure 1 Information of GABA and glutamate pathways

Left Figure: The production, transport of gamma-aminobutyric acid (GABA). GABA is synthesized from glutamate (Glu) by glutamic acid decarboxylase (GAD). After the GABA is released from presynaptic nerve terminals, the synaptic effects of GABA in the brain are mediated through two major receptor subtypes, termed GABA_A and GABA_B, respectively.

Right Figure: The production, transport, and reuptake of Glu. Glu is synthesized from glutamine (Gln) by glutaminase (GLS). Glu is packaged into presynaptic vesicles by the vesicular Glu transporters (VGLUT) and released from the neuron. Glu is cleared from the extracellular space by glial cells. In glial cells, Glu is converted into Gln and is then transported back to the neuron.

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 was calculated by $10^{10} \times E^{-C_t}$ ($E=10^{-(1/\text{slope})}$) (Kamphuis et al., 2001). The geNorm analysis revealed that the transcript level of all reference genes determined in the ACC and DLPFC could be included in the calculation of the normalization factor (More details are shown in the Supplementary Material S1 and S-Table 1).

Statistical analysis

The differences in gene expression levels between groups were determined by Student's *t*-test. Because of multiple testing, a stricter level of $P \leq 0.01$ was considered to be statistically significant. All tests were two-tailed.

RESULTS

In the ACC, no significant difference between MDD and BD patients was found for the GABAergic pathway markers, with the exception of GABRB1, which showed a trend towards a significant decrease in MDD compared to BD (values of $0.01 < P \leq 0.05$ are considered to be a trend). The data from MDD and BD were subsequently pooled for further analysis. GABRB2 transcript levels were decreased in the pooled depression group compared to the matched controls (Table 2). In the DLPFC, no difference in GABA related gene expression was found, either in the subgroups or in the pooled group, except for GABRB3 which showed a trend towards a significant increase in depression (Table 2).

In the ACC, VGluT1 expression showed a trend towards a significant decrease in MDD compared to BD, while PSD-95 transcript level was significantly lower in the pooled depression group (Table 2). In the DLPFC, NR1 expression was decreased and VGluT1 and mGluR2 transcription showed a trend towards a significant decrease in MDD. After pooling MDD and BD, no significant glutamate related gene expression change was found (Table 2).

The main changes, i.e. reduced GABRB2 and PSD-95 expression, were also present when MDD or BD was compared with their respective controls, for more details see Supplementary Material S-Table 2.

Table 2 Result of gene expression changes in depression (MDD+BD)

Gene	anterior cingulate cortex						Fold
	Mean ± SEM			P-values			
	MDD	BD	Depression	Control	MDD vs. BD	Depression vs. Control	
GABRA1	1.346±0.080	1.117±0.075	1.213±0.063	1.153±0.090	0.066	0.593	1.206
GABRA2	1.035±0.081	1.311±0.121	1.196±0.086	1.196±0.060	0.115	0.998	0.789
GABRA3	1.030±0.076	0.867±0.053	0.935±0.049	0.952±0.048	0.098	0.807	1.189
GABRA4	0.549±0.051	0.508±0.039	0.525±0.030	0.482±0.032	0.525	0.345	1.082
GABRA5	0.718±0.115	0.801±0.047	0.766±0.054	0.778±0.048	0.471	0.869	0.896
GABRB1	2.53±0.172	3.186±0.195	2.914±0.161	2.794±0.122	0.039* ↓	0.557	0.795
GABRB2	0.765±0.139	0.847±0.112	0.813±0.084	1.278±0.081	0.652	0.001** ↓	0.903
GABRB3	3.220±0.239	3.362±0.134	3.303±0.122	3.289±0.105	0.591	0.931	0.958
GABRG2	1.756±0.100	1.696±0.142	1.721±0.090	1.711±0.080	0.760	0.937	1.035
GABRD	0.141±0.022	0.129±0.011	0.134±0.011	0.149±0.013	0.606	0.373	1.095
GABRE	0.016±0.005	0.024±0.005	0.020±0.003	0.020±0.003	0.277	0.951	0.669
GABRQ	0.028±0.006	0.039±0.005	0.034±0.004	0.036±0.004	0.184	0.799	0.712
GABBR1	1.422±0.131	1.480±0.102	1.456±0.077	1.639±0.074	0.733	0.102	0.961
GABBR2	1.154±0.095	1.170±0.063	1.163±0.051	1.337±0.092	0.881	0.113	0.986
GAD1	1.006±0.129	0.837±0.119	0.908±0.088	1.014±0.063	0.368	0.334	1.201
GAD2	0.965±0.099	0.799±0.137	0.868±0.090	0.873±0.080	0.389	0.967	1.207
GLS	0.459±0.070	0.526±0.054	0.498±0.042	0.519±0.050	0.459	0.749	0.873
VGLUT1	1.277±0.157	2.024±0.241	1.713±0.186	1.816±0.237	0.040* ↓	0.736	0.631
VGLUT2	0.0007±0.000	0.0012±0.000	0.0010±0.000	0.0014±0.000	0.285	0.164	0.547
Glur1	0.168±0.005	0.182±0.009	0.176±0.006	0.175±0.012	0.280	0.929	0.924
Glur2	0.616±0.049	0.656±0.047	0.639±0.033	0.659±0.042	0.577	0.711	0.939
Glur3	0.142±0.013	0.165±0.021	0.155±0.013	0.160±0.012	0.427	0.791	0.861
Glur4	0.259±0.019	0.281±0.021	0.272±0.015	0.308±0.026	0.473	0.232	0.920
Glur5	0.052±0.005	0.062±0.005	0.058±0.004	0.064±0.004	0.205	0.325	0.835
NRI	0.365±0.056	0.475±0.030	0.429±0.032	0.423±0.016	0.089	0.860	0.769

Table 2 Continued, Result of gene expression changes in depression (MDD+BD)
anterior cingulate cortex

Gene	Mean \pm SEM			P-values			Fold	
	MDD	BD	Depression	Control	MDD vs. BD	Depression vs. Control		MDD/BD
NR2A	0.154 \pm 0.013	0.182 \pm 0.018	0.170 \pm 0.012	0.182 \pm 0.013	0.276	0.508	0.850	0.935
NR2B	0.163 \pm 0.013	0.151 \pm 0.016	0.156 \pm 0.010	0.146 \pm 0.015	0.605	0.582	1.078	1.071
mGluR1	0.050 \pm 0.008	0.070 \pm 0.009	0.062 \pm 0.007	0.058 \pm 0.008	0.122	0.741	0.704	1.058
mGluR2	0.052 \pm 0.004	0.068 \pm 0.009	0.062 \pm 0.006	0.062 \pm 0.004	0.192	0.999	0.762	1.000
mGluR3	0.169 \pm 0.011	0.189 \pm 0.024	0.181 \pm 0.014	0.237 \pm 0.025	0.532	0.060	0.898	0.761
PSD-93	0.646 \pm 0.067	0.866 \pm 0.077	0.775 \pm 0.060	0.849 \pm 0.073	0.070	0.444	0.746	0.913
PSD-95	0.111 \pm 0.006	0.117 \pm 0.010	0.114 \pm 0.006	0.170 \pm 0.012	0.639	0.001**\downarrow	0.946	0.671

Gene	Mean \pm SEM			P-values			Fold	
	MDD	BD	Depression	Control	MDD vs. BD	Depression vs. Control		MDD/BD
GABRA1	0.240 \pm 0.040	0.255 \pm 0.018	0.250 \pm 0.018	0.225 \pm 0.014	0.708	0.280	0.943	1.110
GABRA2	0.037 \pm 0.005	0.037 \pm 0.004	0.037 \pm 0.003	0.033 \pm 0.004	0.969	0.410	0.993	1.115
GABRA3	0.070 \pm 0.013	0.062 \pm 0.004	0.065 \pm 0.005	0.051 \pm 0.005	0.470	0.064	1.128	1.264
GABRA4	0.047 \pm 0.011	0.051 \pm 0.004	0.050 \pm 0.005	0.053 \pm 0.005	0.652	0.612	0.911	0.937
GABRA5	0.025 \pm 0.004	0.035 \pm 0.003	0.031 \pm 0.003	0.026 \pm 0.002	0.084	0.139	0.704	1.200
GABRB1	0.363 \pm 0.039	0.441 \pm 0.050	0.413 \pm 0.036	0.349 \pm 0.025	0.311	0.155	0.822	1.182
GABRB2	0.071 \pm 0.014	0.078 \pm 0.007	0.076 \pm 0.007	0.082 \pm 0.005	0.633	0.470	0.910	0.925
GABRB3	0.156 \pm 0.017	0.168 \pm 0.013	0.164 \pm 0.010	0.136 \pm 0.006	0.603	0.028*\uparrow	0.931	1.201
GABRG2	0.212 \pm 0.033	0.263 \pm 0.025	0.245 \pm 0.020	0.223 \pm 0.012	0.241	0.351	0.806	1.101
GABRD	1.469 \pm 0.151	1.215 \pm 0.086	1.306 \pm 0.081	1.261 \pm 0.086	0.141	0.708	1.208	1.035
GABRE	0.0003 \pm 0.000	0.0005 \pm 0.000	0.0004 \pm 0.000	0.0003 \pm 0.000	0.137	0.309	0.559	1.274
GABRQ	0.0005 \pm 0.000	0.0007 \pm 0.000	0.0007 \pm 0.000	0.0008 \pm 0.000	0.152	0.671	0.611	0.864
GABRI	0.045 \pm 0.010	0.058 \pm 0.003	0.054 \pm 0.004	0.057 \pm 0.005	0.161	0.621	0.775	0.944

Table 2 Continued, Result of gene expression changes in depression (MDD+BD) dosalateral prefrontal cortex

Gene	Mean ± SEM			P-values			Fold	
	MDD	BD	Depression	Control	MDD vs. BD	Depression vs. Control		MDD/BD
GABBR2	0.058±0.012	0.081±0.010	0.073±0.008	0.066±0.004	0.171	0.462	0.713	1.103
GAD1	0.040±0.006	0.037±0.004	0.038±0.003	0.037±0.004	0.664	0.830	1.086	1.029
GAD2	0.120±0.017	0.107±0.015	0.112±0.011	0.116±0.013	0.61	0.796	1.117	0.961
GLS	0.054±0.013	0.069±0.008	0.064±0.007	0.070±0.007	0.319	0.544	0.784	0.916
VGLUT1	0.344±0.049	0.527±0.039	0.462±0.038	0.481±0.053	0.015* ↓	0.775	0.652	0.961
VGLUT2	0.0003±0.00	0.0004±0.00	0.0003±0.00	0.0004±0.00	0.659	0.336	0.854	0.789
GluR1	0.020±0.004	0.029±0.002	0.026±0.002	0.024±0.002	0.068	0.590	0.685	1.066
GluR2	0.171±0.022	0.227±0.022	0.207±0.017	0.180±0.011	0.130	0.202	0.755	1.151
GluR3	0.060±0.009	0.074±0.005	0.069±0.005	0.062±0.005	0.177	0.318	0.812	1.116
GluR4	0.027±0.005	0.033±0.004	0.031±0.003	0.026±0.002	0.409	0.255	0.831	1.163
GluR5	0.005±0.001	0.006±0.000	0.006±0.000	0.005±0.000	0.609	0.846	0.927	1.022
NR1	0.045±0.008	0.083±0.006	0.070±0.007	0.081±0.007	0.003** ↓	0.293	0.541	0.865
NR2A	0.017±0.003	0.020±0.002	0.019±0.002	0.0178±0.001	0.418	0.486	0.849	1.084
NR2B	0.008±0.003	0.011±0.002	0.010±0.002	0.011±0.001	0.483	0.365	0.728	0.832
mGluR1	0.005±0.001	0.009±0.001	0.007±0.001	0.006±0.001	0.071	0.378	0.572	1.200
mGluR2	0.007±0.001	0.013±0.002	0.011±0.001	0.010±0.001	0.036* ↓	0.390	0.544	1.140
mGluR3	0.016±0.004	0.019±0.001	0.018±0.002	0.020±0.002	0.455	0.297	0.866	0.884
PSD-93	0.060±0.009	0.075±0.007	0.069±0.006	0.066±0.005	0.225	0.648	0.796	1.052
PSD-95	0.026±0.005	0.031±0.003	0.029±0.003	0.032±0.002	0.484	0.425	0.856	0.908

Abbreviations: BD, bipolar disorder; MDD: major depressive disorder. For abbreviations of gene see S-Table 1.

The differences between gene expression levels were determined by Student's *t*-test. Because of multiple testing, a stricter level of $P \leq 0.01$ was considered to be statistically significant.

↓ decrease, ↑ increase.

DISCUSSION

We observed alterations in the GABAergic and glutamatergic pathways in the PFC in depression. A significant decrease in transcript levels of two genes was found, i.e. of GABRB2, one of the subunits of the GABA-A receptor, and of PSD-95, a scaffold protein related to the glutamatergic pathway, which indicates diminished activity in these pathways in depression. These alterations were only present in the ACC and not in the DLPFC. The possible confounders - age, CSF-pH and PMD - did not affect our conclusions, for more details see Supplementary Material S2.

As the GABRB2 subunit contains the GABA binding site (Choudary et al., 2005; Blednov et al., 2003; Sur et al., 2001), the decreased expression in depression may have functional implications. Our experiments did not confirm the altered expression of several GABA receptor subunits in the ACC found by microarray (Sequeira et al., 2009). It should be noted, however, that in the latter study only male MDD patients who committed suicide were examined. We did not confirm the increase in GABRB2/3 in the DLPFC of BD subjects either (Ishikawa et al., 2004) but only showed an increased trend in GABRB3. In our study, however, the subjects were much older than in the Ishikawa study (73 ± 3 vs 48 ± 15 years of age).

The postsynaptic scaffold protein PSD-95 directly binds to the NMDA receptor. The observed decreased PSD-95 expression may thus cause a disturbed NMDA receptor clustering, anchoring or receptor-mediated intracellular signaling (Lau and Zukin, 2007; Ziff, 1997). This may also affect synaptic plasticity since PSD-95 can indirectly bind to AMPA receptors, determine the density of AMPA receptors at synapses (Schnell et al., 2002) and participate in synaptic plasticity (Gardoni et al., 2009).

Our finding of only a minor impairment of GABA and glutamate neurotransmission is in accordance with *in vivo* studies in younger patients (Berrettini et al., 1986; Roy et al., 1991; Kaufman et al., 2009). In younger depressed suicide victims, more extensive changes have been reported for GABA and glutamate receptor subunit genes in the PFC (Sequeira et al., 2009), in particular of calbindin GABAergic neurons (Chana et al., 2003; Cotter et al., 2002; Rajkowska et al., 2007). In addition, in the PFC of younger depressed suicide victims several alterations were reported in glutamate and GABA receptor subunits (Choudary et al., 2005) and in GABA-A/benzodiazepine binding sites (Cheetham et al., 1988). These studies were not suited to distinguish between the alterations in GABA and

glutamate that are related to depression and those related to suicide because of the high proportion of suicide cases in the depressed patient groups. The strong alterations in the GABAergic and glutamatergic pathways may thus rather be related to depression at a younger age or to suicide.

Several limitations of our study should be mentioned. Firstly, the groups are relatively small in size. However, the frozen brain material for Q-PCR analysis was collected from clinically well-documented patients and well-matched control subjects with a relatively short post-mortem time and good quality of RNA. Samples meeting these criteria are extremely difficult to obtain. Furthermore, one of the inherent potential confounding factors in a postmortem study is medication. Mood-stabilizer effects on cortical GABA levels have not been studied in patients with mood disorders. However, our study showed no differences regarding the significantly changed genes between the depressed patients treated with mood-stabilizers (benzodiazepine and carbamazepine) and the depressed patients who never took mood-stabilizers (GABRB2: $P = 0.662$; PSD-95: $P = 0.164$) or took them only during their last 3 months (GABRB2: $P = 0.502$; PSD-95: $P = 0.186$). Studies evaluating the impact of electroconvulsive therapy and serotonin reuptake inhibitors suggest that both treatments eliminate the GABA deficit in depressed patients (Sanacora et al., 1998; Sanacora et al., 2000). However, our data did show decreased GABA related gene expression in depressed patients, so the differences might have been even more pronounced without this type of medication. In addition, an MRS study showed no significant changes in the glutamatergic and GABAergic systems in the ACC after lithium treatment (Shibuya-Tayoshi et al., 2008), while PSD-95 showed a significant up-regulation by antipsychotic-mood stabilizing treatment in ACC (Tomasetti et al., 2010). Our study showed decreased PSD-95 expression in depressed patients, which means that the difference in PSD-95 might also have been more pronounced without this type of medication. Moreover, we did not find that the significantly changed genes in our study were any different in the depressed patients who were treated by antipsychotic medication compared to the depressed patients who never received antipsychotic treatment (GABRB2: $P = 0.169$; PSD-95: $P = 0.958$) or received them only during their last 3 months (GABRB2: $P = 0.696$; PSD-95: $P = 0.885$). Since postmortem tissue of mood disorder patients who did not receive medication is hard to come by, additional information will have to come from in vivo imaging studies of medication-free patients. Another possible confounding effect is the different causes of death which might have affected brain metabolism for extended periods.

However, the CSF-pH value is a good indicator of ante-mortem events in brain tissue (Monoranu et al., 2008) and with the groups so well-matched it is highly unlikely that differences in agonal state influenced the data.

In conclusion, the present study shows that markers of the GABAergic and glutamatergic neurotransmission in the ACC are differentially expressed in elderly non-suicidal depression. The possibility should be considered that the more extensive alterations in the GABAergic and glutamatergic pathways in mood disorder as reported in the literature might be related to younger age and suicide rather than to depression per se. The present results support the possibility that the GABA and glutamate pathways may be useful therapeutic targets in depression (Hashimoto, 2011; Sanacora and Saricicek et al., 2007).

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Supplementary Material

Gene	Official full name	Primer sequences (Forward)	Primer sequences (Reverse)	Accession numbers	Amplicon length (bp)
GABA related molecules					
GABRA1	gamma-aminobutyric acid (GABA) A receptor, alpha 1	ATGCCCAACAAACTCCTGCG	ATAGGAAAGTCCTCCAAATGC	NM_000806 NM_001127643	103
GABRA2	gamma-aminobutyric acid (GABA) A receptor, alpha 2	CTTTGGGATGGGAAGAGTGTAGT	GTTCTGTATCATAACGGGAAGCCT	NM_000807 NM_001114175	64
GABRA3	gamma-aminobutyric acid (GABA) A receptor, alpha 3	GCCCCGACTGAGACCAAGACC	ATGGCAAAAGAGCACAGGAAAGA	NM_000808	81
GABRA4	gamma-aminobutyric acid (GABA) A receptor, alpha 4	TGGGCAAACCGTATCAAGTG	GAGGTGGAAAGTAAACCGTCATAA	NM_000809	74
GABRA5	gamma-aminobutyric acid (GABA) A receptor, alpha 5	CAGTCTTGTTGGGCACTTTCA	GAGAGGGGGTCCTTTTAT	NM_000810	80
GABRB1	gamma-aminobutyric acid (GABA) A receptor, beta 1	AGCCAGAGTGGCACTAGGAAT	CCAGCAGAGCCAGGAACAC	NM_000812	148
GABRB2	gamma-aminobutyric acid (GABA) A receptor, beta 2	CGGTGGATAGACTCCTG AA	TGGCAATGTCAAATGTTCAATC	NM_000813	91
GABRB3	gamma-aminobutyric acid (GABA) A receptor, beta 3	TGACAAACCATCAACACCCACC	CAAAGACGAAAGCAGCCCATAA	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	GCTCCTCTCCATGACTTCCCT	NM_004961	123
GABRG2	gamma-aminobutyric acid (GABA) A receptor, gamma 2	CGTCCTATCCTGGGTGCTCTTTC	CAATGGTGCTGAGGGTGCTC	NM_000816 NM_198903	102
GABRQ	gamma-aminobutyric acid (GABA) receptor, theta	CCTGGGAAGGACGATTACT	CCCTCTGAACCTGGAACTT	NM_018558	85

S-Table 1 Information of Gene Selection, sequence of the primers, gene bank accession numbers, amplicon length for the target genes and reference genes

S-Table 1 Continued, Information of Gene Selection, sequence of the primers, gene bank accession numbers, amplicon length for the target genes and reference genes

Gene	Official full name	Primer sequences (Forward)	Primer sequences (Reverse)	Accession numbers	Amplicon length (bp)
GABBR1	gamma-aminobutyric acid (GABA) B receptor, 1	ATTACCGACCAAAATCTACCG	CGCTGGCATCAAACACC	NM_021904 NM_021903	78
GABBR2	gamma-aminobutyric acid (GABA) B receptor, 2	TCACGGGTCAAAGTTGTATTCC	CCTTACACCTCCCCTGCTGTCT	NM_005458	83
GAD1	glutamate decarboxylase 1 (brain, 67kDa)	CGGCTAAGAACGGTGAGGA	CTTGGCGGACATAGTTGAGGAGT	NM_000817 NM_013445	76
GAD2	glutamate decarboxylase 2 (pancreatic islets and brain, 65kDa)	CCACACAAGATGATGGAGTC	TGCTGAAAAGAGGTAGGAGGC	NM_000818 NM_001134366	106
Glutamate related molecules					
GLS	glutaminase	GCTTTCATGTTGGTCTTCC	ACACTGTTGCCCAATCTTATCC	NM_014905	118
VGLUT1 (SLC17A7)	solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 7	TCTTCTACGGGGICTTTGCIT	CACACTTCTCCTCGCTCAICT	NM_020309	74
VGLUT2 (SLC17A6)	solute carrier family 17 (sodium-dependent inorganic phosphate cotransporter), member 6	TAAAAAAGCTAACATCAGACCCC	ATCCAGAATAGACAAAATACAC-CAA	NM_020346	102
GluR1 (GRIA1)	glutamate receptor, ionotropic, AMPA 1	ATCCACAGCAATCCATCAA	ACCCGACCAATTCCTCCAC	NM_000827 NM_001114183	100
GluR2 (GRIA2)	glutamate receptor, ionotropic, AMPA 2	ATTGTTGGAGGTGTGTGGTG	TAAAGTTAGCCGTGTAGGAGGA	NM_000826 NM_001083619	62
GluR3 (GRIA3)	glutamate receptor, ionotropic, AMPA 3	TACTTGTGGGAGGTCTGGG	GTGAGTTTTCATGCGGTTTGG	NM_000828 NM_007325	90
GluR4 (GRIA4)	glutamate receptor, ionotropic, AMPA 4	TCACTACGAATGGAACTGTTTT	AGTATCGAGTATCCCCTGTCTG	NM_001077244 NM_000829	59
GluR5 (GRIK1)	glutamate receptor, ionotropic, kainate 1	CCCAGGAAGACACAACAAGAA	GGCAGCCAGAACAAATGAAGAT	NM_000830 NM_175611	74

S-Table 1 Continued, Information of Gene Selection, sequence of the primers, gene bank accession numbers, amplicon length for the target genes and reference genes

Gene	Official full name	Primer sequences (Forward)	Primer sequences (Reverse)	Accession numbers	Amplicon length (bp)
NR1 (GRIN1)	glutamate receptor, ionotropic, N-methyl D-aspartate 1	ATCCAGATGGCTCTGTCCGGT	GGTGGAGTGAAGTGGTCCGT	NM_007327 NM_021569 NM_000832	101
NR2A (GRIN2A)	glutamate receptor, ionotropic, N-methyl D-aspartate 2A	TCAAGAAGTAATGGCACCG	CATCATCACCCAGACAGAGG	NM_000833 NM_001134407	71
NR2B (GRIN2B)	glutamate receptor, ionotropic, N-methyl D-aspartate 2B	CTGAGAATAAAACAGACGAGG	AAGGATGTCAAATACAGAACCC	NM_000834	70
mGluR1 (GRM1)	glutamate receptor, metabotropic 1	CCTACGGCTCTGTCAATCTGC	CTCTGGCTTGCTTGCTTTTCT	NM_000838 NM_001114329	91
mGluR2 (GRM2)	glutamate receptor, metabotropic 2	GCAAGTATGTTGGGCTCGC	CTCGTTGAAGTTTTCGGGG	NM_000839 NM_001130063	98
mGluR3 (GRM3)	glutamate receptor, metabotropic 3	AITGCCCTGTCTGGGTTTAA	AAGGGTGTGTTGTTGTGCT	NM_000840	70
PSD-93 (DLG2)	discs, large homolog 2	TATTCCTCTGTTGAGTGTGA	ACGAGTTGCGGTGCTATGT	NM_001364 NM_001142699	119
PSD-95 (DLG4)	discs, large homolog 4	GGGTAACCTCAGGCTCGGC	CTTGGTGATGAAAATGGATGG	NM_001365 NM_001128827	84
Reference genes					
RN18S ¹	18S ribosomal RNA	TTCTGATTGCGCCGGCTAGA	TGGCAAAATGCTTTCCGCTCT	NR_003286	70
ACTβ ^{1,2}	actin, beta	CCGAGCGATGTACGTTGCTA	TCACCGGAGTCCATCACGAT	NM_001101	65
GAPDH ²	glyceraldehyde-3-phosphate dehydrogenase	CAATTCATGGCACCGTC	TCGCTCCTGGAAAGATGGT	NM_002046	62
HPRT1 ¹	hypoxanthine phosphoribosyltransferase 1	GGACAGGACTGAACGTCTTGC	ATAGCCCCCTTGAGCACAC	NM_000194	88
TUBα ^{1,2}	tubulin, alpha 1b	CTTTGAGCCAGCCAACCAGA	GTACAACAGGCAGCAAGCCAT	NM_006082	72
UBC ¹	ubiquitin C	GCTGCTATAAGACTCGGCC	GTCACCCAAGTCCCCTCCTA	NM_021009	70
TUBβ ^{4,2}	tubulin, beta 4	GGGCCAAGTTTGGGAGGT	CACTGTCCCCATGGTATGTGC	NM_006087	71

¹The normalization factors used in ACC. ²The normalization factors used in DLPFC.

Supplementary Material S1: Normalization strategy

Quantification of the set of 7 different reference genes (reflecting different cellular processes) showed no statistically significant differences between controls and depression in both ACC ($P \geq 0.24$) and DLPFC ($P \geq 0.34$). Yet there was a good overall correlation between the expression levels of the reference genes showing that the variation observed was correlated and at least partly caused by variations in the amount of cDNA in the samples which is compensated for by the normalization factor derived from the geomean value of the 7 reference genes. Furthermore, we tested the effect of the normalization procedure on the variation of the target genes; the overall variation of the raw data was 40.7% and after normalization this was reduced to 30.2% ($P = 0.001$). So, although effective, the reduction of variation was modest probably as a result of the fact that we started all cDNA reactions with exactly the same amount of RNA and that the quality of the RNA (RIN-values) were within a narrow range.

S-Table 2 Significant level of the comparison between different groups

Gene	MDD vs. Control	BD vs. Control	Depression vs. Control
GABRB2	0.068	0.006	0.001
PSD-95	0.002	0.019	0.001

Supplementary Material S2: Possible confounders in the current post-mortem study

Age, CSF-pH and PMD were very carefully matched between patients and controls. There was no significant difference between two groups (see Table 1 in the manuscript). We have also correlated these factors to every single target gene in our study. In the DLPFC, none of these factors was correlated to any of the target gene; In the ACC, none of the target gene was correlated with PMD. However several target genes (GABRA4, GABRB1, GLS, mGluR1 and PSD-93) correlated either with age or CSF-pH. Therefore we performed Univariate test with these genes and put age or CSF-pH as covariance (depending on which factor was correlated with target gene). As shown in the S-Table 3, the Univariate test of all these five target genes showed no significance between depression and control groups, just as same as Student's *t*-test. Concluding, age, CSF-pH and PMD did not influence our results.

S-Table 3 Comparison of significant level between different statistics

Gene	Student's <i>t</i> -test	Covariance	Univariate test
GABRA4	0.345	age	0.676
GABRB1	0.557	CSF-pH	0.517
GLS	0.749	CSF-pH	0.781
mGluR1	0.741	CSF-pH	0.734
PSD-93	0.444	CSF-pH	0.443