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Prefrontal changes in the glutamate-glutamine cycle and neuronal/glia glutamate transporters in depression with and without suicide



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ABSTRACT

There are indications for changes in glutamate metabolism in relation to depression or suicide. The glutamate-glutamine cycle and neuronal/glia glutamate transporters mediate the uptake of the glutamate and glutamine. The expression of various components of the glutamate-glutamine cycle and the neuronal/glia glutamate transporters was determined by qPCR in postmortem prefrontal cortex. The anterior cingulate cortex (ACC) and the dorsolateral prefrontal cortex (DLPFC) were selected from young MDD patients who had committed suicide (MDD-S; $n = 17$), from MDD patients who died of non-suicide related causes (MDD-NS; $n = 7$) and from matched control subjects ($n = 12$). We also compared elderly depressed patients who had not committed suicide ($n = 14$) with matched control subjects ($n = 22$). We found that neuronal located components (EAAT3, EAAT4, ASCT1, SNAT1, SNAT2) of the glutamate-glutamine cycle were increased in the ACC while the astroglia located components (EAAT1, EAAT2, GLUL) were decreased in the DLPFC of MDD-S patients. In contrast, most of the components in the cycle were increased in the DLPFC of MDD-NS patients. In conclusion, the glutamate-glutamine cycle - and thus glutamine transmission - is differentially affected in depressed suicide patients and depressed non-suicide patients in an area specific way.

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1. Introduction

Suicide is an important public health problem that has a strong association with psychopathology, in particular with mood disorders (Hawton and van Heeringen, 2009). A large body of evidence suggests that neurobiological factors play an important role in the predisposition to suicide (Ernst et al., 2009b; Mann and Currier, 2012). Although there is some overlap, this neurobiological predisposition appears to differ considerably from the predisposition to mood disorders per se, especially in the case of major depressive disorder (MDD) (Egeland and Sussex, 1985; Mann and Currier, 2010; Turecki, 2014).

The prefrontal cortex (PFC) is one of the major brain structures to modulate the stress response. It can be stimulatory or inhibitory,

depending on specific sub-regions or stressor types. The PFC has also been implicated in mood disorders and there is a negative correlation between the severity of post-stroke depression and the distance between the brain injury site and the frontal pole (Narushima et al., 2003). Furthermore, dysfunctioning anterior cingulate cortex (ACC) and dorsolateral prefrontal cortex (DLPFC) were shown by functional and structural imaging in MDD (Drevets, 2000; Drevets et al., 2008) as well as altered glucose metabolism and blood flow (Drevets, 1999; Mayberg, 2003; Seminowicz et al., 2004). Also postmortem data support PFC involvement in MDD: e.g. reduced glial cell density and neuronal size were observed in the ACC and DLPFC (Cotter et al., 2001, 2002; Rajkowska and Miguel-Hidalgo, 2007; Rajkowska et al., 1999).

Recent large-scale gene array studies in post-mortem subjects have provided strong support for alterations in GABAergic and glutamatergic neurotransmission in the PFC of depression (Bernard et al., 2011; Choudary et al., 2005; Duric et al., 2013). However, these postmortem studies consisted largely of psychiatric patients who had committed suicide, whereas none of the subjects in the control group had a psychiatric disease during their lifetime or

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committed suicide. Therefore it remains unclear whether these changes in the PFC were related to depression or to suicide per se.

In a recent study we found that only few GABA and glutamate-related genes were different in the ACC of elderly depressed patients who had not committed suicide (Zhao et al., 2012), while there were obvious changes in depressed patients who had committed suicide (Zhao et al., 2015). The present study therefore studied whether changes in the ACC and DLPFC occur in terms of the glutamate-glutamine cycle and glutamate transporters, in relation to depression with and without suicide.

Glutamate is synthesized from glutamine by glutaminase in neurons. After glutamate is released from the synaptic terminal, it is taken up by astrocytes through high affinity sodium-dependent neuronal glutamate transporters, such as the excitatory amino acid transporter (EAAT) 1 and EAAT2 (Bar-Peled et al., 1997; Chaudhry et al., 1995; Kugler and Schmitt, 2003; Milton et al., 1997), where it is converted back into glutamine by glutamine synthetase (Martinez-Hernandez et al., 1977). Glutamine is then transported back to the neurons by the neutral amino acid transporter (SNAT) 1 and SNAT2, ready for reuse (Melone et al., 2004). Interestingly, brain-derived neurotrophic factor (BDNF)-induced increases in SNAT1 expression are required for the regulation of dendritic length and neuronal complexity during development (Burkhalter et al., 2007), but BDNF is also clearly linked to depression and suicide (Dwivedi et al., 2003; Ernst et al., 2009b; Grah et al., 2014; Qi et al., 2013). This suggests that the glutamate-glutamine cycle is not only an important constituent of the glutamatergic neurotransmission system in physiology, but may also be a vulnerable pathway in psychiatric disorders due to its linkage with both BDNF and glutamate and GABA neurotransmission. Intrasympaptic glutamate can also be taken up by EAAT3 and EAAT4, which are located on neurons (Danbolt, 2001; Rothstein et al., 1994; Yamada et al., 1997). The EAAT family of glutamate transporters shows homology to the neutral amino acid transporters (ASCT) 1 and ASCT2 (Arriza et al., 1993; Utsunomiya-Tate et al., 1996). Studies showed that ASCT1 in glutamate transmission mediates the efflux of glutamate from the neuron into the synaptic junction via calcium-dependent release, and that ASCT2 activity plays an important role in the glutamine-glutamate cycle between neurons and glia by facilitating the efflux of glutamine from glial cells (Broer et al., 1999).

In the present study, we hypothesized that the glutamate-glutamine cycle is impaired in the brains of depressed individuals who committed suicide and that the neuronal and glial glutamate

transporters associated with this cycle are dysregulated. Thus, we used real-time quantitative PCR (qPCR) to compare the gene expression patterns in the PFC of the astrocytic glutamate-glutamine cycle and neuronal/glial glutamate transporters in depressed patients in relation to suicide.

2. Experimental procedures

2.1. Subjects from the Stanley Medical Research Institute (SMRI)

Brain material was obtained from the SMRI, after obtaining permission for a brain autopsy and for the use of the brain material and clinical data for research purposes. Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV diagnoses of MDD were made independently by two senior psychiatrists based on the medical records and, when necessary, telephone interviews with family members. This systematized procedure was carried out as described before (Torrey et al., 2000). The demographic information and medical data, including a life-long use of psychotropic medication and a history of drug abuse provided by SMRI, is shown in [Supplementary Table S1](#).

The SMRI frozen brain samples contained ACC (Brodmann area 24) and DLPFC (Brodmann area 46) and were obtained from MDD patients who died of suicide (MDD-S; $n = 17$), MDD patients who died from other causes than suicide (MDD-NS; $n = 7$), and matched control subjects without a history of suicidal behavior or any other psychiatric or neurological diagnosis ($n = 12$). These three groups were matched for race, age, gender, brain weight, postmortem delay (PMD), brain pH and hemispheric side (see [Table 1A](#)) as much as possible. All analyses were performed by investigators blind to the diagnosis.

2.2. Subjects from the Netherlands brain bank (NBB)

Frozen ACC (Brodmann area 24) and DLPFC (Brodmann area 9) brain material was obtained from the NBB and consisted of elderly depressed patients who died from non-suicidal causes MDD, $n = 5$; bipolar disorder (BD, $n = 10$) and matched controls who did not have a psychiatric or neurological disease ($n = 22$). The MDD versus BD subgroup and the combined depression group (DEP) versus the control group were well matched for age, gender, brain weight, clock time and month of death, PMD and brain pH. Demographic information and P -value of parameter matches are given in [Table 1B](#), further clinico-pathological information has been

Table 1A
Demographic information for the SMRI subjects.

	MDD-S	MDD-NS	Ctr	F or χ^2	p
Age (years, range)	40.41 (24–63)	46.43 (36–56)	46.83 (24–63)	1404	0.260
Gender (M/F)	10/7	3/4	8/4	1034	0.596
Race	16W, 1H	7W	11W, 1H	0.529	0.744
PMD (hours, range)	29.59 (13–65)	29.86 (15–52)	25.25 (9–40)	0.541	0.587
Brain pH	6.7 (6.36–6.88)	6.6 (6.3–6.9)	6.6 (6.31–6.91)	0.565	0.574
Brain weight (gram, range)	1480 (1170–1780)	1441 (1270–1590)	1444 (1200–1595)	0.295	0.747
Side of brain frozen (L/R)	10/7	5/2	6/6	0.838	0.658
Psychotic feature ^a	9	3	–	0.100	0.752
Alcohol hx	11	5	6	1029	0.598
Severity of alcohol abuse ^b	2.18 (0–5)	1.29 (0–5)	2.08 (0–5)	0.509	0.606
Drug hx	5	3	4	0.403	0.817
Severity of substance abuse ^b	0.88 (0–4)	1.43 (0–4)	0.75 (0–4)	0.471	0.629
Fluphenazine (lifetime, range) ^c	1041 (0–6500)	1314 (0–3000)	–	0.121	0.732

Abbreviation: Ctr, control; F, female; hx, history; L, left; M, male; MDD-S, major depressed patients who committed suicide; MDD-NS, major depressed patients who died of other cause than suicide; PMD, postmortem delay; R, right.

^a Psychotic Feature tested without controls.

^b Substance abuse and alcohol abuse was rated on a scale of 0–5.

^c Fluphenazine tested without controls.

Table 1B
Demographic information for the NBB subjects.

	ACC				DLPFC			
	Dep	Ctr	F or χ^2	p	Dep	Ctr	F or χ^2	p
Age (years, range)	74.67 (45–93)	79.50 (56–96)	0.964	0.338	72.86 (45–90)	79.50 (56–96)	0.025	0.680
Gender (M/F)	9/3	9/3	0.000	1.000	10/4	10/4	0.000	1.000
Race	12W	12W	0.000	1.000	14W	14W	0.000	1.000
PMD (hours, range)	7.4 (4.3–16.8)	7.1 (4.5–10.3)	0.149	0.773	7.3 (4.3–16.8)	8.2 (2.7–24.7)	1327	0.504
CTD (hh:mm, range) ^a	13:27 (2:30–23:00)	07:06 (2:00–21:30)	–	0.230	12:09 (2:30–23:15)	07:35 (0:01–17:45)	–	0.810
MTD (month, range) ^a	5 (1–10)	7 (2–12)	–	0.820	7 (1–11)	6 (3–11)	–	0.570
Brain pH (range)	6.43 (6.26–6.64)	6.58 (5.80–7.16)	5153	0.238	6.44 (6.26–6.82)	6.66 (5.80–7.39)	3309	0.113
Brain weight (gram, range)	1306.92 (1080–1488)	1350.58 (1125–1568)	0.015	0.469	1296.36 (1080–1490)	1319.00 (1054–1590)	0.195	0.685
ApoE (4x) ^b	4	2	1.245	0.980	3	4	0.560	1.000
Braak stage (range) ^b	1.5 (0–3)	1.5 (0–3)	0.400	1.000	1.5 (0–3)	1.1 (0–3)	2.700	0.690
Side of brain frozen (L/R)	12/0	12/0	0.000	1.000	12/2	14/0	2.154	0.142

Abbreviation ACC, anterior cingulate cortex; Ctr, control; CTD, clock time of death; Dep, depressed patients who died of cause other than suicide; DLPFC, dorsolateral prefrontal cortex; F, female; g, gram; hh, hour; L, left; M, male; mm, minute; MTD, month time of death; PMD, postmortem delay; R, right; W, white caucasian.

^a Mardia-Watson-Wheeler test.

^b Kolmogorov-Smirnov test.

described in a previous study (Zhao et al., 2012) and is provided in [Supplementary Table S2](#).

2.3. Quantitative real-time PCR

RNA isolation, cDNA synthesis and qPCR reactions were performed as described before (Wang et al., 2008; Zhao et al., 2012). The genes detected were Excitatory Amino Acid Transporter (glial high affinity glutamate transporter): EAAT1, 2; Excitatory Amino Acid Transporter (neuronal high affinity glutamate transporter): EAAT3, 4; Sodium-coupled neutral amino acid transporter: SNAT1, 2; Glutamine synthase: GLUL; Solute Carrier Family 1 (Glutamate/Neutral Amino Acid Transporter), member 4 (SLC1A4) and member 5 (SLC1A5): ASCT1 and ASCT2. Additional information on all tested genes and the sequences for each primer pair are shown in [Supplementary Table S3](#).

2.4. Normalization strategy

To remove sampling-related differences (RNA quantity), a normalization strategy was used (Vandesompele et al., 2002). The expression of target genes was normalized using the sets of stable reference genes mentioned below. The information of reference genes is as follows: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), actin-beta (ACT β), tubulin-alpha (TUB α), tubulin-beta 4 (TUB β 4), hydroxymethylbilane synthase (HMBS), ubiquitin C (UBC) for the SMRI-ACC/DLPFC study, GAPDH, ACT β , HPRT1 (hypoxanthine phosphoribosyltransferase 1), UBC (ubiquitin C), TUB α , TUB β 4, HMBS for the NBB-ACC/DLPFC study.

2.5. Statistical analysis

For the demographic data analyses (age, brain weight, PMD, brain pH) we used the Mann-Whitney test for 2 sample situations and the Kruskal-Wallis test with multiple comparisons for 3 sample situations. Gender and race differences were determined using the Chi-square test (For more details, see [Table 1A](#) and [B](#)). For the statistical analyses of gene expression data TIBCO S+ software (version 8.2.0, TIBCO, Seattle, WA, USA) was used. Before processing, the expression data from target and reference genes were ¹⁰log-transformed. The statistics used to describe data obtained from studies using precious human brain material (with limited availability and consequently small samples) may not conform to the approximate theoretical distributions (e.g. Student-t) usually assumed in hypothesis testing. The stability of *P*-values obtained

with parametric statistical tests may, therefore, be insufficient to provide reliable conclusions about the differences of gene expression in different psychiatric patient groups or about the correlations between genes within a patient group. Resample procedures (without replacement) may help to shed light on this problem. To keep the resample procedures simple and easy to interpret we resorted to two-group comparisons (like in *t*-tests) and correlations per patient group. We used 999 replicates of permuted gene expression data to generate permutation NULL distributions of the appropriate test statistics that correspond to NULL hypotheses implying that the mean differences between 2 groups or the mean correlation coefficients between 2 genes within a group are zero (Davison and Hinkley, 1997). For the difference between groups we used:

$$T = \frac{(\text{mean}(x_2) - \text{mean}(x_1))}{\text{sd} * (\text{df})^{-(1/2)}}$$

Here, sd is the pooled standard deviation and df denotes the Welch modified degrees-of-freedom. For normally distributed data with unknown, unequal variances, *T* is approximately distributed as a *t*-distribution with df degrees-of-freedom. The simulated *T*'s are called permutation *t*-values and the *T* corresponding to the observed difference of expression is called the observed *t*-value. The following statistic

$$\text{cor} = \frac{\text{sum}[(x_1 - \text{mean}(x_1)) * (x_2 - \text{mean}(x_2))]}{[\text{sum}(x_1 - \text{mean}(x_1)) * \text{sum}(x_2 - \text{mean}(x_2))]^{(1/2)}}$$

was calculated for correlation problems. For pairs of normally distributed data this statistic has an approximate *t*-distribution with *n*–2 degrees-of-freedom (*n* = number of observations). The observed test statistic and permutation test statistics are called observed and permutation *cor*-values, respectively. In each procedure the observed test statistic was combined with the permutation test statistics and ordered. Subsequently, the number of statistics equal to or smaller than and the number of statistics equal to or larger than the observed value were counted. These numbers were divided by 1000 (999 + 1) to yield the left (*p*-left) and right (*p*-right) probabilities. Two-sided permutation *P*-values were calculated as 2 times the minimum of *p*-left and *p*-right. All presented tests for the gene expression data are 2-sided and *p*-values were corrected for multiple testing using the Benjamini-Hochberg criterion (Benjamini and Hochberg, 1995) and were considered statistically significant if their value was less than 0.05.

3. Results

3.1. Changes in glutamate-glutamine cycle gene expression are related to suicide, not to depression

Brain material obtained from the SMRI was analyzed for changes in the genes involved in the glutamate-glutamine cycle and neuronal/glial glutamate transporters. We determined the RNA integrity value (RIN) to assess whether the quality of the human post-mortem tissue RNA isolated from the ACC or DLPFC did not differ between patient groups (Stan et al., 2006). The RIN did not show any significant differences between the diagnostic groups in the SMRI material (RIN value of the ACC from MDD-S patients: 7.69 ± 0.66 ; MDD-NS patients: 7.49 ± 0.56 and the control group: 7.73 ± 0.95 , mean \pm SEM; RIN value of the DLPFC from MDD-S: 7.69 ± 0.66 , MDD-NS patients: 7.49 ± 0.56 and the control group: 7.73 ± 0.95 , mean \pm SEM).

In ACC, the expression of neuronal glutamate transporters ASCT1, EAAT3 and EAAT4 were significantly increased in MDD-S compared with MDD-NS patients ($P = 0.024$, $P = 0.008$ and $P = 0.008$ respectively) or with the controls (ASCT1: $P = 0.015$, Fig. 1A; EAAT3: $P = 0.028$, Fig. 1C; EAAT4: $P = 0.008$, Fig. 1D). In addition, neuronal located glutamine transporter SNAT1 and SNAT2 transcription levels were elevated in MDD-S patients compared with MDD-NS patients ($P = 0.008$ and $P = 0.020$ respectively) or with control subjects ($P = 0.028$, Fig. 1E; and $P = 0.028$, Fig. 1F;

respectively). ASCT2 transcript level was also increased in the MDD-S patients compared to MDD-NS patients ($P = 0.008$, Fig. 1B). However, none of these genes showed difference between MDD-NS and control subjects. In contrast, we found that ASCT1 and glial related genes (EAAT1, EAAT2 and GLUL) were decreased in the MDD-S compared with MDD-NS patients in DLPFC ($P = 0.042$, Fig. 1G; $P = 0.038$, Fig. 1H; $P = 0.038$, Fig. 1I; $P = 0.038$, Fig. 1K; respectively). Furthermore, the transcript levels of these genes, and of EAAT3 ($P = 0.038$, Fig. 1J) SNAT1 ($P = 0.038$, Fig. 1L) and SNAT2 ($P = 0.041$, Fig. 1M) were increased in the MDD-NS patients compared with control subjects (Table 2).

To explore what aspects of suicide may cause gene transcription alterations in the MDD patients, the SMRI MDD-S group was divided into two subgroups: violent suicide method (hanging, jumping, shooting and stabbing, $n = 12$) and non-violent method (overdose, $n = 5$). However, we did not find any significant difference in the expression of the genes studied in either ACC ($P > 0.58$) or DLPFC ($P > 0.28$).

3.2. Changes in glutamate-glutamine cycle gene expression are not related to suicide ideation

We then examined ACC and DLPFC samples from the NBB (RIN value of the ACC from the elderly depressed patients: 7.29 ± 0.55 and the control group: 7.24 ± 1.15 , mean \pm SEM; RIN value of the DLPFC from the elderly depressed patients: 7.51 ± 0.66 and the

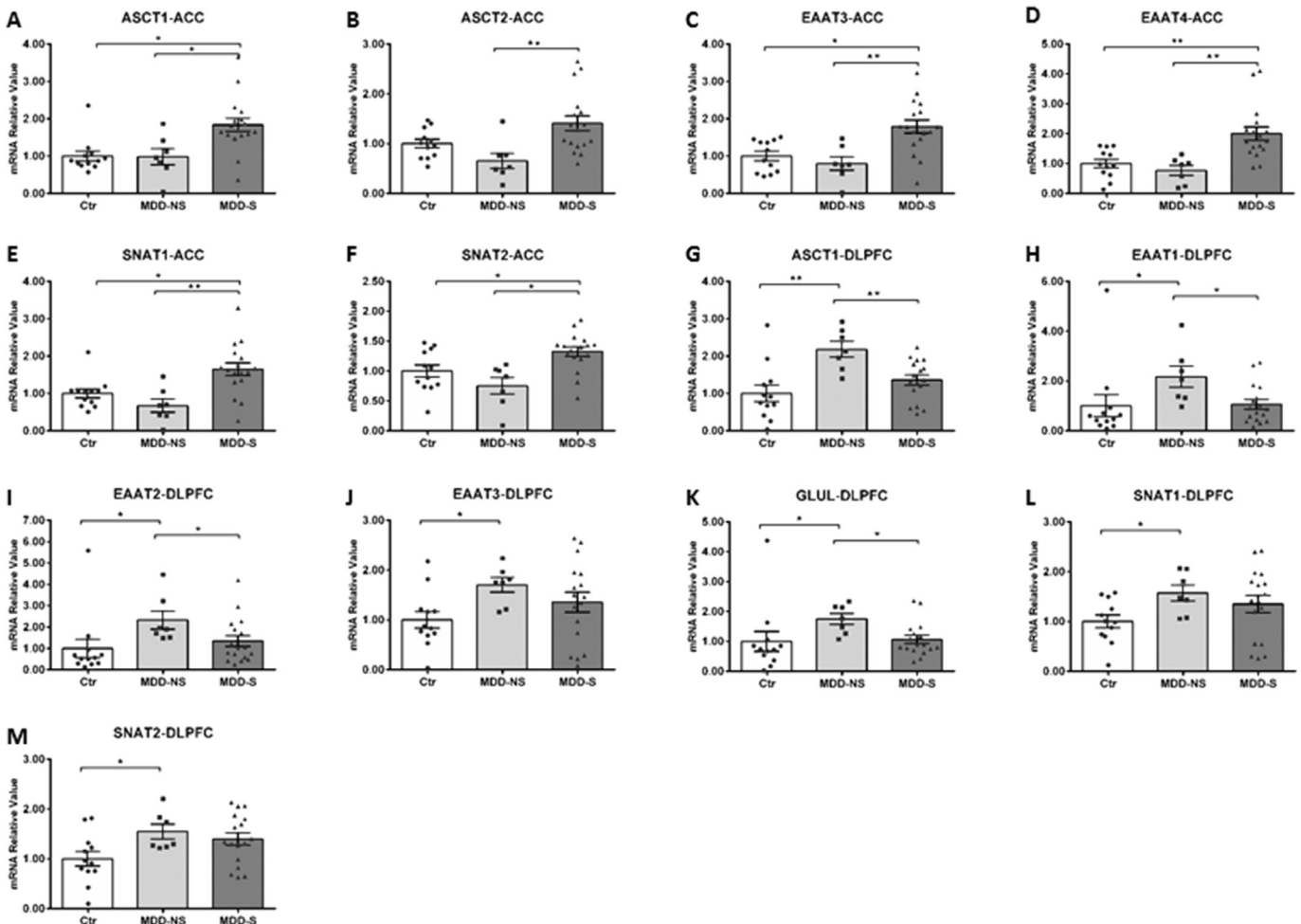


Fig. 1. Significant expression of genes in ACC and DLPFC of the SMRI patients. Abbreviations: ACC, anterior cingulate cortex; Ctr, control; DLPFC, dorsolateral prefrontal cortex; MDD-S, major depressed patients who committed suicide; MDD-NS, major depressed patients who died from causes other than suicide. For abbreviations of genes see SI Table 3.

Table 2
SMRI and NBB results in expression of target gene in the ACC and DLPFC.

	SMRI cohort									NBB cohort		
	Fold changes			p.perm			Bhadj.p			Fold changes	p.perm	Bhadj.p
	MDD-S/MDD-NS	MDD-S/Ctr	MDD-NS/Ctr	MDD-S/MDD-NS	MDD-S/Ctr	MDD-NS/Ctr	MDD-S/MDD-NS	MDD-S/Ctr	MDD-NS/Ctr			
ACC												
EAAT1	1.3	1.9	1.5	0.488	0.058	0.420	0.537	0.101	0.478	0.9	0.612	0.918
EAAT2	1.5	1.9	1.3	0.332	0.072	0.614	0.406	0.113	0.614	1.1	0.774	0.934
EAAT3	3.0	1.8	0.6	0.002**	0.012*	0.292	0.008**	0.028*	0.371	1.0	0.182	0.918
EAAT4	3.0	2.2	0.8	0.002**	0.002**	0.392	0.008**	0.008**	0.462	1.1	0.228	0.918
GLUL	1.6	1.3	0.8	0.088	0.228	0.602	0.132	0.314	0.614	1.1	0.592	0.918
SNAT1	3.3	1.6	0.5	0.002**	0.010**	0.068	0.008**	0.028*	0.112	1.0	0.578	0.918
SNAT2	2.1	1.4	0.7	0.006**	0.012*	0.200	0.020*	0.028*	0.287	0.8	0.318	0.918
ASCT1	2.4	1.8	0.7	0.008**	0.004**	0.552	0.024*	0.015*	0.588	1.0	0.830	0.934
ASCT2	2.3	1.3	0.6	0.002**	0.040*	0.034	0.008**	0.073	0.066	1.0	0.986	0.986
DLPFC												
EAAT1	0.4	1.5	3.5	0.014*	0.304	0.006**	0.039*	0.372	0.039*	1.7	0.710	0.948
EAAT2	0.5	1.7	3.7	0.014*	0.130	0.004**	0.039*	0.195	0.039*	1.6	0.948	0.948
EAAT3	0.6	1.3	2.2	0.154	0.522	0.008**	0.212	0.538	0.039*	1.3	0.764	0.948
EAAT4	0.8	1.3	1.7	0.140	0.126	0.028*	0.201	0.195	0.058	1.6	0.840	0.948
GLUL	0.6	1.6	2.9	0.014*	0.232	0.008**	0.039*	0.306	0.039*	1.3	0.794	0.948
SNAT1	0.7	1.3	1.8	0.260	0.392	0.014*	0.330	0.446	0.039*	1.5	0.734	0.948
SNAT2	0.8	1.6	1.8	0.352	0.058	0.016*	0.415	0.106	0.041*	1.0	0.028	0.252
ASCT1	0.6	1.8	3.1	0.018*	0.080	0.004**	0.042*	0.132	0.039*	1.1	0.940	0.948
ASCT2	1.0	1.6	1.5	0.760	0.012*	0.066	0.760	0.039*	0.115	1.1	0.706	0.948

The differences among MDD-S, MDD-NS and Ctr in the SMRI, Dep-NS and Ctr in the NBB were tested with permutation tests respectively. The permutation *P*-values were corrected for multiple testing using the false discovery rate. *P* < 0.05 were considered significant and marked as bold italic style, * indicates *P* < 0.05, ** indicates *P* < 0.001. Fold changes were calculated using the mean gene expression values.

Abbreviations: ACC, anterior cingulate cortex; Bhadj.p, Benjamini-Hochberg adjusted *p*-values; Ctr, control; Dep-NS, depressed patients who died of non-suicidal cause; DLPFC, dorsolateral prefrontal cortex; MDD-S, major depressive disorder who committed suicide; MDD-NS, major depressive disorder who died of non-suicidal cause; NBB, Netherlands Brain Bank; p.perm, *p*-values obtained using permutation tests; SMRI, Stanley Medical Research Institute. For abbreviations of genes see [SI Table 3](#).

control group: 7.66 ± 0.88 , mean \pm SEM).

None of the depressed patients from the NBB had committed suicide. Consistent with our results from the SMRI subjects, we did not observe significant changes in the ACC of depressed patients and controls. Based on their clinical records, we divided the DEP group into subgroups of patients that either did or did not have a record of suicidal thoughts and ideation. To assess whether suicidal ideation contributes to gene transcription in this area, the same set of markers related to glutamate-glutamine cycle and neuronal glutamate transporters were tested. We did not find any significant differences in the ACC (*P* > 0.95) nor in the DLPFC (*P* > 0.16) with respect to suicide ideation, either for neuronal located genes (ACC: *P* > 0.95; DLPFC: *P* > 0.45) or astrocytic genes (ACC: *P* > 0.95; DLPFC: *P* > 0.16). These findings suggest that suicide itself, but not mere suicide ideation, may cause changes in the expression of genes involved in the glutamate-glutamine cycle.

3.3. Correlation with BDNF/TrkB

As both the SMRI and NBB patient cohorts of the present study had also been investigated in our previous studies on the expression of BDNF/TrkB-related genes (Qj et al., 2013; Zhao et al., 2015), we investigated the putative relationship between truncated receptor of BDNF (TrkB.T1) and BDNF with the present target genes here. There appeared to be a positive correlation between the TrkB.T1 and astrocytic genes (EAAT1, EAAT2, GLUL), and also neuronal located genes (EAAT3, SNAT1, SNAT2, ASCT1) in the MDD-S individuals (For more details of *P*-values, see [Table 3](#)). TrkB.T1 only correlated with astrocytic genes in the MDD-NS group, while there was no such correlation in the control group in the ACC. We found that TrkB.T1 was correlated with both astrocytic genes (EAAT2, GLUL) and neuronal located genes (EAAT3, SNAT1, SNAT2, ASCT1) in the MDD-S patients, but not in the MDD-NS nor control

Table 3
Correlation between BDNF, TrkB.T1 and target genes in the SMRI cohort.

	ACC						DLPFC					
	BDNF			TrkB.T1			BDNF			TrkB.T1		
	MDD-S	MDD-NS	Ctr	MDD-S	MDD-NS	Ctr	MDD-S	MDD-NS	Ctr	MDD-S	MDD-NS	Ctr
EAAT1	0.513	0.079	0.330	0.046*	0.036*	0.819	0.399	0.784	0.374	0.062	0.511	0.420
EAAT2	0.546	0.111	0.339	0.036*	0.036*	0.406	0.507	0.990	0.374	0.048*	0.378	0.374
EAAT3	0.336	0.036*	0.330	0.046*	0.079	0.984	0.602	0.784	0.948	0.048*	0.805	0.748
EAAT4	0.148	0.224	0.288	0.336	0.111	0.900	0.782	0.990	0.748	0.399	0.978	0.748
GLUL	0.866	0.175	0.330	0.018*	0.036*	0.454	0.399	0.511	0.374	0.036*	0.624	0.468
SNAT1	0.404	0.036*	0.406	0.018*	0.111	0.392	0.915	0.990	0.910	0.036*	0.805	0.748
SNAT2	0.513	0.072	0.441	0.018*	0.203	0.643	0.944	0.511	0.948	0.036*	0.990	0.748
ASCT1	0.404	0.072	0.330	0.018*	0.079	0.406	0.862	0.378	0.748	0.036*	0.784	0.948
ASCT2	0.120	0.203	0.586	0.095	0.121	0.330	0.122	0.978	0.374	0.399	0.990	0.948

Permutation *P*-values of the correlations of BDNF and TrkB with the target genes. The *P*-values were corrected for multiple testing. *P* < 0.05 were considered significant and marked as bold italic style, * indicates *P* < 0.05.

Abbreviations: ACC, anterior cingulate cortex; Ctr, control; DLPFC, dorsolateral prefrontal cortex; MDD-S, major depressive disorder who committed suicide; MDD-NS, major depressive disorder who died of non-suicidal cause; SMRI, Stanley Medical Research Institute. For abbreviations of genes see [SI Table 3](#).

subjects in the DLPFC (For more details of *P*-values, see Table 3). BDNF is correlated with EAAT3 and SNAT1 in the ACC of MDD-NS patient group, while there was no correlation in either MDD-S or control subjects. In addition, we did not find any correlation in the DLPFC (For more details of *P*-values, see Table 3).

3.4. Possible confounding factors

The possible confounders in our study, such as age, gender, brain pH, brain weight, PMD, hemisphere side, ethnicity, history of substance abuse, severity of substance abuse and psychotic feature, did not differ between the groups (Table 1A and B), implying that they will not have affected our conclusions. Concerning the possible differences between MDD and BD in the NBB cohort, calculation for all the target genes proved that there was no significance difference between these two subgroups (ACC: $P > 0.30$; DLPFC: $P > 0.05$). Furthermore, we have shown earlier that there is no significant difference between MDD and BD patients on the GABA/glutamate related genes in the PFC in our previous study (Zhao et al., 2012). Thus, there is no indication that pooled MDD-BD in the NBB cohort would affect our conclusion. Since psychosis was present in some of the subjects in the SMRI patient cohort, all differentially expressed genes were compared for the presence or absence of psychosis. None of the comparisons were found to be significant in ACC ($P > 0.94$). In the DLPFC, there is a significantly lower EAAT3 expression in non-psychotic MDD patients ($P = 0.036$), the expression of the other genes is not different between psychotic and non-psychotic patients ($P > 0.11$). A potentially contributing confounding factor in all postmortem studies is medication. However, this does not seem to be a confounder for our data, as medication (SSRI, SARI, TCA) did not affect any of the gene expression in ACC ($P > 0.48$) or in DLPFC ($P > 0.51$). SNRI could not be tested because one group only had 2 observations.

4. Discussion

In the present study we found in the SMRI cohort that ASCT2 and neuronal located molecule transcript levels were significantly higher in the ACC of MDD-S patients than in the MDD-NS patients or the control subjects. In contrast, decreased ASCT1 and astroglia located molecule transcript level were found in the DLPFC of MDD-S patients compared to MDD-NS patients. Furthermore, most of the target genes were increased when comparing MDD-NS with control subjects in the same brain area. We did not find any difference in expression level in the NBB cohort. Our results suggest that the changes in ACC synaptic glutamate cycle are rather related to suicide than to depression per se, and that they are neuronal located. For DLPFC, suicide related changes are more astroglia located and the depression related changes are from both neuronal and astroglia. In both brain areas, the molecular changes in suicide and depression seem to go into an opposite direction.

Glutamine serves as a substrate in both glutamatergic and GABAergic neurons (Gao and Bao, 2011). The uptake of glutamine into neurons is facilitated by system A amino-acid transporters (Mackenzie and Erickson, 2004; Ogura et al., 2006) and these transporters can thus alter both GABA and glutamate functioning. SNAT1 (also termed SLC38A1) is a system A transporter localized in neurons. It shows a high affinity for glutamine released by astroglia (Broer, 2014; Schioth et al., 2013) and is present at the start of the glutamate-glutamine cycle in neurons. Although the 11 human transmembrane domains of SNAT1 show a high homology with the murine ortholog of this protein (Wang et al., 2000), their functional and kinetic properties in humans are still poorly understood (Pochini et al., 2014). Like SNAT1, SNAT2 (also termed SLC38A2) is also expressed in neurons, and acts as a system A transporter

(Sundberg et al., 2008; Varoqui et al., 2000; Yao et al., 2000). One of the differences between these two transporters is that SNAT1 prefers glutamine (Varoqui et al., 2000), while SNAT2 carries several neutral amino acids, including glutamine and alanine, with alanine transport being approximately four times as efficient as glutamine transporter (Yao et al., 2000). Alanine can be converted into glutamate later. Glutamine is a precursor of glutamate, which is further converted into GABA by glutamate decarboxylase (GAD), which is present in GABAergic neurons. The increased SNAT1 and SNAT2 in our study may thus cause more glutamine influx into neurons and over-activate GABA synthesis. This is in line with our previous study, which showed increased GAD levels in the ACC of MDD-S patients (J. Zhao et al., in preparation). Furthermore, animal experiments have shown that SNAT1 is significantly up-regulated by diazepam (a GABA-A receptor agonist) treatment (Sundberg et al., 2008), and the transcript levels of both GABA-A and GABA-B receptor transcript were up-regulated in the MDD-S patients (J. Zhao et al., in preparation), suggesting that increases in SNAT1 might be involved in the dysfunction of GABA receptors in the ACC of MDD patients who committed suicide. Five depressed patients in the NBB cohort were treated with benzodiazepine (BDZ) during the last 3 months of their life, but we did not find significant differences in SNAT1 mRNA between the BDZ treated and non-BDZ treated depressed patients. No such comparison could be performed in the SMRI cohort, as there was insufficient information on their BDZ use. Another animal study showed that SNAT1 and/or SNAT2 are altered in a post-traumatic epilepsy model. Moreover, exogenous glutamine enhanced the abnormal evoked and spontaneous activity characteristic of the injured neocortex. This effect could be blocked by inhibition of SNAT1 and SNAT2 in the post-traumatic epilepsy model (Tani et al., 2007), suggesting that the increased SNAT1 and SNAT2 in the MDD-S patients (ACC) and in the MDD-NS patients (DLPFC) are promising novel pharmaceutical target for suicide and depression treatment.

The main candidates to mediate glutamate efflux from neurons are EAAT3, EAAT4 and ASCT1 that are significantly increased in the ACC of MDD-S individuals. In contrast, we found that ASCT1 mRNA expression is decreased in the DLPFC, while also the expression level of EAAT3 and EAAT4 were decreased although they did not reach significance. This shows that impaired glutamate transport in suicide shows opposite changes in ACC and DLPFC. Furthermore, when comparing MDD-NS to control subjects, most of the target genes are increased, which strongly indicates that suicide and depression also show opposite changing patterns in the DLPFC. This pattern was supported by our observations in ACC. We found that all the suicide related changes were upregulated, while the depression related changes were decreased according to the fold changes, although they did not reach significance. Although the mechanism by which EAAT3, EAAT4 and ASCT1 expression is altered in the different brain areas is unknown, as functional consequence, extracellular and synaptic glutamate levels could be elevated in the ACC and decreased in the DLPFC in MDD-S patients. In clinical investigations, NMDA-antagonists, such as ketamine, turned out to be potent and rapid acting antidepressant agents and also to be effective in suppressing suicide ideations (Ballard et al., 2014; Chen and Lipton, 2006; Zarate et al., 2006) while they also modify glutamate signaling via AMPA-receptors (Maeng and Zarate, 2007), a possibility supported by our previous study (Zhao et al., in preparation).

We did not find any similar changes in the NBB cohort, which is in line with our finding from SMRI cohort that the changes in ACC synaptic glutamate cycle are rather related to suicide than to depression per se in ACC. For DLPFC, it seems there is a discrepancy between SMRI and NBB results. Since the age difference between SMRI cohort (average: 43.72 years old) and NBB cohort (average:

75.42 years old), also the PMD difference (SMRI: 28.19 h and NBB: 7.55 h), we performed further calculation and found that all adjusted permutation *P*-values of the correlation between target gene expression and age are > 0.05 , and >0.98 for PMD in DLPFC of SMRI cohort. This suggests that age and PMD did not affect our result and did not contribute to the discrepancy. It has also to be noted that the DLPFC in the SMRI and NBB cohorts represented different Brodmann areas (i.e. BA 46 or BA 9 respectively). This may explain why DLPFC-SMRI showed numbers of changes while DLPFC-NBB did not show any significant change.

The BDNF/TrkB system has been linked to depression and suicide (Dwivedi et al., 2003; Ernst et al., 2009b; Qi et al., 2013). BDNF regulates dendritic length and neuronal complexity during development, which requires BDNF-induced increases in SNAT1 expression (Burkhalter et al., 2007). Interestingly, in a previous genetic study an association was observed between altered expression of SNAT1 and suicidal behavior (Ernst et al., 2009a). In our current study we found that BDNF is positively correlated with SNAT1 and EAAT3, and the truncated receptor of BDNF (TrkB.T1) was positive correlated with EAAT1, EAAT2 and GLUL in the ACC of MDD-S and MDD-NS group respectively. No such correlation was found in the control group of the SMRI cohort. Since the ACC suicide related changes are more neuronal located (see before), they may be closely linked to BDNF. In the DLPFC only TrkB.T1 was correlated with most of the target genes in the MDD-S patient group, but not with BDNF. Furthermore, there was no correlation observed in either MDD-NS group or control group. This, and gene expression comparisons (see above) suggests that the suicide changes in the DLPFC are both, neuronal and astroglia related.

In conclusion, we observed that the neuronal located components of the glutamate-glutamine cycle were increased in the ACC while the astroglia located components were decreased in the DLPFC of MDD-S patients. In contrast, most of the components were increased in the DLPFC of MDD-NS patients. These data suggest that glutamine transmission in the glutamate-glutamine cycle is affected differentially in depressed suicide patients and depressed non-suicide patients in an area specific way.

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Contributors

J.Zhao, P.J.Lucassen and D.F.Swaab designed the experiments and prepared the manuscript. J.Zhao, D.J. van Wamelena, X-R Qi and S-F Gao performed the experiments. J.Zhao collected data and together with R. W.H.Verwer did Statistical analysis. D.F.Swaab obtained funding.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jpsychires.2016.06.017>.

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