



UvA-DARE (Digital Academic Repository)

Prefrontal gene expression changes in mood disorders and suicide

Zhao, J.

Publication date

2018

Document Version

Other version

License

Other

[Link to publication](#)

Citation for published version (APA):

Zhao, J. (2018). *Prefrontal gene expression changes in mood disorders and suicide*. [Thesis, externally prepared, Universiteit van Amsterdam].

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, P.O. Box 19185, 1000 GD Amsterdam, The Netherlands. You will be contacted as soon as possible.



CHAPTER 5

DIFFERENT STRESS-RELATED GENE EXPRESSION IN DEPRESSION AND SUICIDE

J. Zhao, X-R. Qi, S-F. Gao, J. Lu, D.J. van Wamelen, W. Kamphuis, A-M. Bao and
D.F. Swaab

J Psychiatr Res. 2015;68:176-85

ABSTRACT

Background: Suicide occurs in some, but not all depressed patients. So far, it remains unknown whether the studied stress-related candidate genes change in depression, suicide or both. The prefrontal cortex (PFC) is involved in, among other things, impulse control and inhibitory behaviour and plays an important role in both suicide and depression.

Materials and Methods: We have employed Q-PCR to study 124 anterior cingulate cortex (ACC) and dorsolateral PFC (DLPFC) brain samples, obtained from two brain banks, from: i) young depressed patients (average age 43 years) who committed suicide (MDD-S) and depressed patients who died from causes other than suicide (MDD-NS) and from ii) elderly depressed patients (average age 75 years) who did not commit suicide (DEP). Both cohorts were individually matched with non-psychiatric non-suicide control subjects. We determined the transcript levels of hypothalamic–pituitary–adrenal axis-regulating molecules (corticotropin-releasing hormone (CRH), CRH receptors, CRH binding protein, mineralocorticoid receptor/glucocorticoid receptor), transcription factors that regulate CRH expression, CRH-stimulating cytokines, chaperone proteins, retinoid signaling, brain-derived neurotrophic factor and tropomyosin-related kinase B, cytochrome proteins, nitric oxide synthase (NOS) and monoamines.

Results: In the MDD-S group, expression levels of CRH and neuronal NOS-interacting DHHC domain-containing protein with dendritic mRNA (NIDD) were increased. Other changes were only present in the DEP group, i.e. decreased NIDD, and increased and 5-hydroxytryptamine receptor 1A (5-HT1A) expression levels. Changes were found to be more pronounced in the ACC than in the DLPFC.

Conclusions: Depressed patients who committed suicide have different stress-related gene expression patterns than depressed patients who died of causes other than suicide.

INTRODUCTION

Both depression and suicide are characterized by alterations in the stress response (Bao et al., 2008; Pandey, 2013) and by complex patterns of alterations in various brain regions and neurotransmitter systems (Gross-Isseroff et al., 1998). Different lines of investigation indicate the involvement of the prefrontal cortex (PFC) in depression, a brain region involved in, among other things, behavioural inhibition and impulse control. There is also 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). Imaging studies have shown altered gray matter volume and thickness, and glucose metabolism and blood flow in the dorsolateral PFC (DLPFC) and anterior cingulate cortex (ACC) of depressed patients (Drevets et al., 2008). Furthermore, morphological studies have shown reduced neuronal and glia cell densities in both the DLPFC and ACC in depression (Cotter et al., 2001; Rajkowska et al., 1999). These alterations in the PFC may contribute to a number of signs and symptoms of depression, such as cognitive decline, negative self-evaluation, and suicidal tendencies (Elliott et al., 2011). Concerning neurochemical alterations, monoaminergic receptors and their transporters (serotonin, norepinephrine and dopamine) (Arranz et al., 1994; Mann, 2003; Shelton et al., 2009) and corticotropin-releasing hormone (CRH) in the hypothalamus: they have been frequently studied in relation to depression and suicide (Jokinen and Nordstrom, 2009; Wang et al., 2008), both of which are closely related to (regulation of) the stress response (Bao et al., 2008). In addition, CRH neurons are also located in most regions of the PFC (Swanson et al., 1983) that are part of the circuitry involved in modulating corticotropine, adrenocorticotrop hormone or cortisol-mediated responsivity to stress (Diorio et al., 1993). Also other neurotransmitters, such as the nitric oxide (NO) system, have drawn a lot of interest due to their changes in depression and suicide (Gao et al., 2013; Xing et al., 2002). Retinoid signaling, brain-derived neurotropic factor (BDNF) and the gene that encodes its high-affinity receptor, tropomyosin kinase B (TrkB), have received considerable attention in human studies of suicidal behavior. Some of these studies have revealed that people who commit suicide have decreased mRNA and/or protein levels of BDNF, TrkB, or both, in the PFC (Ernst et al., 2009; Maussion et al., 2014; Pandey et al., 2008). Their decreased expression correlated with hypermethylation of the BDNF promoters in exons 4 and 9 (Roth et al., 2009).

However, there is still no agreement on a particular molecular "signature", which allows for distinguishing expression changes in depression *per se* from depression with suicide. One problem that affects the interpretation of post-mortem studies on this topic is the occurrence of suicide in many of the depression cohorts reported in literature. For instance, postmortem studies that claimed to have determined molecular alterations in relation to *depression* had in fact selected, in the diagnosis group, depressed patients who (nearly) all committed suicide, and compared them with control subjects without any psychiatric disorder and who did not commit suicide (Bernard et al., 2011; Cotter et al., 2001; Dwivedi et al., 2006b; Martins-de-Souza et al., 2012; Rajkowska et al., 1999; Shelton et al., 2009). Vice versa, the studies focused on *suicide* appeared to compare suicide cases to matched controls without any psychiatric disorder (Dwivedi et al., 2006a; Dwivedi et al., 2006b; Poulter et al., 2008; Thalmeier et al., 2008). Such methodological aspects have so far made it impossible to distinguish neurochemical alterations due to depression *per se* from those due to suicide.

We therefore investigated whether a series of stress-related candidate molecules differ in their expression in depressed patients in relation to suicide. We studied a Stanley Medical Research Institute (SMRI) patient cohort consisting of three groups: major depressive disorder patients who committed suicide (MDD-S), MDD patients who died of non-suicidal causes (MDD-NS), and control subjects without a psychiatric disorder, to answer the question whether suicide is responsible for the altered gene expression. In addition, elderly depressed patients who did not commit suicide (DEP) and their matched controls from the Netherlands Brain Bank (NBB) were studied to see what the alterations related to depression were, confirming the findings about young MDD-NS from SMRI in elderly depressed non-suicide patients.

EXPERIMENTAL PROCEDURES

Subjects from the Stanley Medical Research Institute (SMRI)

All patients whose brain material was obtained from SMRI had given permission for a brain autopsy and for the use of their brain material and clinical data for research purposes. MDD diagnoses were based on the Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV and were made independently by two senior psychiatrists based on 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 S-Table 1.

The SMRI frozen brain samples contained ACC (Brodmann area 24) and DLPFC (Brodmann area 46) of MDD patients who died of suicide (MDD-S, N = 17); MDD patients who died of non-suicidal causes (MDD-NS, N = 7); and matched control subjects without a history of suicidal behaviour, or any psychiatric or neurological diagnosis (N = 12). These 3 groups were matched for age, sex, race, brain weight, postmortem delay (PMD), brain pH and hemispheric side (see Table 1A). All analyses were performed by investigators blind to the source of the tissues and diagnosis.

Table 1A Demographic information for the SMRI subjects

	MDD-S	MDD-NS	Ctrl	F or χ^2	P
Age (years, range)	40 (24-63)	46 (36-56)	47 (24-63)	1.404	0.260
Gender (M/F)	10/7	3/4	8/4	1.034	0.596
Race	16W, 1H	7W	11W, 1H	0.529	0.744
PMD (hours, range)	29.6 (13-65)	29.9 (15-52)	25.3 (9-40)	0.541	0.587
Brain pH	6.67 (6.36-6.88)	6.60 (6.30-6.90)	6.64 (6.31-6.91)	0.565	0.574
Brain Weight (gram, range)	1480 (1170-1780)	1441 (1270-1590)	1444.83 (1200-1595)	0.295	0.747
Side of Brain Frozen (L/R)	10/7	5/2	6/6	0.838	0.658
Psychotic Feature ¹	9	3	-	0.100	0.752
Alcohol hx	11	5	6	1.029	0.598
Severity of Alcohol abuse ²	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 ²	0.88 (0-4)	1.43 (0-4)	0.75 (0-4)	0.471	0.629
Fluphenazine (lifetime) ³	1041.18 (0-6500)	1314.29 (0-3000)	-	0.121	0.732

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

¹ Psychotic Feature tested without controls

² Substance abuse and alcohol abuse were rated on a scale of 0-5

³ Fluphenazine tested without controls

Subjects from the Netherlands Brain Bank (NBB)

Frozen ACC (Brodmann area 24) and DLPFC (Brodmann area 9) brain material was obtained from the NBB from elderly depressed patients who died of 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 DEP group versus the control group were well-matched for age, sex, brain weight, clock time, month of death, PMD and brain pH. Demographic information and P-value of parameter matches are given in Table 2B, further clinico-pathological information has been described in our previous study (Zhao et al., 2012) and is provided in S-Table 2.

Quantitative Real-time PCR (Q-PCR)

RNA isolation, cDNA synthesis and QPCR reactions were performed as described before (Wang et al., 2008; Zhao et al., 2012). RNA integrity number (RIN), an indicator of human postmortem tissue RNA quality, did not show any significant difference between diagnostic groups in either the SMRI material or in the NBB material. The genes detected were as follows, based upon previous studies by our own group (Gao et al., 2013; Qi et al., 2013, 2015) hypothalamic-pituitary-adrenal (HPA) axis regulation related genes, i.e. corticotropin-releasing hormone (CRH), CRH receptor 1/2, CRH binding protein (CRHBP), mineralocorticoid receptor/glucocorticoid receptor (MR/GR), vasopressin receptor-1 α (AVP1 α), urocotin 3 (UCN3); transcription factor that regulates CRH expression: estrogen receptor α/β (ER α/β), androgen receptor (AR), cAMP-response element-binding protein (CREB); CRH-stimulating cytokines: interleukin-1 β (IL1 β) and tumor necrosis factor- α (TNF α); chaperone proteins: heat shock protein 70 (HSP 70) and HSP 90. Also, monoamine related genes: monoamine oxidase A (MAOA) and MAOB, serotonin receptor 1A and 2A (5-HT1A and 5-HT2A), catechol-O-methyltransferase (COMT), adrenoreceptor alpha 1a/2a, beta 1 (ADRA1A, ADRA2A, ADRB1), dopamine receptor D1 and 2 (DRD1, DRD2); NOS related genes: NOS1, NOS2, NOS3, NOS1-interacting DHHC domain-containing protein with dendritic mRNA (NIDD); retinoic acid (RA) synthesizing enzymes: retinaldehyde dehydrogenase 1,2,3 (RALDH 1,2,3); RA metabolic enzymes/cytochrome P450 protein: cytochrome P450, family 26, A1, B1, C1 (CYP26A1, B1, C1); RA cellular transport proteins: cellular RA binding protein 1, 2 (CRAPB1, 2); receptors mediating RA function: RAR α , β , γ and retinoid X receptor α , β , γ (RXR α , β , γ); BDNF; TrkB Total, targeting all TrkB isoforms; TrkB full-length isoform (TrkB.FL); and TrkB-truncated isoform 1 (TrkB.T1).

Table 1B Demographic information for the NBB subjects

	ACC				DLPFC			
	DEP	Ctrl	F or χ^2	P	DEP	Ctrl	F or χ^2	P
Age (years, range)	75 (45-93)	80 (56-96)	0.964	0.338	73 (45-90)	80 (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)	1.327	0.504
CTD (hh:mm, range) ¹	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) ¹	5 (1-10)	7 (2-12)	-	0.820	7 (1-11)	6 (3-11)	-	0.570
Brain pH	6.43 (6.26-6.64)	6.58 (5.80-7.16)	5.153	0.238	6.44 (6.26-6.82)	6.66 (5.80-7.39)	3.309	0.113
Brain Weight (gram, range)	1307 (1080-1488)	1351 (1125-1568)	0.015	0.469	1296 (1080-1490)	1319 (1054-1590)	0.195	0.685
ApoE (4x) ²	4	2	1,245	0.980	3	4	0,560	1,000
Braak Stage (stage, range) ²	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; Ctrl, control; CTD, clock time of death; DEP, depressed patients who did not die of 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.

¹Mardia-Watson-Wheeler test

²Kolmogorov-Smirnov test

Additional information on all tested genes and the sequences for each primer pair used is shown in S-Table 3. Analysis of the qPCR data was carried out as described before (Wang et al., 2008; Zhao et al., 2012). In short, after the geNorm analysis, the geomean of the absolute amount of the following genes was calculated to determine a reliable normalization factor that did not differ between the diagnostic groups and their controls, to control for unspecific and non-disease-related changes (Vandesompele et al., 2002): Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), actin-beta (ACT β), tubulin-alpha (TUB α), tubulin-beta 4 (TUB β 4) for the SMRI-ACC/DLPFC study, GAPDH, ACT β , HPRT1 (hypoxanthine phosphoribosyltransferase 1), UBC (ubiquitin C), TUB α , TUB β 4 for the NBB-ACC study and GAPDH, ACT β , HMBS, HPRT1, TUB α , TUB β 4 for the NBB-DLPFC study. The absolute amount of transcript obtained from the target genes was divided by the normalization factor to get the normalized relative value for the final statistical analysis.

Statistical Analysis

Statistical analysis was conducted with SPSS (version 16.0, SPSS Incorporation, Chicago, IL). The Kolmogorov-Smirnov test was used to check whether the data were normally distributed. Gene expression values appeared to be normally distributed in most cases, otherwise log-transformation or subsequently square root transformation or reciprocal transformation were performed. For three-group comparisons, data were analyzed for multiple comparisons using one-way analyses of variance (ANOVAs) for repeated measures where appropriate, followed by *post-hoc* Least Significant Difference test. The two-group comparisons were performed with the unpaired Student's *t*-test. Benjamini and Hochberg's correction was followed for multiple testing. To rule out possible confounders, the analysis of covariance (ANCOVA) was conducted if there was correlation between confounders and gene expression. The ANCOVA model, used for the expression levels of each gene, revealed the diagnostic group as a main effect and PMD and/or age as covariates. Tests were 2-tailed and values of $P \leq 0.05$ were considered significant. Fold changes were calculated using the mean gene expression values.

RESULTS

Altered mRNA Expression Levels in Suicide and Depression

In the ACC of SMRI patients, CRH transcripts were up-regulated in the MDD-S patients and compared to MDD-NS patients (1.98 fold changes, $P = 0.003$) and to the control group (1.56 fold changes, $P = 0.008$), while no differences were observed between MDD-NS patients and control subjects ($P = 0.405$) (Figure 1A). In the DLPFC, we could not find any significant differences between MDD-S, MDD-NS, and control subjects ($P = 0.487$, Figure 1B). In the NBB cohort, there was no significant change in CRH expression between DEP and control subjects either in the ACC ($P = 0.104$, Figure 1C) or DLPFC ($P = 0.855$, Figure 1D). IL1 β showed a trend for an increased expression in the ACC of MDD-S patients ($P = 0.076$).

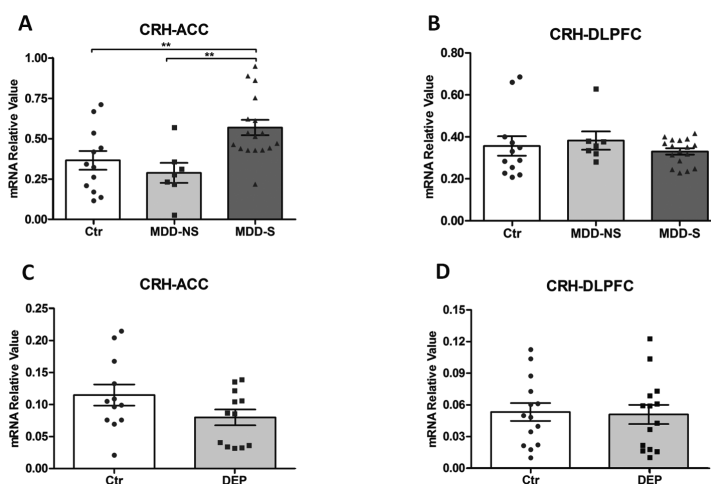


Figure 1 Alteration in CRH in the ACC/DLPFC in the Stanley Medical Research Institute (SMRI) and Netherlands Brain Bank (NBB) patient cohorts In the ACC of SMRI patient cohort, a significant increase was found for CRH mRNA expression in MDD patients who had committed suicide compared to the MDD patients who died of non-suicidal causes (or control subjects) (A), while in the DLPFC there was no significant change (B). In the NBB patient cohort, there was no significant difference between elderly depression patients who did not commit suicide and control subjects, either in the ACC (C) or DLPFC (D). All data are mean \pm SEM. Asterisk indicates $P \leq 0.05$.

Table 2A Continued, Results of expression of stress-related target genes in the ACC and DLPFC between the diagnostic groups and their matched control groups.

		SMRI-DLPFC													
		SMRI-ACC				Mean				Fold changes				P-values	
		Mean		Fold changes		P-value*		MDD-S NS		MDD-S /Ctr		MDD-S NS /Ctr		P-values	
		Ctr	MDD-S NS	MDD-S /Ctr	MDD-S NS /Ctr	P-value	Ctr	MDD-S NS	MDD-S /Ctr	MDD-S NS /Ctr	MDD-S NS /Ctr	MDD-S NS /Ctr	MDD-S NS /Ctr		
TrkB.T1		0,22	0,28	0,25	1,13	1,30	1,15	0,073	0,57	0,59	0,95	0,99	1,04	0,875	
RARa		0,22	0,27	0,20	1,32	1,24	0,94	0,060	0,28	0,33	1,00	1,17	1,17	0,448	
RARb		0,33	0,40	0,41	0,98	1,22	1,24	0,267	0,64	0,69	0,83	1,07	1,29	0,271	
RARv		0,60	0,62	0,66	0,94	1,02	1,09	0,784	0,61	0,56	0,74	0,92	1,24	0,068	
RXRa		0,32	0,34	0,37	0,93	1,08	1,17	0,519	0,30	0,38	0,40	0,97	1,31	0,394	
RXRb		0,54	0,47	0,55	0,86	0,88	1,03	0,787	0,77	0,66	0,73	0,90	0,96	0,474	
RXRv		0,20	0,21	0,20	1,05	1,03	0,99	0,901	0,53	0,52	0,67	0,98	1,25	0,410	
CRABP1		2,20	2,36	2,03	1,16	1,07	0,92	0,641	1,84	1,82	1,97	0,99	1,07	0,475	
CRABP2		0,11	0,10	0,16	0,63	0,96	1,51	0,319	0,53	0,52	0,67	0,98	1,25	0,126	
RALDH1		0,63	0,62	0,60	1,03	0,98	0,96	0,970	1,76	1,82	1,86	1,03	1,06	0,445	
RALDH2		0,42	0,46	0,35	1,32	1,09	0,83	0,338	0,37	0,37	0,41	0,92	1,01	0,840	0,459
RALDH3		0,98	0,84	0,83	1,01	0,85	0,85	0,062	0,14	0,12	0,16	0,86	1,11	0,375	0,680
cytochrome P450 protein															
P450A		0,20	0,24	0,25	0,93	1,18	1,27	0,402	0,44	0,45	0,49	1,03	1,13	0,666	0,506
P450B		0,65	0,67	0,44	1,53	1,03	0,67	0,144	0,77	0,88	0,73	1,21	0,96	0,384	0,708
P450C		0,84	0,90	1,03	0,87	1,06	1,22	0,729	0,34	0,29	0,31	0,93	0,90	0,466	
NOS related genes															
NOS1		0,49	0,51	0,55	0,93	1,04	1,12	0,673	0,578	0,578	0,45	0,99	1,07	0,833	
NOS2		0,84	0,86	0,90	0,95	1,02	1,07	0,957	0,17	0,18	0,24	0,73	1,00	0,359	
NOS3		0,67	0,65	0,77	0,84	0,97	1,15	0,604	0,10	0,10	0,12	0,83	1,00	0,652	
NIDD		0,45	0,56	0,38	1,47	1,24	0,84	0,016	0,22	0,25	0,21	1,19	1,12	0,721	0,554
Monoamine related genes															
MAOA		0,51	0,87	0,87	1,00	1,69	1,69	0,113	0,39	0,36	0,49	0,74	0,93	1,26	0,535
MAOB		0,49	0,67	0,82	0,82	1,38	1,68	0,094	1,38	1,32	1,40	0,94	0,96	1,01	0,376

Table 2A Continued, Results of expression of stress-related target genes in the ACC and DLPPFC between the diagnostic groups and their matched control groups.
SMRI-ACC SMRI-DLPFC

	Mean		Fold changes		Mean		Fold changes		P-values	P-values*				
	Ctrl	MDD-S	MDD-S/NS	MDD-S/Ctrl	MDD-S	MDD-S/NS	MDD-S/NS	MDD-S/Ctrl						
COMT	1,25	0,93	1,23	0,76	0,75	0,99	0,240	1,12	0,88	1,10	0,80	0,78	0,98	0,200
5HT1A	0,20	0,23	0,23	1,02	1,16	1,14	0,757	0,36	0,39	0,32	1,22	1,08	0,89	0,577
5HT2A	0,24	0,34	0,27	1,23	1,40	1,13	0,337	0,14	0,14	0,14	1,03	1,05	1,02	0,931
ADRA1A	0,50	0,51	0,54	0,95	1,04	1,09	0,837	2,20	2,52	2,20	1,15	1,15	1,00	0,249
ADRA2A	1,13	1,23	1,02	1,21	1,08	0,90	0,103	0,23	0,23	0,26	0,86	1,01	1,17	0,733
ADRB1	1,02	1,74	1,71	1,02	1,71	1,68	0,122	0,81	0,86	0,88	0,98	1,06	1,08	0,910
DRD1	1,65	1,99	1,68	1,18	1,21	1,02	0,488	0,21	0,20	0,27	0,75	0,95	1,28	0,395
DRD2	0,28	0,31	0,29	1,05	1,08	1,02	0,904	2,66	1,67	2,16	0,77	0,63	0,81	0,156

The differences among MDD-S, MDD-NS and Ctrl groups were analyzed for multiple comparisons using one-way analyses of variance (ANOVAs) followed by *post-hoc* Least Significant Difference test. The ANCOVA model, used for the expression levels of each gene, revealed the diagnostic group as a main effect and PMD and age as covariates. Tests were 2-tailed and values of $P \leq 0.05$ were considered significant (typed in italic bold front for recognition). Fold changes were calculated using the mean gene expression values. Asterisk indicates corrected P from ANCOVA model, and the blank means there is no correlation between target gene expression and PMD/age. Abbreviations: ACC, anterior cingulate cortex; Ctrl, control; DLPPFC, dorsolateral prefrontal cortex; MDD-S, major depressive disorder who died of suicide; MDD-NS, major depressive disorder who died of non-suicide cause; SMRI, Stanley Medical Research Institute. For abbreviations of gene see S-Table 3.

Table 2B Results of expression of stress-related target genes in the ACC and DLPFC between the diagnostic groups and their matched control groups.

	NBB-ACC Genes				NBB-DLPFC					
	Mean		Fold changes		Mean		Fold changes		P-values	P-value*
	Ctrl	DEP	DEP / Ctrl		Ctrl	DEP	DEP / Ctrl			
NOS related genes										
NIDD	0,81	0,52	0,64	0,026	0,49	0,53	1,08	0,556	0,794	
Monoamine related genes										
MAOA	0,11	0,11	1,00	0,986	0,72	0,88	1,23	0,236		
MAOB	0,65	0,81	1,25	0,177	0,39	0,46	1,18	0,427		
COMT	1,22	1,18	0,97	0,716	0,90	1,13	1,25	0,238		
5HT1A	0,50	0,68	1,35	0,037	0,27	0,28	1,04	0,695		
5HT2A	2,04	2,16	1,06	0,099	0,39	0,41	1,06	0,656		
ADRA1A	0,11	0,09	0,88	0,567	0,65	0,64	0,99	0,954	0,922	
ADRA2A	0,14	0,17	1,21	0,199	1,17	1,17	1,00	0,994		
ADRB1	0,89	0,82	0,93	0,690	1,02	1,05	1,03	0,624	0,652	
DRD1	0,98	1,15	1,17	0,065	0,92	0,97	1,06	0,631		
DRD2	0,18	0,20	1,13	0,690	0,37	0,17	0,46	0,062		

To find the differences between DEP and Ctrl groups tests were performed with the unpaired Student's t-test. The ANCOVA model, used for the expression levels of each gene, revealed the diagnostic group as a main effect and PMD and age as covariates. Tests were 2-tailed and values of $P \leq 0.05$ were considered significant (typed in italic bold front for recognition). Fold changes were calculated using the mean gene expression values. Asterisk indicates corrected P from ANCOVA model, and the blank means there is no correlation between target gene expression and PMD/age.

Abbreviations: ACC, anterior cingulate cortex; Ctrl, control; DLPFC, dorsolateral prefrontal cortex; DEP, depression patients who died of non-suicide cause; NBB, Netherlands Brain Bank. For abbreviations of gene see S-Table 3.

The transcript level of NIDD, a protein involved in the NO signaling pathway, was increased in the MDD-S patients compared to MDD-NS patients (2.18 fold changes, $P = 0.009$) and to control subjects (1.52 fold changes, $P = 0.045$) in the ACC-SMRI cohort, while there was no difference between MDD-NS patients and controls ($P = 0.337$, Figure 2A). A decreased NIDD transcript level in the ACC-NBB was found in the DEP patients compared to the control subjects (36% reduction, $P = 0.026$, Figure 2C), NIDD transcript levels were unchanged in the DLPFC-NBB patients ($P = 0.556$, Figure 2D). In the ACC-NBB cohort, we found a significant increase in 5-HT1A mRNA levels in DEP patients compared to the matched controls (1.35 fold changes, $P = 0.037$, Figure 3C), while no such difference was found in the DLPFC ($P = 0.695$, Figure 3D). In the SMRI patient cohort, we did not find any significant change of 5-HT1A among the diagnosis groups in either ACC ($P = 0.757$, Figure 3A) or DLPFC ($P = 0.577$, Figure 3B). Other monoamine-related genes (MAOA, MAOB, COMT, 5-HT2A, ADRA1A, ADRA2A, DRD1, DRD2), whether from the SMRI or the NBB collection, did not show any significant difference in their mRNA expression (Table 2A, 2B). All these changes remained significant after ANCOVA testing to control for PMD and age, except NIDD-SMRI transcript levels, which became a trend ($P = 0.060$).

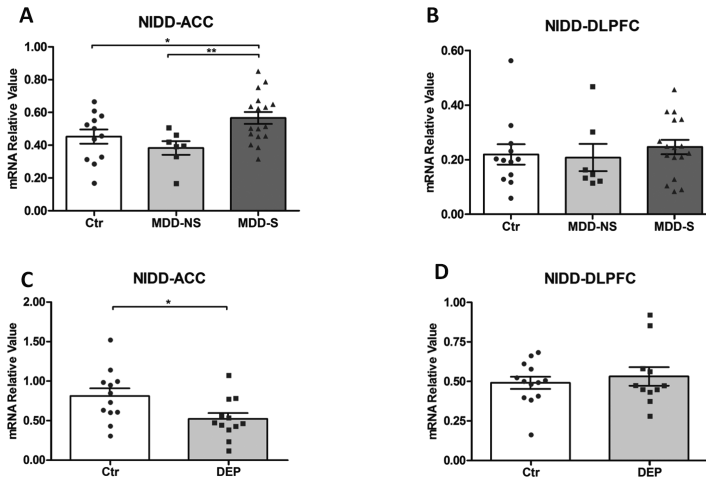


Figure 2 Alteration in NIDD in the ACC/DLPFC in the SMRI and NBB patient cohorts. In the ACC of SMRI patient cohort, a significant increase was found for NIDD mRNA expression in the MDD patients who committed suicide compared to the MDD patients who died of non-suicidal causes (or control subjects) (A), while in the DLPFC there was no significant change (B). In the NBB patient cohort, there was a significant decrease of NIDD mRNA expression in elderly depression patients who did not commit suicide compared to control subjects in the ACC (C), but not in the DLPFC (D). All data are mean \pm SEM. Asterisk indicates $P \leq 0.05$.

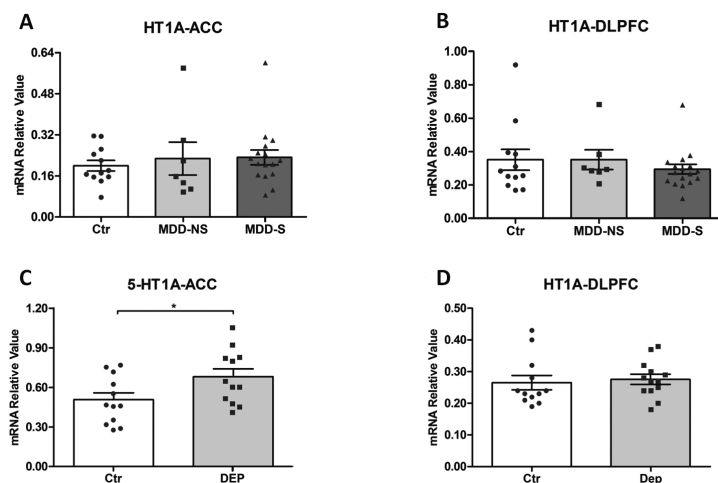


Figure 3 Alteration in 5HT1A in the ACC/DLPFC in the SMRI and NBB patient cohorts. In the ACC of the SMRI patient cohort, there were no significant changes in the 5HT1A mRNA expression among the MDD patients who died of suicide, the MDD patients who died of non-suicidal causes and control subjects, neither in the ACC (A) nor in the DLPFC (B). In the NBB patient cohort, there was a significant increase of 5HT1A mRNA expression in elderly depression patients who did not die of suicide compared to control subjects in the ACC (C), but not in the DLPFC (D). All data are mean \pm SEM. Asterisk indicates $P \leq 0.05$.

There was no significant difference in transcript levels of RA/TrkB-related genes among MDD-S patients, MDD-NS patients and control subjects in either ACC- or DLPFC-SMRI. See Table 2A for the transcript expression of all target genes.

To explore what aspects of suicide cause gene transcription changes in the ACC, further analysis was carried out on the SMRI material to assess whether the suicide method played a role. The SMRI depressed suicide victims were divided into two subgroups: violent suicide (hanging, jumping, shooting and stabbing, $N = 12$) and non-violent suicide (drug overdose, $N = 5$). Subsequently the significant genes (CRH, NIDD, and 5-HT1A) were compared between these two subgroups. Higher mRNA expression levels of CRH were present in the violent suicide subgroup compared to the suicide victims who died of non-violent methods ($P = 0.013$).

After the Benjamini and Hochberg correction for multiple testing, the significant changes in ACC-SMRI CRH, ACC-NBB NIDD and 5HT1A survived. Only the significance in ACC-SMRI NIDD was lost.

Different Pattern of Gene Expression Changes in Suicide and Depression

When we listed all significant changes in either NBB or SMRI patient cohort (table 3) for the CRH-, retinoid signaling-, BDNF-, TrkB- and NOS-related genes of the present and of our previous studies on the same NBB material (Gao et al., 2013; Qi et al., 2013; Qi et al., 2015), it became clear that suicide and depression clearly have different patterns of significant changes in these genes.

Table 3 Significant changes of stress-related target genes in suicide (SMRI) and depression (NBB).

Gene	SMRI-ACC	SMRI-DLPFC	NBB-ACC	NBB-DLPFC
CRH related genes (A)				
CRH	↑	=	=	=
ER2	=	=	=	↑ ¹
MR	=	=	↓ ¹	↓ ¹
IL1β	=	=	=, ↓ ¹	=
Retinoid signaling, BDNF and TrkB related genes (B)				
CRABP1	=	=	↓ ²	=
RALDH1	=	=	↓ ²	↓ ²
RALDH3	=	=	↓ ²	↓ ²
RXRα	=	=	=	↓ ²
RXRβ	=	=	=	↓ ²
TrkB.FL	=	=	↓ ²	↓
TrkB.T1	=	=	↓ ²	↓ ²
BDNF	=	=	↓ ²	↓ ²
NOS related genes (C)				
NOS1	=	=	↓ ³	=
NIDD	↑	=	↓	=
Monoamine related genes (D)				
5HT1A	=	=	↑	=

Abbreviations: ACC, anterior cingulate cortex; Ctr, control; DLPFC, dorsolateral prefrontal cortex; DEP, depression patients who died of non-suicide cause; MDD-S, major depressive disorder who died of suicide; MDD-NS, major depressive disorder who died of non-suicide cause; NBB, Netherlands Brain Bank; SMRI, Stanley Medical Research Institute. For abbreviations of genes see S-Table 3.

Significant genes determined in SMRI and NBB material from the present study and previous studies (genes A¹, B² and C³), are listed according to Brain Bank and brain area. ↑ increase when compared MDD-S to MDD-NS, or MDD-S to Ctr in the SMRI patient cohort, or DEP to Ctr in the NBB patient cohort; ↓ decrease when compared DEP to Ctr; = no significant difference when compared MDD-S to MDD-NS, or MDD-S to Ctr in the SMRI patient cohort, or DEP to Ctr in the NBB patient cohort.

¹ Qi, X.R., Kamphuis, W., Wang, S., Wang, Q., Lucassen, P.J., Zhou, J.N., Swaab, D.F., 2012. Aberrant stress hormone receptor balance in the human prefrontal cortex and hypothalamic paraventricular nucleus of depressed patients. *Psychoneuroendocrinology*. 38: 863-70.

² Qi, X.R., Zhao, J., Liu, J., Fang, H., Swaab, D.F., Zhou, J.N., 2015. Abnormal Retinoid and TrkB Signaling in the Prefrontal Cortex in Mood Disorders. *Cereb Cortex*. 25: 75-83.

³ Gao, S.F., Qi, X.R., Zhao, J., Balesar, R., Bao, A.M., Swaab, D.F., 2013. Decreased NOS1 expression in the anterior cingulate cortex in depression. *Cereb Cortex*. 23: 2956-64.

Potentially Confounding Effects

No significant differences were found between the groups in terms of age, gender, brain pH, brain weight, PMD, hemisphere side, ethnicity, history of substance abuse, severity of substance abuse or psychotic features (Table 1A and 1B), indicating that these variables will not have affected our conclusions. The average age of the patient groups in the SMRI cohort, however, was lower compared to the NBB cohort (SMRI: 44 years and NBB: 75 years). There was also a big difference in PMD between the two cohorts (SMRI: 28.19 h and NBB: 7.55 h). The ANCOVA model with age and PMD as covariates used in our study further controlled for these two possible confounders (see below). As to psychosis, which was present in some of the subjects in the SMRI patient cohort, differentially expressed genes were compared by with/without psychosis, and none of the comparisons were significant ($P \geq 0.300$). Additionally, we have combined MDD and BD in the NBB collection, since these disorders share similarities with regard to their neurobiological underpinnings (Bao et al., 2008) and with respect to some structural and functional cortical alterations (Drevets et al., 2008). Furthermore, when we compared gene expression levels between subgroups with BD and MDD for each brain area, there was no significant difference (Gao et al., 2013; Qi et al., 2015). Therefore, MDD and BD patient data were pooled.

While possible effects of medication on gene expression are always a limitation in post-mortem human brain studies, we do not think it has been a major confounder in our results, even with our small group sizes. For instance, for the 5-HT1A gene in the NBB collection, no difference was found in mRNA levels between patients taking antipsychotics (02-051, 06-011, 06-026) and those not taking antipsychotics or antidepressants in the last 3 months. The same holds for the patients taking benzodiazepines (00-074, 06-021, 06-026, 07-033, 07-060). One patient who was taking selective serotonin reuptake inhibitors (SSRI, 01-074) and two patients who were taking lithium (06-021, 06-075) were within the middle range of the observed expression levels. For the CRH and NIDD genes in the SMRI material, no differences were found in mRNA levels between patients taking SSRIs (DC3, DC7, DC12, DC16, DC20, DC21) and those taking no SSRIs. The same holds for the patients taking serotonin antagonist and reuptake inhibitors (SARI, DC12, DC13, DC24, D25, D30). It should be noted that the patients who were taking tricyclic antidepressants (TCA, DC1, DC14, DC16, DC25, DC32, DC34) showed significantly lower CRH and NIDD expression levels. Consequently, should such medication have influenced our data, it would rather be expected

to have decreased, not increased, CRH and NIDD levels in the suicide group, as we had observed in our present study. The CRH and NIDD mRNA levels of two patients who were taking serotonin–norepinephrine reuptake inhibitors (DC17, DC31) were intermingled with the other data points, and one patient taking monoamine oxidase inhibitors (DC14) was within the middle range.

DISCUSSION

In an attempt to understand the biological processes associated with depression and suicide, one approach has been to study molecular changes in brain tissue obtained from patients that suffered from these disorders. So far, many post-mortem studies have searched for candidate genes, but there is considerable inconsistency in the reported results, which limits our understanding of the pathophysiology of these disorders. We show here that the composition of cohorts, i.e. the proportion of suicide patients in the material studied for depression, and the absence of appropriate disease controls in the material studied for suicide may explain at least some of the discrepancies in the literature. Studies comparing depressed suicide victims with healthy control subjects that did not suffer from psychiatric disorders cannot distinguish whether the changes found are related to the underlying psychiatric disorders or to the suicide itself. Our present study shows that there are indeed alterations in stress-related gene expression levels that are only present in patients who committed suicide (increased CRH and NIDD), or only present in relation to depression without suicide (increased 5-HT1A and decreased NIDD) in the ACC. One important conclusion therefore is that appropriate disease controls such as depressed, non-suicide patients and, if possible, non-depressed suicide victims, are necessary to better understand the full spectrum of molecular alterations in depression and suicide.

CRH plays a central role in the regulation of the HPA axis, which is a central system in the stress response. The action of CRH on ACTH release results in an increase in cortisol, while MR and GR are involved in the negative feedback. Depression, both with and without suicide, is characterized by alterations in stress systems (Bao et al., 2008; Braquehais et al., 2012; Wang et al., 2008). Recently, we have shown in the NBB cohort of depression patients that did not commit suicide that there is an aberrant MR and GR balance in the ACC (Qi et al., 2013). In the present study, MR or GR mRNA did not show significant differences in relation

to suicide when we compared the ACC of the MDD-S compared to MDD-NS patients, but we did observe up-regulated CRH-mRNA expression in the MDD-S patients. There was no difference in CRH-mRNA between the MDD-NS patients and controls, while CRH mRNA expression remained unaltered also in elderly depressed patients who did not commit suicide (Qi et al., 2013). Merali and his colleagues reported elevated CRH levels in several frontal cortical brain regions of depressed suicide victims (Merali et al., 2004). However, due to the absence of a disease control group, they could not draw the conclusion whether these changes were due to depression or to suicide. Bringing all data together, increased CRH-mRNA expression in the ACC seems thus to be related to suicide rather than to depression *per se*. One may thus speculate that the depression-like effects observed following intra-cerebroventricular injection of CRH in rats (Holsboer, 2001) or the overexpression of CRH in transgenic mice (Lu et al., 2008) may show a greater resemblance to alterations found in depressed patients who died of suicide than to major depression *per se*. Moreover, we found that there was increased CRH-mRNA expression in those patients who died of violent suicide. Interestingly, an animal model showed that the acute stress increased the CRH mRNA expression in the PFC (Meng et al., 2011). Whether the increased cortical CRH is related to the cause of violent suicide or to the stress of violent suicide should be studied in the future. In addition, there was a trend towards increased expression of IL1 β , a powerful CRH-stimulating pro-inflammatory cytokine, in the MDD-S patient group, but not in the depression patients who did not commit suicide, which thus also seems to be related to suicide rather than to depression.

NO generated by NOS1 plays a significant role in modulating the activity of the stress system during acute stress. NIDD is a NOS1-interacting protein, which increases the NOS1 enzyme activity by targeting it to the synaptic membrane (Saitoh et al., 2004). We found an increased NIDD transcript level in the ACC of MDD-S patients, while no significant change of NOS1 mRNA was present. This was different from the decreased NIDD gene expression in the NBB patient cohort, who were older, did not commit suicide, and had a diminished NOS1-mRNA expression as we reported before (Gao et al., 2013). These findings indicate that both NIDD and NOS1 have their own specific expression patterns in depression and suicide. Seeing the role that NIDD plays in synaptic function, our data suggest that differences may exist in synaptic plasticity in the ACC of suicide and depression patients.

Monoaminergic systems are considered to be crucial for the stress response and determine, at least partly, the vulnerability to mood disorders and suicidal behavior as a response to depression (Mandelli and Serretti, 2013). It was therefore remarkable to find no significant change in any monoamine-related transcript in the ACC/DLPFC in the MDD patients with or without suicide. In the elderly NBB cohort, we found elevated 5-HT1A mRNA levels in the ACC of DEP cohort. This extends a previous finding of increased 5-HT1A binding in the raphe nucleus in elderly depression patients (Meltzer et al., 2004). The present finding is also consistent with a genetic meta-analysis showing that the G allele in the 5HT1A gene, which is associated with higher expressions of 5-HT1A receptors (Lemonde et al., 2003), is related to depression (Kishi et al., 2013). Recent studies have also shown that there are alterations in 5-HT1A levels in depressed/stressed subjects (Cheetham et al., 1990; Jovanovic et al., 2011; Miller et al., 2013). This is in line with a transgenic mouse model that is modified to produce increases in 5-HT1A autoreceptor levels and displaying increased depressive-like behaviors (Richardson-Jones et al., 2010). In the current study, the MDD-NS group from the SMRI cohort is relatively small ($N = 7$). However, the larger group of non-suicide patients from the NBB cohort, which also consisted of patients who did not commit suicide, can compensate for this limitation. It is true that the average age of these two cohorts is different: SMRI ($y = 44 \pm 12$) and NBB ($y = 77 \pm 12$). Some postmortem studies (Arango et al., 1997; Dillon et al., 1991), but not others (Palego et al., 1997), have found aging effects on 5-HT1A receptor density and we therefore checked the correlation between 5-HT-1A and age, and found an inverse correlation in the ACC of the MDD-S patients ($r = -0.619$, $P = 0.011$). This is in line with PET studies showing that 5-HT1A binding declines with age (Tauscher et al., 2001). However, such a correlation was absent from the MDD-NS group, DEP group and from the control groups. As the group size of MDD-NS was so small, there might be a chance that age had an effect on the data. We thus corrected for age together with PMD, the other major difference between the two cohorts. With age and PMD as covariates in the ANCOVA model the changes of 5-HT1A appeared to occur independently of these two factors. Our finding of increased 5-HT1A mRNA levels in the ACC in relation to depression but not to suicide illustrates again the problem with the interpretation of the discrepancies reported in the literature on 5-HT1A alterations in postmortem material where depressed and suicide patients are mixed in order to study depression (Lowther et al., 1997; Yates and Ferrier, 1990).

Retinoid signaling is involved in the stress response (Hu et al., 2013). In our previous study we found that mRNA expression of key components of retinoid signaling, such as RALDH1 and 3, RXR α and β , and CRABP1, and the mRNA levels of BDNF and TrkB isoforms were significantly reduced in the ACC of non-suicide depressive patients in the NBB patient-cohort (Qi et al., 2015). The same genes were now detected on the SMRI material, but we did not find any of these changes, either between MDD-S and MDD-NS patients, or between MDD-NS patients and control subjects, which suggests that these alterations are related to depression rather than to suicide in elderly individuals.

In conclusion, different expression changes were present in relation to depression and suicide. These changes were observed in the ACC, but not in the DLPFC. In our study, different genes (increased CRH and NIDD) were linked to suicide, while increased 5-HT1A and decreased NIDD were related to depression. These two disorders thus seem to have their own specific expression changes. This makes it necessary to include appropriate disease controls to disentangle the molecular differences between depression and suicide.

ACKNOWLEDGMENTS

Postmortem brain tissue was donated by The Stanley Medical Research Institute Brain Collection and Netherlands Brain Bank. We are indebted to them for providing us with the brain material and patient information. We thank Dr. R.W.H Verwer for statistical assistance, A. Sluiter for her technical advice, and W. Verweij for secretarial help.

REFERENCES

- Arango, V., Underwood, M.D., Mann, J.J., 1997. Postmortem findings in suicide victims. Implications for in vivo imaging studies. *Ann N Y Acad Sci.* 836, 269-87.
- Arranz, B., et al., 1994. Brain 5-HT_{1A}, 5-HT_{1D}, and 5-HT₂ receptors in suicide victims. *Biol Psychiatry.* 35, 457-63.
- Bao, A.M., Meynen, G., Swaab, D.F., 2008. The stress system in depression and neurodegeneration: focus on the human hypothalamus. *Brain Res Rev.* 57, 531-53.
- Bernard, R., et al., 2011. Altered expression of glutamate signaling, growth factor, and glia genes in the locus coeruleus of patients with major depression. *Mol Psychiatry.* 16, 634-46.
- Braquehais, M.D., et al., 2012. Hypothalamic-pituitary-adrenal axis dysfunction as a neurobiological correlate of emotion dysregulation in adolescent suicide. *World J Pediatr.* 8, 197-206.
- Cheetham, S.C., et al., 1990. Brain 5-HT₁ binding sites in depressed suicides. *Psychopharmacology (Berl).* 102, 544-8.
- Cotter, D., et al., 2001. Reduced glial cell density and neuronal size in the anterior cingulate cortex in major depressive disorder. *Arch Gen Psychiatry.* 58, 545-53.
- Dillon, K.A., et al., 1991. Autoradiographic analysis of serotonin 5-HT_{1A} receptor binding in the human brain postmortem: effects of age and alcohol. *Brain Res.* 554, 56-64.
- Diorio, D., Viau, V., Meaney, M.J., 1993. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *J Neurosci.* 13, 3839-47.
- Drevets, W.C., Price, J.L., Furey, M.L., 2008. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct Funct.* 213, 93-118.
- Dwivedi, Y., et al., 2006a. Differential and brain region-specific regulation of Rap-1 and Epac in depressed suicide victims. *Arch Gen Psychiatry.* 63, 639-48.
- Dwivedi, Y., et al., 2006b. ERK MAP kinase signaling in post-mortem brain of suicide subjects: differential regulation of upstream Raf kinases Raf-1 and B-Raf. *Mol Psychiatry.* 11, 86-98.
- Elliott, R., et al., 2011. Affective cognition and its disruption in mood disorders. *Neuropsychopharmacology.* 36, 153-82.
- Ernst, C., et al., 2009. Alternative splicing, methylation state, and expression profile of tropomyosin-related kinase B in the frontal cortex of suicide completers. *Arch Gen Psychiatry.* 66, 22-32.
- Gao, S.F., et al., 2013. Decreased NOS1 expression in the anterior cingulate cortex in depression. *Cereb Cortex.* 23, 2956-64.
- Gross-Isseroff, R., et al., 1998. The suicide brain: a review of postmortem receptor/transporter binding studies. *Neurosci Biobehav Rev.* 22, 653-61.
- Holsboer, F., 2001. Stress, hypercortisolism and corticosteroid receptors in depression: implications for therapy. *J Affect Disord.* 62, 77-91.
- Hu, P., et al., 2013. All-trans retinoic acid-induced hypothalamus-pituitary-adrenal hyperactivity involves glucocorticoid receptor dysregulation. *Transl Psychiatry.* 3, e336.
- Jokinen, J., Nordstrom, P., 2009. HPA axis hyperactivity and attempted suicide in young adult mood disorder inpatients. *J Affect Disord.* 116, 117-20.
- Jovanovic, H., et al., 2011. Chronic stress is linked to 5-HT(1A) receptor changes and functional disintegration of the limbic networks. *Neuroimage.* 55, 1178-88.
- Kishi, T., et al., 2013. The serotonin 1A receptor gene confer susceptibility to mood disorders: results from an extended meta-analysis of patients with major depression and bipolar disorder. *Eur Arch Psychiatry Clin Neurosci.* 263, 105-18.
- Lemondé, S., et al., 2003. Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. *J Neurosci.* 23, 8788-99.
- Lowther, S., et al., 1997. 5-HT_{1A} receptor binding sites in post-mortem brain samples from depressed suicides and controls. *J Affect Disord.* 42, 199-207.
- Lu, W., et al., 2008. Correlates of adverse childhood experiences among adults with severe mood disorders. *Psychiatr Serv.* 59, 1018-26.
- Mandelli, L., Serretti, A., 2013. Gene environment interaction studies in depression and suicidal behavior: An update. *Neurosci Biobehav Rev.* 37, 2375-97.
- Mann, J.J., 2003. Neurobiology of suicidal behaviour. *Nat Rev Neurosci.* 4, 819-28.

- Martins-de-Souza, D., et al., 2012. Identification of proteomic signatures associated with depression and psychotic depression in post-mortem brains from major depression patients. *Transl Psychiatry*. 2, e87.
- Maussion, G., et al., 2014. Functional DNA methylation in a transcript specific 3'UTR region of TrkB associates with suicide. *Epigenetics*. 9, 1061-70.
- Meltzer, C.C., et al., 2004. Serotonin 1A receptor binding and treatment response in late-life depression. *Neuropsychopharmacology*. 29, 2258-65.
- Meng, Q.Y., et al., 2011. Stress and glucocorticoids regulated corticotropin releasing factor in rat prefrontal cortex. *Mol Cell Endocrinol*. 342, 54-63.
- Merali, Z., et al., 2004. Dysregulation in the suicide brain: mRNA expression of corticotropin-releasing hormone receptors and GABA(A) receptor subunits in frontal cortical brain region. *J Neurosci*. 24, 1478-85.
- Miller, J.M., et al., 2013. Positron emission tomography quantification of serotonin transporter in suicide attempters with major depressive disorder. *Biol Psychiatry*. 74, 287-95.
- Narushima, K., Kosier, J.T., Robinson, R.G., 2003. A reappraisal of poststroke depression, intra- and inter-hemispheric lesion location using meta-analysis. *J Neuropsychiatry Clin Neurosci*. 15, 422-30.
- Palego, L., et al., 1997. Apparent absence of aging and gender effects on serotonin 1A receptors in human neocortex and hippocampus. *Brain Res*. 758, 26-32.
- Pandey, G.N., et al., 2008. Brain-derived neurotrophic factor and tyrosine kinase B receptor signalling in post-mortem brain of teenage suicide victims. *Int J Neuropsychopharmacol*. 11, 1047-61.
- Pandey, G.N., 2013. Biological basis of suicide and suicidal behavior. *Bipolar Disord*. 15, 524-41.
- Poulter, M.O., et al., 2008. GABAA receptor promoter hypermethylation in suicide brain: implications for the involvement of epigenetic processes. *Biol Psychiatry*. 64, 645-52.
- Qi, X.R., et al., 2013. Aberrant stress hormone receptor balance in the human prefrontal cortex and hypothalamic paraventricular nucleus of depressed patients. *Psychoneuroendocrinology*. 38, 863-70.
- Qi, X.R., et al., 2015. Abnormal retinoid and TrkB signaling in the prefrontal cortex in mood disorders. *Cereb Cortex*. 25, 75-83.
- Rajkowska, G., et al., 1999. Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biol Psychiatry*. 45, 1085-98.
- Richardson-Jones, J.W., et al., 2010. 5-HT1A autoreceptor levels determine vulnerability to stress and response to antidepressants. *Neuron*. 65, 40-52.
- Roth, T.L., et al., 2009. Lasting epigenetic influence of early-life adversity on the BDNF gene. *Biol Psychiatry*. 65, 760-9.
- Saitoh, F., et al., 2004. NIDD, a novel DHHC-containing protein, targets neuronal nitric-oxide synthase (nNOS) to the synaptic membrane through a PDZ-dependent interaction and regulates nNOS activity. *J Biol Chem*. 279, 29461-8.
- Shelton, R.C., et al., 2009. Elevated 5-HT 2A receptors in postmortem prefrontal cortex in major depression is associated with reduced activity of protein kinase A. *Neuroscience*. 158, 1406-15.
- Swanson, L.W., et al., 1983. Organization of ovine corticotropin-releasing factor immunoreactive cells and fibers in the rat brain: an immunohistochemical study. *Neuroendocrinology*. 36, 165-86.
- Tauscher, J., et al., 2001. Serotonin 5-HT1A receptor binding potential declines with age as measured by [11C]WAY-100635 and PET. *Neuropsychopharmacology*. 24, 522-30.
- Thalmeier, A., et al., 2008. Gene expression profiling of post-mortem orbitofrontal cortex in violent suicide victims. *Int J Neuropsychopharmacol*. 11, 217-28.
- Torrey, E.F., et al., 2000. The stanley foundation brain collection and neuropathology consortium. *Schizophr Res*. 44, 151-5.
- Vandesompele, J., et al., 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol*. 3, RESEARCH0034.
- Wang, S.S., et al., 2008. Gene expression analysis in the human hypothalamus in depression by laser microdissection and real-time PCR: the presence of multiple receptor imbalances. *Mol Psychiatry*. 13, 786-99, 741.
- Xing, G., et al., 2002. Decreased calcium-dependent constitutive nitric oxide synthase (cNOS) activity in prefrontal cortex in schizophrenia and depression. *Schizophr Res*. 58, 21-30.
- Yates, M., Ferrier, I.N., 1990. 5-HT1A Receptors in major depression. *J Psychopharmacol*. 4, 69-74.
- Zhao, J., et al., 2012. Gene expression of GABA and glutamate pathway markers in the prefrontal cortex of non-suicidal elderly depressed patients. *J Affect Disord*. 138, 494-502.

SUPPLEMENTARY MATERIALS

S-Table 1 Clinico-pathological information of patients with MDD and control subjects (SMRI collection)

	SMRI code	Sex	Age (y)	PMD (h)	Brain pH	BW (g)	RIN	Brain Region	Medication	Psychotic feature	Cause of Death
MDD	DC1	F	48	24	6.36	1330	8,0	L DLPFC, L ACC	TCA	Yes	SUIC: OD
MDD	DC3	F	40	49	6.72	1450	8,8	L DLPFC, L ACC	SSRI	Yes	SUIC: HANGING
MDD	DC5	F	56	15	6.59	1370	8,4	L DLPFC, L ACC	antidepressant*	No	BURNS
MDD	DC7	M	28	26	6.7	1780	6,9	R DLPFC, R ACC	SSRI	Yes	SUIC: HANGING
MDD	DC9	M	35	19	6.6	1335	8,2	R DLPFC, R ACC	-	Yes	SUIC: HANGING
MDD	DC10	F	32	19	6.7	1280	8,9	L DLPFC, L ACC	-	Yes	SUIC: HANGING
MDD	DC12	F	32	19	6.8	1470	8	R DLPFC, R ACC	non-TCA, SSRI	No	SUIC: HANGING
MDD	DC13	M	63	31	6.6	1540	7,8	L DLPFC, L ACC	non-TCA	Yes	SUIC: HANGING
MDD	DC14	F	51	36	6.3	1440	7,3	L DLPFC, L ACC	MAOI, TCA	Yes	UNKNOWN
MDD	DC15	M	35	36	6.6	1710	6,3	L DLPFC, L ACC	-	Yes	SUIC: GSW
MDD	DC16	M	44	24	6.52	1550	7,9	R DLPFC, R ACC	TCA, SSRI	No	CARDIAC
MDD	DC17	M	56	38	6.59	1365	7,3	L DLPFC, L ACC	SNRI, anxiolytics	No	SUIC: OD
MDD	DC18	M	33	25	6.86	1640	7,6	R DLPFC, R ACC	-	No	SUIC: HANGING
MDD	DC20	M	34	24	6.79	1425	8,2	R DLPFC, R ACC	SSRI	No	SUIC: JUMPED
MDD	DC21	F	45	29	6.9	1350	7,4	L DLPFC, L ACC	SSRI	No	CARDIAC
MDD	DC22	M	53	21	6.64	1520	7,5	L DLPFC, L ACC	-	No	CARDIAC
MDD	DC24	M	62	65	6.57	1490	7,5	R DLPFC, R ACC	non-TCA	ND	SUIC: STABBED
MDD	DC25	F	36	32	6.74	1270	7,3	R DLPFC, R ACC	SARI, TCA	Yes	PULM EMBOL
MDD	DC27	M	40	52	6.48	1590	6,6	L DLPFC, L ACC	-	Yes	OD
MDD	DC29	F	28	40	6.68	1430	7,1	L DLPFC, L ACC	-	Yes	SUIC: OD

S-Table 1 Continued, Clinico-pathological information of patients with MDD and control subjects (SMRI collection)

SMRI code	Sex	Age (y)	PMD (h)	Brain pH	BW (g)	RIN	Brain Region	Medication	Psychotic feature	Cause of Death
MDD DC30	M	45	29	6.75	1514	7,9	L DLPFC, L ACC	SARI	No	SUIC: HANGING
MDD DC31	M	24	21	6.61	1737	7,4	R DLPFC; R ACC	SNRI	No	SUIC: OD
MDD DC32	F	45	13	6.58	1170	7,2	L DLPFC; L ACC	TCA	No	SUIC: OD
MDD DC34	F	47	25	6.88	1495	7,7	L DLPFC; L ACC	TCA	No	SUIC: GSW
Ctr DC2	M	48	12	6.51	1410	8,6	R DLPFC; R ACC	-	No	CARDIAC
Ctr DC4	F	50	35	6.31	1520	7,9	L DLPFC; L ACC	anxiolytics	No	CARDIAC
Ctr DC6	M	50	11	6.5	1530	8,6	L DLPFC; L ACC	-	No	CARDIAC
Ctr DC8	M	63	37	6.5	1530	8	L DLPFC; L ACC	-	No	CARDIAC
Ctr DC11	M	24	17	6.6	1595	8,1	L DLPFC; L ACC	-	No	MVA
Ctr DC19	M	44	27	6.82	1410	7,2	R DLPFC; R ACC	-	No	ACUTE ALCO-HOL POISONING
Ctr DC23	M	35	31	6.59	1520	8	R DLPFC; R ACC	-	No	MVA
Ctr DC26	M	63	40	6.91	1410	8,2	R DLPFC; R ACC	-	No	CARDIAC
Ctr DC28	M	34	9	6.56	1535	5,9	R DLPFC; R ACC	-	No	MVA
Ctr DC33	F	56	29	6.78	1278	8,9	L DLPFC; L ACC	-	No	CARDIAC
Ctr DC35	F	56	31	6.66	1400	6,1	L DLPFC; L ACC	-	No	OBESITY
Ctr DC36	F	39	24	6.88	1200	7,3	R DLPFC; R ACC	-	No	CARDIAC

* Type uncertain

Abbreviations: ACC, anterior cingulate cortex; E/ZD, benzodiazepine; BW, brain weight; Ctr, control; DLPFC, dorsolateral prefrontal cortex; F, female; GSW, gunshot wound; L, left; MAOI, MAO inhibitor; MDD, major disorder depression; M, male; MVA, motor vehicle accidents; OD, overdose; PMD, postmortem delay; R, right; RIN, RNA integrity number; SARI, Serotonin antagonist and reuptake inhibitors; SMRI, Stanley Medical Research Institute; SNRI, Serotonin-norepinephrine reuptake inhibitors; SSRI, selective serotonin reuptake inhibitor; SUI, suicide; TCA, tricyclic antidepressant.

S-Table2 Clinico-pathological information of patients with MDD, BD and control subjects (NBB collection)

NBB number	Sex	Age (y)	PMD (h)	CTD (h)	MTD	Brain pH	BW (g)	RIN (ACC)	RIN (DLPFC)	Brain Region	ApoE	Braak stage	Medication in the past	Medication (last 3 months)	Suicide Ideation	Cause of death
MDD 01-074	M	45	7:00	02:30	6	6.55	1427	7.0	8.0	R DLPFC LACC	43	0	BZD, SSRI	SSRI	No	Pontine hemorrhage
MDD 02-051	M	81	6:00	15:30	6	6.50	1345	8.0	7.3	L DLPFC, LACC	43	3	antipsycho- sis, BZD, TCA	antipsychosis	No	Renal insufficiency
MDD 06-011	F	60	4:20	16:10	1	ND	1080	8.1	8.9	L DLPFC, LACC	33	1	antipsy- chosis, an- tiestrogen, BZD, Mo, SSRI	antipsycho- sis, cortico- steroid	No	Euthanasia due to carcinoma
MDD 06-026	M	70	7:15	08:00	3	6.50	1415	7.9	8.4	L DLPFC, LACC	43	1	antipsycho- sis, BZD, corticoste- roid	antipsycho- sis, BZD, Mo	Yes	Respiratory insufficiency
MDD 07-033	M	88	6:37	21:15	5	6.26	1225	7.0	7.3	L DLPFC, LACC	43	2	anticonvul- sant, BZD, Li, Mo	anticonvul- sant, BZD, Mo	Yes	Multiple epileptic seizures
BD 00-074	M	78	7:35	23:00	6	6.27	1227	6.7	7.3	L DLPFC, LACC	33	0	antipsycho- sis, BZD, Li	BZD	Yes	Colon cancer
BD 00-088	M	73	5:15	09:30	7	6.38	1260	7.5	7.2	R DLPFC, LACC	33	2	BZD, CBZ, ECT, Li	Mo	Yes	Dehydration
BD 00-111	M	70	4:50	02:45	10	6.26	1490	-	6.9	L DLPFC	33	1	antipsycho- sis, BZD, ECT, Li	antipsycho- sis, Mo	No	Cardiac arrest, ileus
BD 02-014	M	68	16:46	00:00	2	6.64	1424	7.2	8.1	L DLPFC, LACC	33	1	antipsycho- sis, CBZ, Li, MAOI	None	No	Subdural hematoma

S-Table 2 Continued, Clinico-pathological information of patients with MDD, BD and control subjects (NBB collection)

NBB number	Sex	Age (y)	PMD (h)	CTD (h)	MTD	Brain pH	BW (g)	RIN (ACC)	RIN (DLPFC)	Brain Region	ApoE	Braak stage	Medication in the past	Medication (last 3 months)	Suicide Ideation	Cause of death
BD 06-021	M	70	6:23	13:07	3	6.53	1488	7.0	7.1	L DLPFC, LACC	33	3	Li	BZD, Li	No	Cerebral Contusion, pneumonia
BD 06-075	F	80	9:30	09:30	10	6.33	1190	6.3	6.3	L DLPFC, LACC	33	1	Li	corticosteroid, Li	No	Acute heart death
BD 07-076	F	79	7:25	03:10	11	6.26	1231	-	7.2	L DLPFC	43	2	BZD, CBZ, ECT	CBZ, Mo	No	ND
BD 97-058	F	90	6:30	10:15	5	ND	1143	7.1	7.4	L DLPFC, LACC	32	3	ECT, Li, MAOI, TCA	MAOI	No	Lung embolism
BD 99-118	M	68	5:55	23:15	10	6.82	1204	-	7.7	L DLPFC	32	1	Li	None	Yes	Cardiac ischemia
BD 07-060	M	93	6:00	21:10	9	6.37	1459	7.7	-	LACC	33	1	BZD, TCA, SSRI	BZD	No	Cachexia, renal failure
Ctrl 00-067	M	73	12:45	00:01	6	ND	1267	-	7.8	L DLPFC	33	0	ND	ND	-	Massive lung embolism
Ctrl 01-033	M	75	6:20	06:10	3	6.18	1180	-	7.3	L DLPFC	43	1	None	Mo	-	Dehydration, adenocarcinoma, pneumonia
Ctrl 01-086	M	88	7:00	03:00	7	6.84	1398	-	8.1	L DLPFC	32	1	BZD, corticosteroid	Mo	-	Heart failure
Ctrl 04-049	F	77	8:20	07:55	7	6.48	1312	5.9	6.1	L DLPFC, LACC	32	1	BZD	TCA	-	Cachexia and uremia
Ctrl 04-057	F	81	6:40	13:10	8	7.16	1164	7.5	8.2	L DLPFC, LACC	33	1	None	Mo	-	Euthanasia

S-Table 2 Continued, Clinico-pathological information of patients with MDD, BD and control subjects (NBB collection)

NBB number	Sex	Age (y)	PMD (h)	CTD (h)	MTD	Brain pH	BW (g)	RIN (ACC)	RIN (DLPFC)	Brain Region	ApoE	Braak stage	Medication in the past	Medication (last 3 months)	Suicide Ideation	Cause of death
Ctr 05-019	M	74	5:00	02:00	4	6.70	1125	8.1	8.3	L DLPFC, LACC	43	3	corticosteroid, Salbutamol	antipsychosis, BZD, Mo	-	Bronchus carcinoma
Ctr 05-034	M	56	14:00	00:01	5	7.03	1323	-	8.5	L DLPFC	33	0	ND	ND	-	Heart failure
Ctr 05-044	M	80	7:15	07:15	6	5.80	1376	5.2	5.7	L DLPFC, LACC	33	0	None	Mo	-	Cachexia and dehydration
Ctr 05-068	M	56	9:15	04:45	10	6.54	1553	7.9	-	LACC	43	0	None	None	-	Acute myocardial infarction
Ctr 06-037	M	66	7:45	17:45	5	6.70	1590	-	7.8	L DLPFC	33	0	corticosteroid	corticosteroid	-	Ruptured abdominal aorta aneurysm
Ctr 96-052	M	73	9:10	11:30	5	ND	1500	-	8.5	L DLPFC	43	2	None	None	-	Cardiac arrest
Ctr 97-039	M	87	4:00	15:00	4	7.39	1506	-	8.3	L DLPFC	33	3	None	None	-	Myocardial infarction
Ctr 97-156	F	77	2:40	08:30	11	6.37	1235	-	8.2	L DLPFC	33	1	None	None	-	Septic shock, pancreas carcinoma
Ctr 98-006	M	50	8:30	11:00	1	6.65	1436	-	7.5	L DLPFC	43	0	None	None	-	Cardiac arrest, septic shock
Ctr 99-111	F	88	5:40	03:05	9	6.67	1054	-	6.9	L DLPFC	33	3	BZD, Prednisolone	None	-	Cardiac decompensation, peritonitis

S-Table 2 Continued, Clinico-pathological information of patients with MDD, BD and control subjects (NBB collection)

NBB number	Sex	Age (y)	PMD (h)	CTD (h)	MTD	Brain pH	BW (g)	RIN (ACC)	RIN (DLPFC)	Brain Region	ApoE	Braak stage	Medication in the past	Medication (last 3 months)	Suicide Ideation	Cause of death
Ctrl	M	87	10:20	04:00	3	6.32	1356	7.5	-	L ACC	33	1	ND	Mo	-	Pneumonia, heart infarction
Ctrl	F	89	6:25	21:30	12	6.46	1210	7.1	-	L ACC	32	2	antipsychosis, BZD	None	-	Ruptured abdominal aorta aneurysm
Ctrl	M	79	6:00	06:10	10	6.51	1392	8.6	-	L ACC	33	1	BZD, steroid drug	None	-	Metastasized adenocarcinoma and lung carcinoma
Ctrl	M	68	10:10	09:05	4	7.08	1547	8.5	-	L ACC	33	2	None	Epinephrine	-	Heart infarction
Ctrl	M	80	4:30	14:30	6	6.22	1400	5.4	-	L ACC	33	2	chemo, Mo, steroid drug	None	-	Renal failure
Ctrl	M	87	6:05	08:05	10	6.96	1568	7.8	-	L ACC	33	3	None	None	-	-
Ctrl	M	96	5:23	13:27	2	6.70	1204	7.4	-	L ACC	33	1	None	Mo	-	Pneumonia

Abbreviations: ACC, anterior cingulate cortex; BD, bipolar disorder; BZD, benzodiazepine; BW, brain weight; CBZ, carbamazepine; CTD, clock time of death; Ctrl, control; DLPFC, dorsolateral prefrontal cortex; MTD, month time of death; ECT, electro-convulsive treatment; F, female; L, left; Li, lithium; MAOI, MAO inhibitor; MDD, major disorder depression; Mo, morphine; M, male; NBB, Netherlands Brain Bank; ND, no data; PMD, postmortem delay; R, right; RIN, RNA integrity number; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant.

S-Table 3 Information on gene and primers

Gene	Official full name	Primer sequences (Forward)	Primer sequences (Reverse)	NCBI reference number	Amplicon length (bp)
CRH related genes					
AR	Androgen receptor	GTCAACTCCAGGATGCTCTACT	AGTGCCCTCATTCGGACA	NM_000044	101
AVPR1a	vasopressin receptor-1 α	CTTTTGTGATCGTGACGGCTTA	TGATGGTAGGGTTTTCCGATTC	NM_000706	75
CREB	cAMP-response element-binding protein	ACCACTGTAACGGTGCCAAC	CTCCTCCCTGGGTAATGG	NM_134442.73	73
CRHR1	Corticotrophin-releasing hormone receptor 1	CGGGTCGTCTCATCTACTTCA	TGGCAGAAGCGGACCTCACT	NM_004382	100
CRHR2	Corticotrophin-releasing hormone receptor 2	CCACATCCGAGACAATCCA	CGAAGAAAGCATGTAGGTGA	NM_001883	87
CRH	Corticotrophin-releasing hormone	CATCCCTGGATCTCACCTTC	AATAATCTCCAATGAGTTTCCTGTTG	NM_000756	109
CRHBP	Corticotrophin-releasing hormone binding protein	AGGTTGCGAGGGGAATAGGAG	GGCGTCATCTTGGAAAGGG	NM_001882	96
ER1	Estrogen receptor 1	TCTTGGACAGGAAACAGGGAA	CGGAACCGAGATGATGTAGCC	NM_000125	82
ER2	Estrogen receptor 2	TGCTTTGGTTTGGGTGATTG	TTCCATGCCCTTGTACTCG	NM_001437	118
GR α	Glucocorticoid receptor α	CCATTGTCAAAGAGGGAAAGGAA	TGTTTGGAAAGCAATAGTTAAGGAG	NM_001018077	119
MR	Mineralocorticoid receptor	AAGTCGTGAAGTGGGCAAAG	CCAAGAATACTGGATTAGGGT	NM_000901	83
HSP70	Heat shock protein 70	AGGCTGGTGAACCACTTCGT	CGGCTCGCTTGTTCCTGGCT	NM_005345	73
HSP90	Heat shock protein 90	CGCTCCTGTCTTCTGGCTTC	TGTTATCATCAGCAGTAGGGTCA	NM_005348	117
IL1 β	Interleukin-1 β	CCGACCACCACTACAGCAA	GGCAGGGAACCCAGCATCT	NM_000576	86
TNF α	Tumor necrosis factor- α	GGCGTGGAGCTGAGAGATA	CAGCCTTGGCCCTTGAAGA	NM_000594	88
UCN3	Urocotin3	CGGGGAGAGATCTGTATGC	GAAGATGGGCTTGGCTTTGTAG	NM_053049	108
Retinoid signaling, BDNF and TrkB related genes					
CRABP1	Cellular retinoic acid-binding protein 1	AGAAGGCTTTGAGGAGGAGA	TCACGGGTCCAGTAGGTTTT	NM_004378	132
CRABP2	Cellular retinoic acid-binding protein 2	ACATCAAAAACCTCCACCACC	CCCATTTCCACCAGGCTCTTA	NM_001878	111

S- Table 3 Continued, Information on gene and primers

Gene	Official full name	Primer sequences (Forward)	Primer sequences (Reverse)	NCBI reference number	Amplicon length (bp)
CYP26A1	Cytochrome P450, family 26, subfamily A, polypeptide 1	GGCCTAAAGCAATCTTCAAC	TTACTCTTCAGCTCTTCTCGC	NM_057157	135
CYP26B1	Cytochrome P450, family 26, subfamily B, polypeptide 1	GTGCCTCTGCCTTATCCC	CGCCTCCAAATCTACCTGA	NM_019885	92
CYP26C1	Cytochrome P450, family 26, subfamily C, polypeptide 1	GCCCTCGACTAATCATTCAC	CACAGCGACTCCTTCAGC	NM_183374	78
RALDH1	Retinaldehyde dehydrogenase 1	ACTTACCTGTCTACTCACCG	TATCTCCTTCTTCTACCTGGC	NM_000689	147
RALDH2	Retinaldehyde dehydrogenase 2	TCACAGGGTCTACTGAGGTT	CACAGCATAGTCCAAGTCAGC	NM_003888	134
RALDH3	Retinaldehyde dehydrogenase 3	AAAGAGCGGAATAGCACCGACT	AAGCCACCAAATGGAGCCTGT	NM_000693	151
RAR α	Retinoic acid receptor α	CTGGGCAAAATACACTACGAAC	TGGCGAACTCCACAGTCTTA	NM_000964	118
RAR β	Retinoic acid receptor β	CTAAAGGTGCAGAGCGTGTA	TTGGGGTCAAGGGTTTCAT	NM_000965	111
RAR γ	Retinoic acid receptor γ	AGACCCTGCCATTCACAGA	GCTCAGCCCTTCTCCTTTTG	NM_000966	104
RXR α	Retinoid X receptor α	AGCGGTTAGCAGTGTTTTGTG	TGCCTTGAGATAITTCATTGGA	NM_002957	134
RXR β	Retinoid X receptor β	GGGCAGAACCCAAAGAACATAAAC	ATGGAGAACCCCGACAAAT	NM_021976	134
RXR γ	Retinoid X receptor γ	TCTCAAACCGCACTATGTGGACTC	CAAGATGGTCAATCGGTTCCCTA	NM_006917	111
TrkB,FL	Tropomyosin-related kinase B, full length isoform	GGAGTAAACACTCCATCTTCTTCG	TGGTGATGCCAAAAGTACTGG	NM_006180	102
TrkB,T1	Tropomyosin-related kinase B, truncated isoform 1	CACTCCAAGTTTGGCAATGAA	CACCACAGCCCCCTTTTCTC	NM_001007097	98
TrkB,total	Tropomyosin-related kinase B, all isoforms	ATGACATCGGGGACACCA	CACAGACGCAATCACACC	NM_006180	113
BDNF	Brain-derived neurotrophic factor	AACCTTCTGCCCCATCCTGT	GCTCCAAACTTGACTTCTCC	NM_170731	85
NOS related genes					
NOS1	Nitric oxide synthase 1, neuronal	AGCAGTTTGGCTCCCTA	GTTCTTGCCCCCATTTCC	NM_000620.2	101
NOS2	Nitric oxide synthase 2, inducible	GCCCTTACTTGACCTCC	AGTTCCATCTTTCACCCAC	NM_000625	133
NOS3	Nitric oxide synthase 3, endothelial cell	CGAGTGAAGGGGACAAT	TCCATACACAGGACCCG	NM_000603	113

S- Table 3 Continued, Information on gene and primers

Gene	Official full name	Primer sequences (Forward)	Primer sequences (Reverse)	NCBI reference number	Amplicon length (bp)
NIDD	Neuronal nitric oxide synthase (nNOS)-interacting DHHC domain-containing protein with dendritic mRNA	TCTCAACAATCGCACAACA	ACCAGTCCTCCTTCGGCTT	NM_173570.3	93
Monoamine related genes					
MAOA	Monoamine oxidase A	TAAATGGTCTCGGGAAGGTG	CCAGAAACAGAGGGCAGGT	NM_000240.3	118
MAOB	Monoamine oxidase B	TCTCTGCTCTCTGGTTCTCTG	TCTCTGTCTCCTCCATTTGTT	NM_000898.4	84
COMT	Catechol-O-methyltransferase	CTGGGAAACGAAAGAGGAGT	GGGGAGTAGGGAAGGAGAT	NM_000754.3	139
5HT1A	5-hydroxytryptamine (serotonin) receptor 1A	GTGAGGCAAGGTGACGAT	GGCACAAAGGGGTAGGAC	NM_000524.3	111
5HT2A	5-hydroxytryptamine (serotonin) receptor 2A	CTACACAGGCAGGAGGACTA	CACCACATCACCCACAACACAG	NM_000621.4	96
ADRA1A	Adrenoceptor α 1A	GACGGATGGCGTTTGIG	CAGGAGGATTTGGTCTTTGG	NM_033304.2	87
ADRA2A	Adrenoceptor α 2A	GTGTTCCCTCTCTCCCTCCA	CCCCATAGTCAAGCACTTTTCG	NM_000681.3	104
ADRB1	Adrenoceptor β 1	AGCCCACAATCCTCGTCTG	CTCCCATCCCTTCCCAAAC	NM_000684.2	88
DRD1	dopamine receptor D1	AAGGCAGTGGCTGAGATTG	ACACAGAGTTGAGGATGGA	NM_000794.3	107
DRD2	dopamine receptor D2	TTTCCCTCTCTCCTGTTTCCC	GATGGTTTTCAGCAGCCTCT	NM_000795.3	80
Reference genes					
RN18S	18S ribosomal RNA	TTCGTATTGGCCCGCTAGA	TGGCAAATGCTTTCCGCTCT	NR_003286	70
ACT β	Actin, beta	CCCAGGGATGACGTTGCTA	TCACGGGAGTCCATCAGGAT	NM_001101	65
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	CAATTCCATGGCACCCGTC	TCTCGCTCCTGGAAGATGGT	NM_002046	62
HPRT1	Hypoxanthine phosphoribosyltransferase 1	GGACAGGACTGAACGCTCTGC	ATAGCCCCCCTTTGAGCACAC	NM_000194	88
TUB α	Tubulin, alpha 1b	CTTTGAGCCAGCCAACCAGA	GTACAA CAGGCAGCAAGCCAT	NM_006082	72
UBC	Ubiquitin C	GCTGTCTATAAGACTCGGCC	GTCAGCCAAAGTCCCGTCTTA	NM_021009	70
TUB β 4	Tubulin, beta 4	GGGCCAAAGTTTTGGGAGGT	CACTGTCCCCATGGTATGTGC	NM_006087	71