The epigenetic effects of antidepressant treatment on human prefrontal cortex BDNF expression

Convergent lines of evidence suggest that major depression is associated with neurotrophin alterations, particularly decreased brain-derived neurotrophic factor (BDNF) levels (Martinowich et al. 2007). The gene that codes for BDNF has distinct splice variants, each one regulated by a specific promoter region (Tsankova et al. 2004). Of these variants, BDNF transcript IV is the most commonly studied and its expression changes have been associated with behavioural responses following antidepressant treatment in animal models of depression (Bredy et al. 2007; Tsankova et al. 2006).

In a recent study investigating mice exposed to chronic social defeat stress, a model of depression, Tsankova and colleagues (2006) reported that a 4-fold increase in histone H3 lysine 27 (H3K27) methylation was associated with repression of BDNF IV expression. Treatment of the chronically defeated mice with imipramine increased expression of BDNF IV to baseline levels, but could not reverse the alteration of H3K27 methylation.

To assess the relationship between major depression, antidepressant medication, BDNF IV expression, and H3K27 methylation at the BDNF IV promoter in humans, we quantified BDNF IV expression and H3K27 tri-methylation levels in prefrontal cortex of control subjects with no psychiatric history (n = 9, Con), major depressive disorder (MDD) subjects without positive toxicology for antidepressants or history of antidepressant use (n = 11, AD –), and MDD subjects with a history of antidepressant use and with antidepressant detected in post-mortem toxicology (n = 7, AD +). Antidepressants used included: fluoxetine (1/7), venlafaxine (2/7), clomipramine (1/7), amitriptyline (1/7), citalopram (1/7) and doxepine (1/7). Toxicological screens were performed using mass spectrometry with known standards for all commercially available antidepressants and psychotropic compounds, as well as illicit drugs. To reduce sample heterogeneity, no subjects with psychotic symptoms, bipolar disorder or with positive toxicology for alcohol or illicit drugs were used in this study, and all subjects were male Caucasians of French Canadian origin. All MDD subjects died by suicide and diagnoses for MDD cases and controls were made using proxy-based SCID-1 interviews complemented by hospital records, as described previously (Dumais et al. 2005). Analysis of covariance revealed no significant associations between post-mortem index, brain pH, age, and BDNF expression or H3K27 methylation. All research was approved by the Internal Review Board of McGill University and informed consent was received from next-of-kin.

After extracting RNA from BA 10 from all subjects and testing RNA quality (Agilent Technologies, USA; RNA integrity number > 7), we performed RT–PCR on a 7500 real-time PCR system (Applied Biosystems, USA) using primers directed at BDNF IV – a homologous region to that used by Tsankova et al. (2006) (5’-GCTGCAGAACAGAAGGTACA-3’; antisense: 5’-GTTCCTCATCCAACAGCTTTCTATC-3’) and two control genes, GAPDH and β-actin. The AD+ group showed higher levels of BNDIV expression than AD – (F = 4.395, p = 0.024; post-hoc Tukey test significance values: C vs. AD – 0.72; C vs. AD + 0.109; AD – vs. AD+ 0.020; Fig. 1 a).

ChIP assays were performed as detailed previously (Huang et al. 2006). Briefly, we extracted DNA from BA 10 from all subjects and immunoprecipitated DNA with an antibody directed at H3K27 tri-methyl residue (Upstate Biotechnology, USA). First, positive (GRIN2A) and negative (haemoglobin gene, HBB) controls were used in all cases and specificity was assessed by using non-immune immunoglobulin and a no-antibody pull-down. Next, input and bound DNA fractions were amplified using primers targeted to the human homolog of the BDNF region used by Tsankova et al. (2006) (5’-GTGAGAACCCTGGGGC-3’; antisense: 5’-ACGGAAAAAGGGAGGTAAGAAG-3’). Input and bound DNA were run on the same plates for all subjects and data were generated by
We found a significant difference in H3K27 trimethylation levels among groups ($F = 9.379$, $p = 0.001$; Fig. 1b). Post-hoc Tukey analysis revealed that the AD+ group presented significantly lower H3K27 methylation levels than AD− and control groups (C vs. AD− 0.914; C vs. AD+ 0.004; AD− vs. AD+ 0.001).

To ensure the specificity of these findings we performed qRT–PCR in identical cDNA as that used for BDNF IV promoter. We assessed expression changes using primers directed at BDNF I (5′-CACCCAGCTCCA-AGTGTGT-3′; antisense: 5′-TAGCAGCTTACACGC-CAAAAG-3′) and BDNF II (5′-CGCTAGGAAGCCA-CCTCAG-3′; antisense: 5′-CTGTGCACCCTAAA-AAGC-3′) as well as TrkC (5′-CCTGGCTCTGCATA-GAAAC-3′; antisense: 5′-AACAGACCTTGGTAA-TGC-3′) with β-actin as internal control. No significant difference was observed between groups (BDNF I: $F = 1.18$, $p = 0.32$; BDNF II: $F = 1.84$, $p = 0.18$; TrkC: $F = 1.60$, $p = 0.22$).

To demonstrate the specificity of the H3K27 trimethylation findings, using identical input and bound DNA as that used for BDNF IV promoter, we assessed primers targeting the promoter region of β-actin (5′-CACGGCTCGTTATGCTGT-3′; antisense: 5′-TGCAAGGAGGAGGTCTTC-3′). We found no significant difference between the three groups when H3K27 tri-methylation status was assessed ($F = 2.639$, $p = 0.10$).

In mice, chronic stress decreases the expression of BDNF IV and increases H3K27 methylation. In humans with major depression not treated with antidepressants, we found no significant difference in BDNF IV expression or H3K27 methylation. The most robust results we observed were in subjects treated with antidepressants. In MDD cases, antidepressants increased BDNF IV expression above baseline level and significantly decreased H3K27 tri-methylation levels. This effect seems to be common to different antidepressant classes (Nibuya et al. 1995), thus the fact that different subjects were taking different antidepressants seems not to be a confounding factor in the present study.

These results differ somewhat from other human brain studies investigating the relationship between BDNF expression and major depression. We did not observe a significant decrease in BDNF I, II, or III expression between subjects with MDD not treated with antidepressants and controls. This may be due to the small sample size used in this study. It is unclear to what extent past human studies reporting a link between decreased BDNF expression in brain and MDD have controlled for antidepressant use, but the present study would suggest that this could be important given that antidepressants appear to increase BDNF IV expression.

Our study should be viewed in the context of its limitations and small sample size. This small sample size should, however, be viewed in the context of reduced sample heterogeneity (all subjects were Caucasian, male, French Canadian, and negative for illicit drugs). As with any human study, we could not control for medication compliance or collect subjects on a single class of antidepressant. Nevertheless, our results are an important complement to other work on antidepressants and BDNF. Our data suggest, in humans, that antidepressants are associated with a
decrease in methylation at H3K27 and an increase in BDNF IV expression.

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Statement of Interest
None.

References


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