(H3K27me3), a modification associated with transcriptional repression. Long-term imipramine treatment reversed BDNF-IV down-regulation to baseline levels.[7] Studies by our group in the postmortem brain of depressed subjects with or without history of antidepressant treatment compared with controls showed an increased expression of BDNF-IV and a decrease of H3K27 trimethylation levels in subjects treated with antidepressants only.[8]

In order to investigate the epigenetic regulation of BDNF in MDD patients according to antidepressant treatment, we conducted a prospective study in 25 treatment-naive MDD patients. All patients had Hamilton Rating Scale for Depression (HAM-D) scores $\geqslant 24$ at baseline ($N = 25$, $X = 29.4 \pm 1.2$). All participants gave written informed consent for this study, which was approved by our Institutional Review Board.

Subjects were excluded from the study if they had comorbidity with other major psychiatric disorders, if they had positive tests for illicit drugs at any point during the study or if they had general medical illnesses. Patients (12 males and 13 females) were treated with citalopram, starting with an initial dose of 10 mg die, which was titrated progressively to a maximum of 60 mg die. All final doses were within the therapeutic range and blood levels of total BDNF and H3K27me3 were measured at baseline (T0) and after 8 weeks (T8) of treatment. Subject treatment compliance was assessed using high-performance liquid chromatography at the end of the trial. All subjects showed detectable plasma citalopram levels and we observed a significant correlation between citalopram dose and plasma concentration (Spearman's $r = 0.54$; $P = 0.005$).

A repeated-measures ANOVA with Bonferroni correction revealed that in line with previous findings, the expression of peripheral BDNF mRNA in depressed patients ($N = 25$) was significantly elevated after 8 weeks of citalopram treatment (FC $= 34\%$; $P < 0.001$; Figure 1a). Subjects were classified into responders (RES) and non-responders (NRES), based on changes to the HAM-D scores. We defined response as 8-week HAM-D scores < 9, whereas non-response was defined as 8-week HAM-D scores $> 50\%$ reduction in baseline HAM-D scores (T8; RES $= 5.6 \pm 0.7$; $N = 15$; NRES $= 17.0 \pm 1.4$; $N = 10$). The RES group showed increased BDNF levels (T8–T0; FC $= 49\%$; $P < 0.001$; Figure 1a) after treatment, whereas there was no significant difference in the NRES group (T8–T0; FC $= 3\%$; $P > 0.05$; Figure 1a). Consistently, RES had higher T8 BDNF levels as compared with NRES (T-test $P < 0.05$; Figure 1c), whereas there was no difference at T0 (T-test $P = 0.98$; Figure 1c). Finally, we found a significant correlation between change in depression severity and change in BDNF expression (Pearson's $r = 0.49$; $R^2 = 0.25$; $P < 0.05$; Figure 1f). These findings indicated a relationship between peripheral BDNF expression and citalopram treatment response.

To investigate the role of chromatin modifications in BDNF expression changes, based on previous findings in rodents,[7] we performed chromatin immunoprecipitation (ChIP) and found a significant decrease in H3K27me3 levels at promoter-IV of the BDNF gene after 8 weeks of citalopram treatment in all patients ($N = 25$) according to a repeated-measures ANOVA with Bonferroni correction (FC $= 31\%$; $P < 0.001$; Figure 1b). However, these results were explained primarily by changes in the RES group (FC $= 43\%$; $P < 0.001$; Figure 1b), and there was no significant difference in the NRES group ($P > 0.05$; Figure 1b). Consistently, we found a significant difference in H3K27me3 levels at T8 between groups (T-test $P < 0.01$; Figure 1d), but no difference at T0 (T-test $P > 0.05$; Figure 1d). Furthermore, we found a significant negative correlation between change in depression severity and change in H3K27me3 expression (Pearson's $r = -0.63$; $R^2 = 0.39$; $P < 0.01$; Figure 1g). Finally, total BDNF and H3K27me3 levels were significantly negatively correlated (Pearson's $r = -0.86$; $R^2 = 0.75$; $P < 0.0001$; Figure 1e).

To our knowledge, this is the first study that translates to human findings previously reported in an animal model of depression,[7] reporting evidence suggesting that antidepressants regulate BDNF expression through alterations of promoter-IV H3K27me3 levels. Our results suggest that changes in H3K27 methylation state of the BDNF promoter IV and BDNF expression levels in peripheral tissue are biomarker correlates of antidepressant response. Finally, these findings are preliminary and await confirmation by larger samples and alternative designs. Moreover, additional work is necessary to better understand the relationship between epigenetic modifications and the exon-specific regulation of BDNF, as this has the potential to lead to new therapeutic options for MDD.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

JP Lopez, F Mamdani, B Labonte, M-M Beaulieu, JP Yang, MT Berlim, C Ernst and G Turecki
McGill Group for Suicide Studies, Douglas Mental Health University Institute, McGill University, Montreal, QC, Canada
E-mail: gustavo.turecki@mcgill.ca

REFERENCES

1 Dwivedi Y. *Neuropsychiatr Dis Treat* 2009; **5**: 433–449.
2 Molendijk ML, Bus BA, Spinhoven P, Penninx BW, Kenis G, Prickaerts J et al. *Mol Psychiatry* 2011; **16**: 1088–1095.
3 Pruunsild P, Kazantseva A, Aid T, Palm K, Timmusk T. *Genomics* 2007; **90**: 397–406.
4 Boulle F, van den Hove DL, Jakob SB, Rutten BP, Hamon M, van Os J et al. *Mol Psychiatry* advance online publication, 6 September 2011; doi:10.1038/mp.2011.107.
5 Tao X, West AE, Chen WG, Corfas G, Greenberg ME. *Neuron* 2002; **33**: 383–395.
6 Martinowich K, Hattori D, Wu H, Fouse S, He F, Hu Y et al. *Science* 2003; **302**: 890–893.
7 Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ. *Nat Neurosci* 2006; **9**: 519–525.
8 Chen ES, Ernst C, Turecki G. *Int J Neuropsychopharmacol* 2011; **14**: 427–429.

Linking *in vivo* brain serotonin type 1B receptor density to phenotypic heterogeneity of posttraumatic stress symptomatology

Brain serotonergic (5-HT) dysfunction has been linked to the neurobiology of posttraumatic stress disorder (PTSD) in both preclinical and clinical studies.[1–3] Animal studies have found that stress exposure reduces serotonin type 1B (5-HT$_{1B}$) receptor function in multiple brain regions, which consequently results in behaviors that resemble chronic anxiety.[4] Recent work from our group has suggested that humans with PTSD have markedly reduced 5-HT$_{1B}$ receptor density in a neural circuit that has been consistently implicated in this disorder, including the caudate, amygdala and anterior cingulate cortex.[2] However, no study of which we are aware has examined how these abnormalities are linked to the most up-to-date and refined phenotypic model of PTSD symptom dimensionality.

Table 1. Analyses evaluating associations between posttraumatic stress disorder (PTSD) symptom clusters and regional [^{11}C]P943-binding potential values in adults with PTSD ($n = 49$)

Spearman's rank correlations

	Reexperiencing symptoms	Avoidance symptoms	Numbing symptoms	Dysphoric arousal symptoms	Anxious arousal symptoms
Clinical variables					
Major depression	**0.30***	**0.30***	0.23	−0.07	0.07
Age of first trauma	**−0.33***	−0.24	−0.27^	−0.10	−0.18
Number of traumas	0.10	0.20	**0.37***	0.15	0.07
[^{11}C]P943-binding potential					
Anterior cingulate cortex	−0.27^	−0.23	−0.21	−0.19	−0.18
Amygdala	−0.06	0.10	−0.24	−0.07	−0.21
Hippocampus	−0.19	−0.21	**−0.32***	0.06	−0.21
Caudate	−0.25	−0.19	−0.13	−0.12	−0.03
Pallidum	−0.25^	−0.12	−0.26^	−0.17	**−0.43****

Stepwise linear regressions

	Reexperiencing symptoms	Avoidance symptoms	Numbing symptoms	Dysphoric arousal symptoms	Anxious arousal symptoms
R^2	0.13	0.10	0.26	—	0.17
Major depression	0.18	**0.31***	—	—	—
Age of first trauma	**−0.36***	—	0.04	—	—
Number of traumas	—	—	**0.34***	—	—
Anterior cingulate cortex	−0.19	—	—	—	—
Amygdala	—	—	—	—	—
Hippocampus	—	—	**−0.35***	—	—
Caudate	—	—	—	—	—
Pallidum	−0.26^	—	−0.18	—	**−0.41****

^$P < 0.10$; *$P < 0.05$; **$P < 0.01$. Statistically significant correlations and regression weights ($P < 0.05$) are highlighted in bold. Regression results are adjusted for clinical variables associated with PTSD symptoms clusters at $p < 10$ level in bivariate analyses.

According to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV), PTSD is comprised of three clusters of reexperiencing, avoidance/numbing and hyperarousal symptoms. However, a growing body of confirmatory factor analytic studies has suggested that a five-factor model of reexperiencing, avoidance, numbing, dysphoric arousal (for example, irritability) and anxious arousal (for example, hypervigilance) symptoms provides a significantly better representation of PTSD symptom structure than the DSM-IV or alternative four-factor models.[5–7]

Although confirmatory factor analysis-derived models of PTSD symptom dimensionality provide a useful means of understanding the clinical phenotype of this disorder, neurobiological correlates of these symptom dimensions are unknown. Thus, in this study, we sought to examine how 5-HT$_{1B}$ receptor expression is related to the five-factor model of PTSD symptom structure in an *a priori*-defined limbic corticostriatal circuit.

Data from 49 individuals with PTSD were analyzed (mean age = 32.0, s.d. = 9.3, range = 19–54; 53.1% male; 46.9% white). Additional details regarding the sample are available elsewhere.[2] PTSD was assessed using the Clinician-Administered PTSD Scale for DSM-IV. None of the participants were receiving psychotropic medication or psychotherapy for at least 4 weeks before scanning. Participants underwent a resting positron emission tomography scan using a recently developed 5-HT$_{1B}$ receptor-selective radio-tracer [^{11}C]P943 to assess regional [^{11}C]P943-binding potential (BP$_{ND}$), a measure of 5-HT$_{1B}$ receptor density.

Table 1 shows results of Spearman's rank correlation and stepwise linear regression analyses of the relation between clinical variables, regional BP$_{ND}$ values, and PTSD symptom clusters; variables associated with dependent variables at the $P < 0.10$ level were entered into the regression analyses. Major depression correlated positively with reexperiencing and avoidance symptoms;

age of first trauma correlated negatively with reexperiencing symptoms and number of traumas correlated positively with numbing symptoms. Greater BP$_{ND}$ values in the anterior cingulate cortex correlated marginally negatively with reexperiencing symptoms; greater BP$_{ND}$ in the hippocampus correlated negatively with numbing symptoms and greater BP$_{ND}$ in the pallidum correlated negatively with anxious arousal symptoms; and marginally negatively with reexperiencing and numbing symptoms; BP$_{ND}$ values in the amygdala and caudate were not associated with any of the symptom clusters. In regression analyses adjusted for clinical characteristics associated with PTSD symptom clusters, BP$_{ND}$ in the hippocampus was independently negatively associated with numbing symptoms and BP$_{ND}$ in the pallidum was independently negatively associated with anxious arousal symptoms. None of the other regional BP$_{ND}$ values were significant in these analyses.

These data further substantiate the role of the 5HT$_{1B}$ receptor in PTSD. Serotonin, via 5-HT$_{1B}$ autoreceptors, modulates cortical inhibitory input onto subcortical structures. In addition, several neuropathways besides 5-HT appear to be involved in the modulation of anxiety states in PTSD, and 5-HT$_{1B}$ heteroreceptors have a critical role in this process. The pallidum and hippocampus regions contain particularly high levels of 5-HT$_{1B}$ heteroreceptors on GABAergic and glutamatergic neurons.[1] Alterations in 5HT$_{1B}$-receptor function might contribute to PTSD-related anxious arousal symptoms by influencing 5-HT input to the pallidum by their role as 5-HT terminal autoreceptors, and glutamatergic activity within the pallidum by heteroreceptors on corticofugal projections.[8] Reduced 5-HT$_{1B}$ receptors in the hippocampus may affect the balance between excitatory and inhibitory neurotrans-mission,[9] which is modulated by 5-HT$_{1B}$ receptors, and ultimately alter modulation of GABAergic outputs,[10] thereby resulting in behavioral inhibition and numbing.

Altogether, herein we show that alterations in 5HT$_{1B}$-receptor density are differentially linked to a novel, five-factor model of PTSD symptoms, and may therefore explain aspects of the clinical phenotype of this disorder. These data have important implications for understanding the typically complex phenomenology of PTSD and may help inform theoretical models of PTSD symptom dimensionality, as well as approaches to assessment and treatment.

CONFLICT OF INTEREST

RH Pietrzak[1,2], S Henry[1,2], SM Southwick[1,2], JH Krystal[1,2] and A Neumeister[3]
[1]National Center for Posttraumatic Stress Disorder, Clinical Neurosciences Division, VA Connecticut Healthcare System, West Haven, CT, USA;
[2]Department of Psychiatry, Yale University School of Medicine, West Haven, CT, USA and
[3]Department of Psychiatry and Radiology, New York University School of Medicine, New York, NY, USA
E-mail: alexander.neumeister@nyumc.org

REFERENCES

1 Sari Y. Neurosci Biobehav Rev 2004; 28: 565–582.
2 Murrough JW, Czermak C, Henry S, Nabulsi N, Gallezot JD, Gueorguieva R et al. Arch Gen Psychiatry 2011; 68: 892–900.
3 Murrough JW, Huang Y, Hu J, Henry S, Williams W, Gallezot JD et al. Biol Psychiatry 2011; 70: 1033–1038.
4 Bibancos T, Jardim DL, Aneas I, Chiavegatto S. Genes Brain Behav 2007; 6: 529–539.
5 Elhai JD, Biehn TL, Armour C, Klopper JJ, Frueh BC, Palmieri PA. J Anxiety Disord 2011; 25: 340–345.
6 Wang L, Zhang J, Shi Z, Zhou M, Li Z, Zhang K et al. J Anxiety Disord 2011; 25: 771–776.
7 Pietrzak RH, Tsai J, Harpaz-Rotem I, Whealin JM, Southwick SM. J Psychiatr Res 2012; 46: 317–322.
8 Morikawa H, Manzoni OJ, Crabbe JC, Williams JT. Mol Pharmacol 2000; 58: 1271–1278.
9 Hoebel BG, Avena NM, Rada P. Curr Opin Pharmacol 2007; 7: 617–627.
10 Lopes AP, da Cunha IC, Steffens SM, Ferraz A, Vargas JC, de Lima TC et al. Behav Brain Res 2007; 184: 142–149.

DRD2/DRD4 heteromerization may influence genetic susceptibility to alcohol dependence

A major limitation of candidate gene studies in psychiatric genetics is the lack of evidence-based hypotheses for the study of gene–gene interactions. An emerging field that may provide such evidence is the analyses of oligomerization of G-protein-coupled receptors such as dopamine receptors (DRs). It has recently been shown that DRD4 and DRD2 are able to form heteromers and that specific variants in both genes may interfere in this process.[1,2] We wondered if this heteromerization process might help to elucidate the controversial but repeated findings regarding DRD4 and DRD2 genes in some behavioral phenotypes.

DRD4 gene has a well-studied 48-bp variable number tandem repeat (VNTR) polymorphism in exon 3. The most common variants are 2-, 4- and 7-repeats (2R, 4R and 7R, respectively), with the last one being frequently associated with psychiatric disorders. DRD2 is expressed in two distinct isoforms, the short variant named D$_{2S}$ (mainly presynaptic) and the long variant named D$_{2L}$ (mainly postsynaptic). The balanced expression of both isoforms may be disrupted by the DRD2 rs2283265 single-nucleotide polymorphism (SNP), where the minor T allele favors the inclusion of the sixth DRD2 exon, switching D$_{2S}$/D$_{2L}$ balance toward the long form.[3] This allele has been associated with reduced working memory and attention control[3] and with cocaine abuse.[4]

Gonzalez et al.,[2] on a recent Molecular Psychiatry article, observed that the short D$_{2S}$ is capable of forming heteromers with DRD4 2R and 4R, but not with the 7R variant. Additionally, Borroto-Escuela et al.[1] showed that the long D$_{2L}$ may form heteromers with all three main DRD4 variants, although it is less effective with the 7R variant. Based on these data, we tested if this functional interaction between molecules could be modulating previous genetic findings regarding alcohol dependence. We hypothesized that the effects of the presence of the DRD4 7R risk allele could be enhanced or attenuated according to the presence of the DRD2 T allele (as in T allele carriers an increased expression of the D$_{2L}$ isoform would be expected).

We tested if there is an interaction effect between the DRD4 7R variant and the DRD2 rs2283265T allele on the susceptibility to alcohol dependence. The DRD4 VNTR genotypes were obtained as described by Roman et al.[5] (where a description of allele frequencies for this sample is available). Subjects with rare alleles (not 2R, 4R or 7R) were excluded from all analyses, because in these cases no information on the heteromization pattern is available. DRD2 rs2283265 SNP was genotyped by Taqman allelic discrimination assay (frequencies for the G and T allele are 0.81 and 0.19, respectively). Subjects were classified according to the presence of DRD4 7R (7R carriers) and DRD2T (T carriers) alleles. All subjects included are Brazilian adult males of European descent, and the final sample size was of 104 patients with alcohol dependence and 259 healthy blood donor controls. A replication sample of 192 attention deficit hyperactivity disorder (ADHD) male patients, with information for comorbid alcoholism was also used. Both samples were diagnosed following DSM-IV criteria (detailed descriptions can be found in Bau et al.[6] and Grevet et al.[7]).

The logistic regression model revealed a significant interaction effect (likelihood ratio test, $P = 0.002$) between the studied polymorphisms. The estimation of odds ratio in the presence of interaction was performed by separating the logits at the two levels of the risk factor, followed by computing the exponentiated value of the difference between the two logits.[8] The odds ratio estimation (95% confidence interval) of alcohol dependence for the presence of DRD4 7R allele in GG homozygotes (T noncarriers) was 1.33 (0.75–2.34). This same estimation in T carriers was 0.26 (0.10–0.66). These results are consistent with the heteromerization-based hypothesis, showing a different effect of DRD4 variants according to the concomitant presence of either the DRD2 GG genotype (balanced expression of D$_{2S}$/D$_{2L}$) or the presence of the T allele (increased expression of D$_{2L}$). Among 7R carriers, 82.5% of patients with alcohol dependence are GG homozygotes (as opposed to 61.9% of controls), whereas among 7R noncarriers, 54.7% of patients present GG genotype (as opposed to 67.8% of controls). No main effect of DRD4 7R variant or DRD2 T allele on alcohol dependence was observed, which may be explained by