Title

Brain-derived neurotrophic factor association with amygdala response in major depressive disorder

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Abstract

Background: Brain-derived neurotrophic factor (BDNF) has an essential role in synaptic plasticity and neurogenesis. BDNF mediates amygdala-dependent learning for both aversive and appetitive emotional memories. The expression of BDNF in limbic regions is posited to contribute the development of depression, and amygdala responsivity is a potential marker of depressive state.

Methods: The present study examined the relationship between platelet BDNF levels and amygdala volume and function in major depressive disorder (MDD). Participants were 23 MDD (mean age 38.9 years) and 23 healthy controls (mean age 38.8 years). All participants were recruited from the community. MDD participants were in a current depressive episode of moderate severity and medication-free. Amygdala responses were acquired during a functional MRI task of implicit emotional processing with sad facial expressions.

Results: Significant correlation was observed between platelet BDNF levels and left amygdala responses, but no significant correlations were found with right amygdala responses or with amygdala volumes.

Limitations: Interactions with neuroprotective as well as neurotoxic metabolites in the kyneurenine pathway were not examined.

Conclusions: Relationship between BDNF levels and amygdala responsivity to emotionally salient stimuli in MDD could reflect the importance of BDNF in amygdala-dependent learning with clinical implications for potential pathways for treatment.

Keywords

BDNF; depression; amygdala; plasticity

Introduction

BDNF protein has an essential role in synaptic plasticity, neurogenesis and neuronal differentiation. BDNF crosses the blood brain barrier, and levels in the central nervous system are highly correlated with peripheral serum BDNF (Karege et al., 2002), which are interrelated with platelet BDNF (Serra-Millàs, 2016). Reduced serum and platelet BDNF levels have been observed in major depressive disorder (MDD), and the neurotrophin model proposes that stress decreases the expression of BDNF in limbic regions contributing to the development of depression (Duman and Monteggia, 2006; Serra-Millàs, 2016).

Amygdala responsivity is a potential marker of depressive state which shows a demonstrated specificity to sad faces with increased activation during a current depressive episode that normalises following treatment (Fu et al., 2004; Arnone et al., 2015). The synaptic plasticity which underlies amygdala-dependent learning for both aversive and appetitive emotional memories is proposed to be mediated by BDNF (Heldt et al., 2014). BDNF is distributed within key regions in the neural circuitry of affective processing, in which the relationship between BDNF levels and amygdala structure and function offers a putative mechanism for regional brain alterations in MDD.

Serum BDNF levels have been positively correlated with bilateral amygdala volumes in adolescents with bipolar disorder (Inal-Emiroglu et al., 2015) as well as with left amygdala volume in healthy adults over 60 years old (Manna et al., 2015). In a mouse model, chronic unpredictable mild stress was associated with increased BDNF expression in the amygdala and reduced resting state blood oxygenation level-dependent (BOLD) activity (Huang et al., 2018). In individuals with combined MDD and anxiety disorders, however, no significant correlations were observed (van Velzen et al., 2016), although the sample may have been confounded by comorbid anxiety disorders and concomitant medications.

The majority of peripheral BDNF is contained within platelets, which correlates with serum BDNF, and platelet-derived BDNF avoids confounds associated with serum BDNF, such as sex and ethnicity mediated by platelet count (Sera-Millàs et al., 2016). The present study sought to examine the relationship between platelet BDNF levels and amygdala volume and function in MDD during an unmedicated, current depressive episode. If BDNF is necessary for the consolidation of emotionally salient events (Heldt et al., 2014), then increased BDNF levels might be associated with increased amygdala responses to emotionally salient stimuli.

Methods

Participants

Cambridgeshire 4 NHS Research Ethics Committee, NHS Health Research Authority, provided ethical approval. All participants provided informed written consent. All participants were recruited from the general community (Fu et al., 2015). MDD participants met criteria defined by Diagnostic Statistical Manual of Mental Disorders, Fourth edition, text revision (DSM-IV-TR) (APA, 200). All MDD participants had first episode or recurrent MDD, in a current depressive episode, at least moderate severity as measured by a minimum score of 18 on 17-item Hamilton Rating Scale for Depression (HRSD). All were free of antidepressant or psychotherapy treatment for a minimum of 4 weeks, or 6 weeks for fluoxetine. Exclusion criteria included comorbid Axis I or II disorders and treatment-resistant depression. Healthy participants had no history of medical or psychiatric disorders. Histories were confirmed from general practitioner records.

Blood sample and BDNF analysis

The blood sample was obtained on the day of the magnetic resonance imaging (MRI) scan, by Vacutainer serum tubes, processed and stored at -80 ºC. Platelets were thawed on ice,

centrifuged, and the pellet was reconstituted in ice cold TME buffer (10mM Tris HCl; 1mM MgCl; 1mM EDTA pH 7.5) and protease inhibitor cocktail (Sigma Aldrich #P2714). Pellet was homogenized with 20 strokes of a hand held, teflon/glass homogenizer. Homogenate was centrifuged 40.000xg for 30 minutes. Supernatant was collected, and protein determination performed using Bradford assay. BDNF was assayed from the supernatant using 100ul of 1mg/mL of each sample in each well of the ELISA (Millipore, CYT306). The analysis consists of MDD participants for whom a valid BDNF determination could be obtained.

MRI data acquisition

High-resolution 3-dimensional sagittal T1-weighted structural images (Magnetization Prepared Rapid Gradient Echo; resolution 1 mm^3) and gradient echo T2*-weighted echoplanar images depicting blood oxygenation level-dependent (BOLD) contrast were acquired. Total of 180 volumes, consisting of 39 oblique axial slices parallel to the intercommissural plane with parameters: slice thickness: 3 mm, slice gap: 0.3 mm, echo time: 30 milliseconds, repetition time: 2000 milliseconds, flip angle: 75°, field of view: 240 mm, and matrix size: 64 × 64 for each volume.

In the functional MRI scan, participants viewed a series of 10 faces (5 female), adapted from Ekman and Friesen's Pictures of Facial Affect morphed to depict varying intensities of sadness: low, medium and high. Participants indicated the gender by button press, with the explicit instruction being gender identification to facilitate implicit emotion processing (Costafreda et al., 2008). Facial stimuli were presented twice at each intensity, 60 faces in total, with 12 baseline trials, consisting of a crosshair, for total of 72 presentations in a pseudo-randomised order. Each stimulus was presented for 3 s, and the interval between

trials varied randomly according to a Poisson distribution, with mean intertrial interval of 5 s, for a total duration of 360 s.

MRI data analysis

Analysis of structural images was performed with Freesurfer 4.5.0 automated longitudinal stream to obtain amygdala volumes. Quality control was performed by visually assessing the brain segmentation overlaid on original T1 image to ensure that cortical reconstructions did not present major anomalies. Medial temporal lobe region was assessed with coronal sections. All reconstructions passed qualitative control, and original Freesurfer outputs were used without manual corrections.

Left and right amygdalae regions of interest were defined according to Harvard-Oxford probability atlas distributed with FMRIB Software Library (FSL) package (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases). Statistical Parametric Mapping (SPM8, Wellcome Department of Imaging Neuroscience, London, UK: http://www.fil.ion.ucl.ac.uk/spm) was used to pre-process and analyse functional MRI data. Images were realigned to correct for motion artefacts, spatially normalized to the Montreal Neurological Institute (MNI) template, and smoothed using an 8 mm full-width at half maximum (FWHM) Gaussian kernel filter. First-level analysis was performed using general linear model, accounting for serial autocorrelations by applying an autoregressive model. Stimuli presentation was modelled as individual events, and the first level analysis produced contrast images depicting overall facial processing capacity (mean difference in response between all facial trials taken together and baseline trials). Region of interest analysis was performed using MarsBar tool in SPM8 (http://marsbar.sourceforge.net/). BOLD responses for left and right amygdalae were extracted separately for each participant in the contrast of interest.

Statistical Analysis

A Pearson's bivariate correlation analysis with BDNF levels was performed for left and right amygdalae separately, using the `stats` package in R statistical software (R Foundation for Statistical Computing, Vienna, Austria.).

Results

Participants were 23 MDD (10 women), mean age 38.9 (standard deviation (SD) 12.1) years and 23 age and IQ matched healthy controls, mean age 38.8 (SD 10.2) years, from African, Asian and Caucasian ethnicities (Table 1). Mean platelet BDNF levels were 89.8 (SD 56.8) ng/mL in MDD and 83.1 (SD 52.7) ng/mL in healthy control participants (Table 1). In MDD, the correlation between left amygdala BOLD response and BDNF levels was statistically significant (r = 0.42, p = 0.043) (Figure 1). The correlation remained significant when BOLD response was normalised by left amygdala volume (r = 0.45, p = 0.031). There were no significant associations between BDNF levels and right amygdala BOLD response (r = 0.07, p = 0.756) or with amygdala volumes (left amygdala, r = - 0.11, p = 0.605; right amygdala, r = 0.31, p = 0.157).

Discussion

Amygdala response was significantly correlated with platelet BDNF levels in a community sample of MDD participants who were unmedicated in a current depressive episode and who did not have treatment-resistant depression. No relationship was found between BDNF levels and amygdala volumes, consistent with the lack of associations in participants with MDD which had been comorbid with anxiety disorders (van Velzen et al., 2016). BDNF signalling is integral for amygdala-dependent learning (Heldt et al., 2014), and the present findings support a role for BDNF in amygdala responsivity in MDD.

BDNF has a critical role in synaptic plasticity, neural growth, differentiation and maintenance. A common single nucleotide functional polymorphism in the BDNF gene which results in a valine to methionine (Val66Met) substitution has been associated with reduced BDNF secretion, and BDNF Val66Met genotype modulates amygdala responsivity (Egan et al., 2003). The BDNF 66Met allele has demonstrated a specific association with increased amygdala activation to emotional faces in adolescents with MDD or anxiety disorders which was not observed in healthy adolescents (Lau et al., 2010). In adults, the BDNF 66Met allele has been associated with persistently elevated amygdala activation to repeated presentations of unpleasant pictures, representing an impairment in habituation, in borderline personality disorder (Perez-Rodriguez et al., 2017).

The pathway from the Val66Met genotype to BDNF gene expression to BDNF protein may be modulated by BDNF methylation which shows an inverse correlation with BDNF levels (Kleimann et al., 2015). DNA methylation is an epigenetic mechanism whereby gene expression can be altered, and peripheral blood cell BDNF methylation has been associated with MDD (Fuchikami et al., 2011). Neural plasticity in the amygdala is dependent on BDNF, and BDNF is essential for the consolidation of both appetitive and aversive learning (Heldt et al., 2014). Sad facial expressions are emotionally salient stimuli for a current depressive episode in MDD, and increased BDNF levels were associated with increased amygdala BOLD responses. Aversive learning increases the probability of amygdala activation (Costafreda et al., 2008). While present task instructions did not explicitly involve learning, the inherent acquisition of associations with the emotionally salient stimuli could have sufficiently engaged amygdala nuclei. The potential as a clinical state marker or prognostic marker though would depend on its sensitivity and specificity at the individual level.

Left hemispheric lateralisation of amygdala responsivity has been reported in MDD to sad fearful and neutral faces expressions that were supraliminally as well as subliminally presented (Korgaonker et al., 2018). In healthy individuals, left lateralization of amygdala

activation is significantly associated with language-based tasks, in which there is a greater reduction in the probability of activation in right amygdala activation (Costafreda et al., 2008). The usual perceptual bias for aversive relative to neutral words is absent with left or bilateral amygdala lesions but not with right amygdala lesions (Anderson and Phelps, 2001). Lateralisation of the relationship between BDNF levels with the left amygdala has been reported in amygdala volume in healthy adults who were 60-76 years, but not in younger cohorts (Manna et al., 2015). We did not find any significant associations with BDNF levels and amygdala volumes in the present sample, which had excluded participants who were over the age of 60 years.

Limitations include the strength of correlation which may be considered to be moderate and that correlation does not indicate causation in which additional mediating factors could be implicated, such as the relationship between BDNF and the kynurenine pathway. Activation of the kynurenine pathway is proposed to partly mediate the effects of inflammatory processes that lead to the development of depression. The kynurenine pathway consists of putative neurotoxic as well as neuroprotective metabolites which impact on N-methyl-D-aspartate (NMDA) receptors and glutamatergic neurotransmission. NMDA is associated with decreased secretion of BDNF, and an inverse relationship has been observed between BDNF and the neurotoxic metabolite 3-hydroxy-kynurenine (3-HK). Levels of kynurenine and its neuroprotective metabolite kynurenic acid (KYNA) are reduced in depression, which shows increased synthesis along with diminished production of the neurotoxic 3-HK metabolite in astroglial cultures following 24 hour incubation with antidepressant medication (Kocki et al., 2012). Kynurenine and KYNA levels are positively correlated with amygdala volume in MDD (Savitz et al., 2015).

In summary, a significant positive correlation was evident between platelet BDNF levels and amygdala activation in participants with recurrent MDD during a current depressive episode that was unmedicated. The relationship between BDNF levels and amygdala responsivity to

emotionally salient stimuli in MDD could reflect the importance of BDNF in amygdala-dependent learning with clinical implications for treatment pathways. Interactions with the neuroprotective as well as neurotoxic metabolites in kyneurenine pathway require further investigation.

Contributors:

CF, SC, MR and LM made substantial contributions to conception and design of the study. CF and SC made substantial contributions to the acquisition of data. CF, RR, and SC made substantial contributions to the data analysis. VL, CF, SC made substantial contributions to the interpretation. All authors contributed to revising the manuscript critically for important intellectual content and final approval.

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The study was funded in part by Eli Lilly and Company. CF and SC had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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Table 1.

	Major Depression Participants	Healthy Participants
Total number	23	23
Number female participants	10	10
Mean age (years)	38.9 (12.1)	38.8 (10.2)
Ethnicity:		
African	3	8
Asian	9	3
Caucasian	11	12
HRSD	21.8 (0.3)	0.3 (1.1)
Verbal IQ	110.42 (10.33)	110.00 (12.6)
Mean platelet BDNF (ng/mL)	89.8 (56.8)	83.1 (52.7)
Right amygdala volume (mm^3)	1705.00 (245.65)	1697.30 (165.64)
Left amygdala volume (mm^3)	1697.30 (24.93)	1491.91 (170.67)

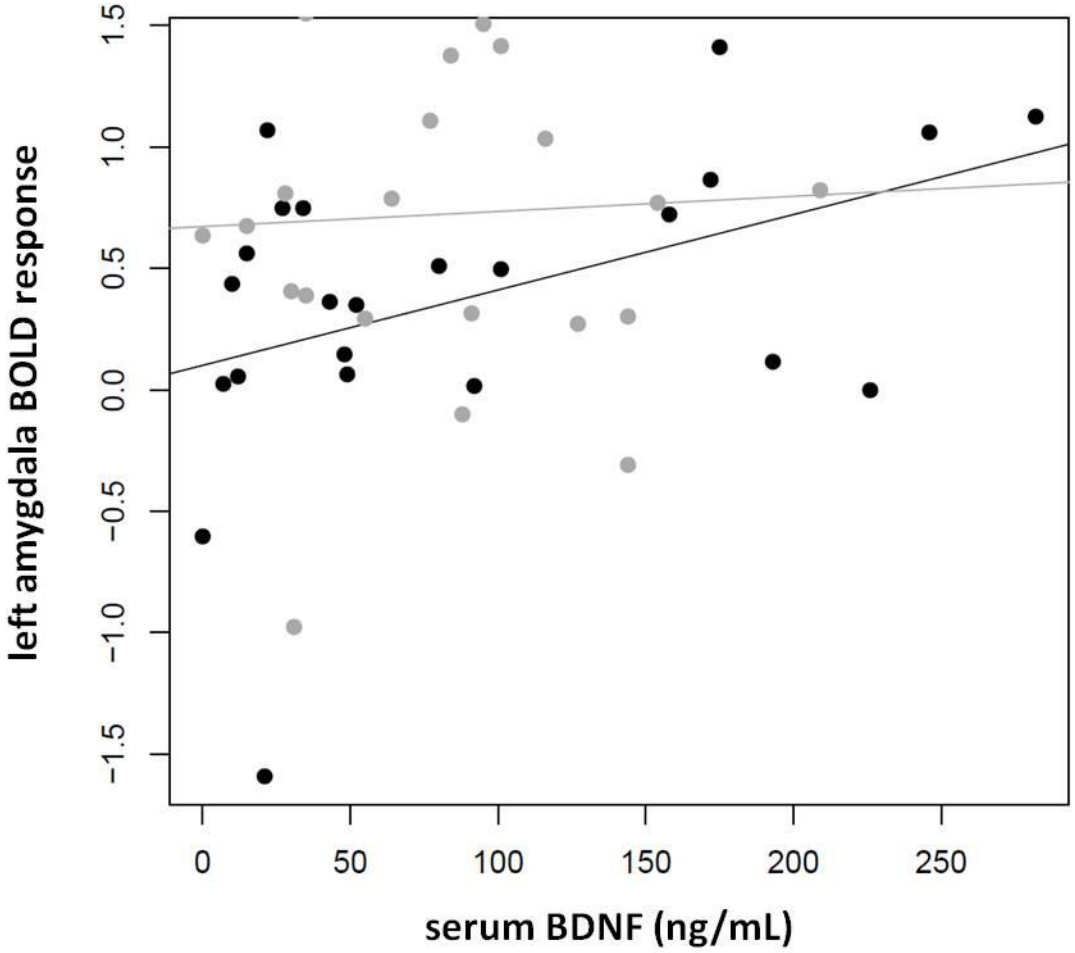
Data presented as mean and standard deviation in parenthesis. HRSD=Hamilton Rating Scale for Depression. There were no significant differences in Verbal IQ (p=0.91), BDNF (p=0.75), right (p=0.92) or left (p=0.44) amygdala volumes.

Figure Legend

Figure 1.

Scatter plot and correlation between platelet BDNF levels (ng/mL) and left amygdala BOLD response in MDD and healthy control participants. There was a significant correlation between platelet BDNF levels and left amygdala BOLD response in MDD (r = 0.42, *p* = 0.043) (black circles), but not in healthy control participants (grey circles).

Figure 1.


left amygdala BOLD response
1.5
1.0
0.5
0.0
−0.5
−1.0
−1.5
0
50
100
150
200
250
serum BDNF (ng/mL)