



Published in final edited form as:

Prog Neuropsychopharmacol Biol Psychiatry. 2008 October 1; 32(7): 1652–1655. doi:10.1016/j.pnpbp.2008.06.009.

Effect of BDNF Val66Met Polymorphism on Age-Related Amygdala Volume Changes in Healthy Subjects

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Abstract

Brain-derived neurotrophic factor (BDNF) has been implicated in the mechanism of age-related regional brain volumetric changes. Healthy volunteers with the valine to methionine polymorphism at codon 66 of the BDNF gene (val66met) exhibit decreased volume of a number of brain structures, including hippocampus, temporal and occipital lobar gray matter volumes, and a negative correlation between age and the volume of bilateral dorsolateral prefrontal cortices. We sought to characterize the relationship between age, BDNF and amygdala volumes among healthy volunteers. We measured amygdala volumes in 55 healthy, right-handed volunteers who underwent structural magnetic resonance imaging (MRI) and were also characterized demographically and genotyped with respect to BDNF. Using an ANCOVA model, we found that amygdala volumes were inversely correlated with age in BDNF val66met carriers but not in non-carriers. This is the first report of age-related BDNF val66met polymorphism effects on amygdala volume.

Keywords

Aging; Amygdala; BDNF; MRI

Introduction

Brain-derived neurotrophic factor (BDNF) is the most abundant member of the neurotrophin superfamily and plays a role in differentiation during development (Alcantara et al., 2006; Engelhardt et al., 2007; Sato et al., 2006), neuronal survival in the adult brain (Morse et al., 1993), and regulation of synaptic function (Lessmann et al., 2003). BDNF signaling also has

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This work was performed at the New York State Psychiatric Institute and Columbia University.

been implicated in learning/memory processes, including episodic memory in humans (Egan et al., 2003). Similarly, in rats, BDNF signaling has a role in the acquisition of conditioned fear (Rattiner et al., 2004) and consolidation of fear extinction (Chhatwal et al., 2006) through binding to TrkB receptors in the amygdala. Murine behavioral expression of conditioned fear has been found to be positively related to amygdala BDNF levels (Yee et al., 2007).

One frequent, nonconservative single nucleotide polymorphism (SNP) has been identified in the human BDNF gene. The SNP (rs6265) at nucleotide 196 (G/A), produces an amino acid substitution of valine to methionine at codon 66 (val66met) in the 5' prodomain. This polymorphism results in decreased distribution of BDNF val66met to neuronal dendrites. It also is associated with lower activity-dependent secretion in neuronal culture (Chen et al., 2005; Chen et al., 2004; Egan et al., 2003) and in ex-vivo Met-Met homozygous knock-in mice (Chen et al., 2006). BDNF val66met has been reported to have an allele frequency of approximately 19% to 25% in Caucasian samples (Egan et al., 2003; Sen et al., 2003) and 41% in Asian samples (Shimizu et al., 2004). Val66Met allele carriers have previously been found to have smaller volumes of temporal and occipital lobar gray matter (Ho et al., 2006), prefrontal cortex (Pezawas et al., 2004), and hippocampus (Bueller et al., 2006; Frodl et al., 2007; Pezawas et al., 2004), and greater white matter hyperintensity volumes (Taylor *et al.*, 2008), presumably due to decreased BDNF neuroprotection. Among healthy volunteers carrying the val66met polymorphism, there are reports of a negative correlation between age and dorsolateral prefrontal cortical (DLPFC) volume (Nemoto et al., 2006). The val66met polymorphism has been associated with a phenotype of increased anxiety-related behaviors in stressful settings in animal studies (Hashimoto, 2007) and higher levels of trait anxiety/anxious temperament (Jiang et al., 2005; Lang et al., 2005) in humans. Additionally, the val66met polymorphism has been associated with age-related decline of non-verbal reasoning ability. (Harris *et al.*, 2006)

Aging strongly affects brain morphology in humans (Nemoto et al., 2006). It has been reported that BDNF levels in plasma –but not platelet levels- decrease significantly with increasing age (Lommatzsch et al., 2005), and normal aging seems to be associated with a decreased BDNF signaling capacity in the brain (Mattson et al., 2004). Considering the previous findings that BDNF is expressed abundantly in several brain regions and that BDNF has a neuroprotective effect, the val66met polymorphism might have an effect on age-related morphological changes in the brain.

We sought to characterize the relationship between age, BDNF and amygdala volumes among healthy volunteers. We hypothesized a negative correlation between age and amygdala volume only in val66met allele carriers.

Methods

Sample

Healthy volunteers (N=55) were recruited from community referrals and advertisements. They were enrolled in the study after giving written informed consent as approved by the Institutional Review Board. All subjects underwent physical examination, screening for Axis I and II psychiatric illness, and routine laboratory screening. Exclusions were pregnancy, neurologic illness, active medical disease, any Axis I psychiatric diagnosis, current substance use, or first-degree relatives with a mood disorder or schizophrenia.

Polymerase chain reaction (PCR)

The BDNF Val66Met polymorphism (GenBank dbSNP: rs6265) was typed by PCR and using the *BsaA I* restriction enzyme. Briefly, the oligonucleotide primers, sense MannBF-1F (5'-

ATCCCGGTGAAAGAAAGCCCTAAC-3') and antisense MannBF-1R (5'-CCCCTGCAGCCTTCTTTTGTGTAA-3'), were used to amplify a PCR fragment of 673 bp length. PCR was carried out in a 20 μ l volume, containing 100 ng DNA, 40 ng of each primer, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2 mM MgCl₂, 0.01% gelatin, 200 μ M of each dNTP, and 0.8 U of REDTAQ Genomic DNA Polymerase (Sigma, MO, USA). Samples were processed in a Robocycler (Stratagene, CA, USA). Thirty temperature cycles, consisting of 30 sec at 95°C, 30 sec at 60°C, and 40 sec at 72°C, were followed by a final extension step of 72°C for 4 min. The PCR fragments were digested with *BsaA I* restriction enzyme (NE Biolab, MA, USA), which produces 3 fragments of 275, 321 and 77 bp when guanine is present at nucleotide 1249, and 2 fragments of 321 and 352 bp if cytosine is present at this position. The digested PCR products were separated on a 1.2% agarose gel.

MRI Acquisition

MRI acquisition and image analysis methods have been described previously (Parsey et al., 2006). MRI scans were acquired on a GE 1.5 T Signa Advantage system. A sagittal scout (localizer) was performed to identify the AC-PC plane (1 min). A transaxial T1 weighted sequence with 1.5 mm slice thickness was acquired in a coronal plane orthogonal to the AC-PC plane over the whole brain with the following parameters: 3-dimensional Spoiled Gradient Recalled Acquisition in the Steady State (SPGR); TR 34 msec; TE 5 msec; flip angle of 45 degrees; zero gap; 124 slices; field of view of 22 \times 16 cm; with 256 \times 192 matrix, reformatted to 256 \times 256, yielding a voxel size of 1.5 mm \times 0.9 mm \times 0.9 mm; time of acquisition 11 min.

Image Analysis

Raw coronal MRI images were cropped to remove non-brain material, utilizing the Exbrain v. 2 utility (Lemieux et al., 2003). Where Exbrain was unable to process an MRI, the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB) Brain Extraction Tool (BET) v1.2 (Smith, 2002) was used along with manual removal of non-brain matter left after utilizing BET. Delineation of the amygdala region of interest (ROI) was performed based on criteria and anatomical landmarks developed by two of the authors (S.R. and V.A.), using an electronic mouse with 3D image analysis software (MEDX, Sensor), on consecutive 1.5-mm-thick coronal slices. Corresponding sagittal and axial views were available to aid in the accuracy of border definition. In anterior slices, the amygdala emerged as a rounded mass of gray matter that increased in size in subsequent slices (Bogerts et al., 1990; Niu et al., 2004). The lateral border consisted of temporal lobe white matter, and the medial border was separated from the entorhinal cortex by the angular bundle (Hammers et al., 2003; Niu et al., 2004). The inferior border was defined by white matter in anterior slices and by the hippocampus and ventricle in more posterior slices. The entorhinal sulcus defined the superior border (Hammers et al., 2003; Pruessner et al., 2000). In the most caudal slices, the amygdala became a thin strip of gray matter located dorsal to the alveus or inferior horn of the lateral ventricle, which serve as boundaries (Convit et al., 1999; Pruessner et al., 2000). The dorsal border was defined with the optic tract and fundus of the inferior portion of the circular sulcus of the insula (Pruessner et al., 2000). The entire rostrocaudal extent of the amygdala was measured for each subject. The areas of all slices were summed, and the sum was multiplied by the slice thickness to obtain volume. Reliability was computed by repeated measurements (two raters traced left and right amygdalae in 9 subjects); the intraclass coefficient (ICC) was 0.87 for left amygdala and 0.92 for right amygdala.

Statistical analyses

Statistical analyses were done with SPSS Version 14. An ANCOVA model with amygdala volumes of both hemispheres integrated as "within-subject variable" was used to examine the

effects of the presence of the BDNF val66met allele. We added age as a covariate and sex as a cofactor to the model. A separate analysis included age as covariate and race as a cofactor.

Results

Genotype distributions in healthy volunteers (Met-Met N=4 [7%], Met-Val N=18 [33%], Val-Val N=33 [60%]) did not deviate from Hardy-Weinberg expectations ($\chi^2 = 0.48$, $df=2$, $p=0.923$). There were no differences among subjects between BDNF val66met allele carriers and non-carriers with regard to total cerebral volume (TCV), mean raw amygdala ROI volumes, amygdala volumes after normalization for TCV, sex or age (see Table 1).

Total amygdala volumes were inversely correlated with age in BDNF val66met carriers, but not in non-carriers [BDNF and age interaction ($F=4.58$; $df=1$; $p=0.037$)], (see Figure 1). Mean volumes of left and right amygdala differed among all subjects (left amygdala, $0.98\% \pm 0.18$ vs. right amygdala, $1.06\% \pm 0.22$; $t = -2.90$, $df = 54$, $p = 0.005$). However, there was no lateralization of the effects of aging or of the BDNF val66met allele on amygdala volume (data not shown). There was no difference between males and females in mean corrected amygdala volume. Nor was there a difference between the sexes in terms of the age effects on amygdala volume, although statistical power to detect such a difference was low. The sex distribution in the sample is illustrated in Figure 1. Upon racial stratification, in our sample lower rates of Val/Met genotype were seen in African (10%) and Hispanic Americans (20%) than in Caucasian (54%) and Asian Americans (54%). We dichotomized the race variable into two categories of similar Val/Met distribution: African and Hispanic Americans vs. Caucasians and Asian Americans. We tested the contribution of race in terms of its effect on the model (entering race as an additive covariate) and in terms of effects that might differ according to age (race and age interaction). In this adjusted model, there was no main effect of race on amygdala volume (data not shown). Although there was a trend for an interaction of race and age ($p=0.09$), including it in the model did not modify the significant interaction between BDNF and age in effects on amygdala volume.

Discussion

We observed an interaction between BDNF and age, such that total amygdala volumes were inversely correlated with age in BDNF val66met carriers but not in non-carriers. Our results in amygdala are consistent with a report of an increased age-related volume reduction of the DLPFC in healthy BDNF val66met carriers (Nemoto et al., 2006), although unlike the latter study, we did not find sex effects associated with amygdala volume or BDNF val66met allele carrier status. On the other hand, smaller hippocampal volumes in BDNF val66met carriers were found to be age and sex-independent. (Pezawas et al., 2004) Perhaps these inconsistencies reflect different mechanisms by which the BDNF val66met variant can affect brain volumes. It has been suggested that the BDNF val66met allele may result in reduced BDNF synthesis during neurodevelopment, causing decreased neuronal proliferation and survival, small soma size and diminished dendritic growth; alternatively, BDNF val66met may exert its influence through modulating synaptic activity in mature neurons. (Ho et al., 2006)

Reduced amygdala volumes could be a marker for abnormal fear-driven responses and/or anxiety levels, affecting the ability of an organism to assess the dangerousness of stimuli and respond appropriately. Future studies might examine relationships between amygdala volumes, BDNF val66met status, and anxiety in the elderly.

This study has limitations due to sample size, and therefore these results should be considered as preliminary, e.g. 1) the sample included relatively few subjects of older age in the BDNF-Met carrier group, with no subjects older than 60 yrs; and 2) there was not adequate statistical

power to detect sex or racial differences in three-way interactions with effect of age and BDNF genotype on amygdala volume.

Conclusions

We observed an inverse correlation between amygdala volumes and age in BDNF val66met carriers but not in non-carriers. Our results support the role of the val66met polymorphism in age-related morphological changes in the brain.

Abbreviations

AC-PC, Anterior commissure – Posterior commissure
 ANCOVA, Analysis of Covariance
 BDNF, Brain-Derived Neurotrophic Factor
 BET, Brain Extraction Tool
 DLPFC, Dorsolateral Prefrontal Cortex
 fMRI, Functional Magnetic Resonance Imaging of the Brain
 ICC, Intraclass Coefficient
 MRI, Magnetic Resonance Imaging
 ROI, Region of Interest
 SNP, Single Nucleotide Polymorphism
 SPGR, Spoiled Gradient Recalled Acquisition in the Steady State
 TCV, Total Cerebral Volume
 TE, Echo time
 TR, Repetition time
 Val66met, Valine to methionine polymorphism at codon 66 of the BDNF gene

Acknowledgments

This study was supported by NIMH grant MH62185. Dr. Baca-Garcia is a Lilly Suicide Scholar at Columbia University. Dr. Sublette's work was supported in part through a T32 National Research Service Award Training Grant MH016434.

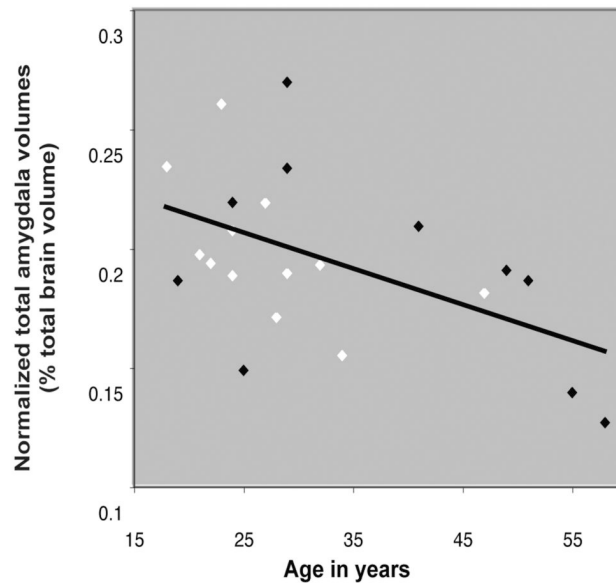
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A. Among Val66met Carriers



B. Among Val-Val Homozygotes

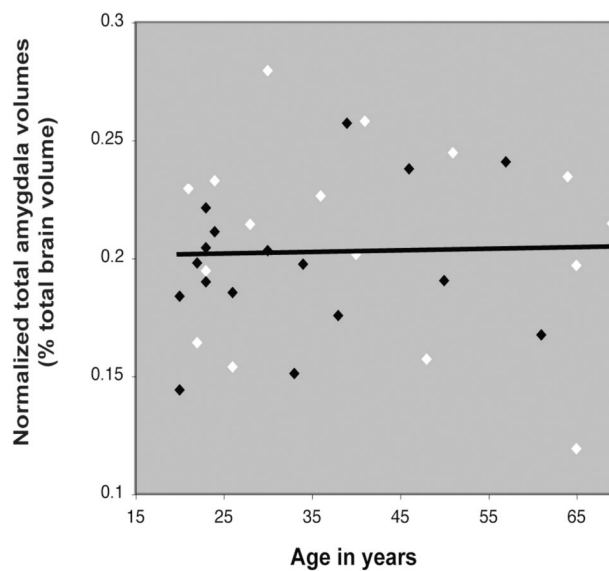


Figure 1.

Scatter plot of the correlation between total amygdala volume (expressed as a percentage of total brain volume) and age. White symbols represent females; black symbols, males. Total amygdala volume declined with increasing age in (A) BDNF val66met carriers ($r=-0.52$, $p=0.014$) but not in (B) val-val homozygous subjects ($r=0.03$, $p=0.873$).

Table 1
 Volumetric and demographic characteristics by val66met status.

Characteristic	Side	Val66met		t	df	p
		+	-			
Mean total cerebral volume, TCv (L ± SD)		1.43 ± 0.12	1.42 ± 0.20	0.07	53	0.946
Mean raw amygdala volumes (cm ³ ± SD)	left	1.44 ± 0.21	1.37 ± 0.31	1.02	53	0.313
	right	1.50 ± 0.34	1.50 ± 0.31	-0.02	53	0.987
Mean amygdala volumes after normalization (% of TCv ± SD)	left	1.02 ± 0.16	1.00 ± 0.19	1.09	53	0.279
	right	1.06 ± 0.24	1.06 ± 0.22	-0.10	53	0.920
Mean age (years ± SD [range])		32.2 ± 12 [40]	37.0 ± 16 [49]	-1.22	53	0.229
				χ^2	df	p
Gender (No. [%] males)		27 [45.5]	28 [51.5]	0.194	1	0.660