BDNF Val<sup>66</sup>Met polymorphism significantly affects \( d' \) in verbal recognition memory at short and long delays

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Abstract

A functional polymorphism at the val66met locus in the BDNF gene has significant effects on the pro-form of the protein in intracellular trafficking and activity-dependent, but not constitutive, secretion. These differences are thought to underlie several findings in humans related to this polymorphism, including markers of neuronal viability, BOLD activation in medial temporal lob regions, and some aspects of behavior.

However, many important questions remain about the impact of BDNF on various mnemonic subprocesses at the behavioral level. In this study, we examined the impact of the val/met polymorphism in a verbal recognition memory paradigm involving manipulation of depth of encoding and differential delays for recall and analyses of hits for previously presented target words and correct rejections of foils. Twenty-four human val homozygous individuals and 24 met carrier individuals comprised the sample. All were healthy controls. IQ between the groups was equivalent. In the encoding phase of the study, words were presented and encoded either by a decision as to whether they were living or nonliving ("deep") or if they contained the letter "A" (shallow). After this phase, recognition was tested immediately, half an hour, and 24 h later. BDNF genotype had significant effects on hits and discriminability (\( d' \)), accounting for at least 10% of the variance, but not on correct rejections or beta. BDNF did not interact with level of encoding, nor did it interact with delay. In sum, BDNF genotypes impacted "hits" in a recognition memory paradigm, findings consistent with the general notion that BDNF plays a prominent role in memory subprocesses thought to engage the medial temporal lobe.

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1. Introduction

Brain-derived neurotrophic factor (BDNF) plays an important role in promoting long-term potentiation (LTP), thought to be a critical component in episodic memory processing, via a number of actions, including presynaptic effects on vesicle docking or postsynaptic effects at the TrkB receptor (Kovalchuk et al., 2002; Tyler et al., 2002; Tyler and Pozzo-Miller, 2001; Xu et al., 2000). BDNF promotes transcription-related protein synthesis with consequent consolidation in late phase LTP (Pang and Lu, 2004). BDNF also can induce local morphological dendritic changes (Horch and Katz, 2002). Furthermore, BDNF is selectively expressed in the medial temporal lobe (MTL) during context episodic memory tasks in rats and is upregulated in brain area IT during visual association learning in primates (Hall et al., 2000; Tokuyama et al., 2000).

Previous work from our group found that a functional polymorphism at the val66met locus (rs 6265) in the BDNF gene has significant effects on the pro-form of the protein in intracellular trafficking, such that the met form aggregates in perinuclear regions while the val form reaches neurites in hippocampal cell cultures (Egan et al., 2003). Moreover, the val form also demonstrates greater activity dependent (i.e., phasic) but not constitutive (i.e., tonic) secretion (Chen et al., 2004). In brief, failures of intracellular trafficking of the met form of the protein result in reductions in BDNF in neurites during conditions in which LTP (a presumptive cellular mechanism of memory) might normally occur. These intracellular trafficking differences are thought to underlie several findings in humans related to this polymorphism (Egan et al., 2003; Hariri et al., 2003), including: (1) NAA levels in the hippocampus are greater in individuals with val alleles in comparison to
individuals who carry met alleles, (2) fMRI BOLD response in the MTL is greater in val/val homozygote individuals during an episodic memory task requiring encoding of visual scenes than in met carriers, and (3) memory for semantically structured, verbally presented stories is superior in val carrier individuals. The latter results have been replicated. Ho et al. (2006) found a significant effect of BDNF genotype on several verbal memory measures, including immediate memory for stories and list learning of unrelated words. Additionally, Dempster et al. (2005) have very recently found a trend level effect (p = .09) of BDNF val/met genotype on memory for stories after a delay that was larger in nonpsychotic relatives of schizophrenic individuals than in probands. Other studies have examined the role of BDNF polymorphisms in different facets of cognition in psychiatric disorders with mixed results (Rybakowski et al., 2003; Strauss et al., 2004; Tan et al., 2005).

However, many important questions remain about the impact of BDNF on various mnemonic subprocesses at the behavioral level. In this study, we examined the impact of the val/met polymorphism in a verbal recognition paradigm involving manipulation of depth of encoding and differential delays for recall. We predicted (1) equivalent effects of the polymorphism on shallow encoding and deep encoding in levels of encoding paradigm, given prior studies that indicated that encoding during memory tasks engages inferior prefrontal cortex, while BDNF effects appear largest in medial temporal lobe regions, (2) an equivalent impact on short and long delays, given BDNF’s probable role in both early and late phase LTP (with val/val individuals maintaining an advantage across delays), and (3) greater impact of BDNF genotype on hits rather than correct rejections of foils during the test phase, given earlier findings that BDNF plays a larger role during acquisition of novel stimuli as opposed to their retrieval or subsequent monitoring (Hariri et al., 2003; Schacter and Stolinick, 2004); we therefore predicted val/val individuals would outperform met carriers for hits but not correct rejections in a recognition memory paradigm. In summary, these predictions are based on the general notion that evidence is strongest for BDNF’s role in medial temporal lobe processing, while evidence is less consistent for BDNF’s impact in non-medial temporal lobe regions during memory processing.

2. Methods

2.1. Participants

Forty-eight normal subjects participated in this study. All were screened by SCID to ensure that they did not have current diagnosable psychopathology. The proportion of males and females differed between the groups (16 females in the val group, 8 in the met carrier group; \( \chi^2 = 5.33, p = .02 \)), but did not impact performance (see Section 3, below). The proportion of non-Caucasian individuals did not differ between the groups and was small (about 10%, \( \chi^2 = .22, p = .64 \)). Mean age of the groups did not differ significantly (val/val 39.3 ± 9.9 years old, met carrier 38.0 ± 10.9 years old; \( t = .41, p = .68 \)). Importantly, IQ of the groups, measured by the WAIS-R, did not differ by t-test (val/val = 109.2 ± 8.2, met carrier = 111.3 ± 8.3, \( t = .39, p = .38 \)).

Of the 48 subjects in the present study, 21 participated in the study of Egan et al. (2003); none had been exposed to the paradigm used in this study. We compared the old and new cohorts on several measures (including \( d’ \), see below) and found that there was no cohort effect (\( F = 2.72, p = .14 \)), nor more critically, no BDNF × cohort interaction (\( F = .01, p = .99 \)).

2.2. Genotyping

Subjects’ DNA was genotyped at the BDNF val66met locus by Taqman assay as previously described (Egan et al., 2003). Twenty-four subjects had val/val genotype, 22 had the val/met genotype, and two had the met/met genotype. Val/met and met/met individuals were combined to form a met carrier group.

2.3. Recognition memory

The task was based on the study of Paul et al. (2005) and derived theoretically from the work of Craik and Tulving (1975). Additional delay conditions were added for this study.

2.3.1. Words

All words were nouns. Frequency and imageability of the targets and foils in the recognition section were matched across lists using the MRC database (psych.rl.cc.uk/MRC_Psych_Db.html). Kucera-Francis mean frequencies were in the 12–18 range for each encoding condition and for targets and foils in the recognition delay conditions. All words had imageability values above 450, which is greater than the mean value in the MRC database.

2.3.2. Encoding task

Participants were presented with a deep encoding instruction (Is it living?) for 1 s, followed by 18 words presented at 2.5 s each, with 2.5 s between presentations. They were asked to accurately respond either “yes” or “no” to each word on a keyboard. The shallow encoding question (Is there an “A” in the word?) then appeared on the screen for 1 s, followed by 18 words presented at 2.5 s each, with 2.5 s between presentations. Participants were asked to respond “yes” if the letter “A” was present and “no” if the letter “A” was absent in the word, again by pressing the same two buttons on the keyboard assigned to either “yes” or “no”. This procedure was repeated two more times. There were 108 words presented in total, with 54 deep and 54 shallow encoding words. Stimuli were presented in the center of a computer monitor.

2.3.3. Recognition tasks

Three recognition tasks were administered following the encoding task, one immediately afterwards, one half an hour later, and one 24 h later. At each time condition, participants were presented with a question (Have you seen this word before?) for 1 s, and were presented with 36 words, one at a time, for 2.5 s with 2.5 s between presentations and responded by pressing buttons of a keyboard. Different words were used for each time condition. Each condition included equal or near equal numbers of words from the deep encoding lists, shallow encoding lists, as well as foils, or words that were never present in the encoding task.

2.4. Scoring

Hits and correct rejections were calculated. A hit was defined as a yes response to an old item; a correct rejection as a no response to a new item. \( D’ \), a measure of discriminability, and beta, a measure of response bias, were calculated using the formula of Underwood (1974). Percent correct was used in Fig. 1a–c for ease of interpretation.

3. Results

First, we examined hits in a repeated measures analysis in which genotype group (val/val vs. met carriers) served as a class variable. Time and encoding type served as within subject variables. These results are illustrated in Fig. 1. Critically, we observed a main effect of genotype group (\( F(1,46) = 4.95, p = .03 \)). The val/val group performed better than the met carrier group. As expected, a significant effect of time was
present, as performance worsened with increasing delay ($F(2.92) = 13.34, p = .0001$). Also, as expected, words deeply encoded were recognized more accurately than words encoded at a shallow level ($F(1.46) = 113.93, p = .0001$). No genotype × encoding ($F = .71, p = .41$) nor genotype × delay ($F = .00, p = .99$) interactions were observed (An encoding × time interaction was nearly significant ($p = .06$); hits for words encoded at a shallow level displayed a steeper decline over time from half an hour to 24 h later, but this was not germane to the focus of the present study). While an unexpected increase in performance at half an hour was noted, performance did not differ significantly from the immediate condition by t-test.

Second, for correct rejections (of foils), we did not observe a significant effect of group ($F(1.46) = 0.0, p = .97$). A near significant time × genotype effect on correct rejections was observed $F(2.92) = 2.91, p = .06$, due to a “cross-over” pattern from half an hour to 24 h later between the groups; however, the interpretation of this is unclear. We observed a significant effect of time, such that the number of correct rejections declined steeply from the immediate condition to 24 h.

Third, $d'$ for the combined high and low encoding conditions was $.71 \pm .04$ for the val/val group and $.67 \pm .06$ for the met carrier groups. These means differed significantly by t-test ($t = 2.81, p = .007$). Beta for the combined deep and shallow encoding conditions was $-.16 \pm .31$ for the val/val group and $-.25 \pm .41$ for the met carrier groups, i.e., both groups were conservative in their response bias. These means did not differ significantly by t-test ($t = .97, p = .34$).

![Fig. 1. Deep and shallow encoding: percentage correct of hits. Val homozygotes had a significant advantage in recognizing previously presented words irrespective of whether they were encoded deeply or superficially compared to met carriers over all delays.](Image)

![Fig. 2. Scatterplot of $d'$ data by genotype group. Outliers were not present in either group (i.e., performances greater or less than 2.5 S.D.s from the group means).](Image)

The effect size of BDNF genotype was large for a signal detection of accuracy (.84) while for response bias (yea saying or nay saying) the effect size was moderate (.28). To assess the impact of outliers a scatterplot is displayed in Fig. 2. As can be observed there were no extreme outliers, i.e., all subjects were within 2.5 S.D.s of their group mean.

Because of differences in the sex ratio between the groups we directly contrasted males and females. Sex main effects were nonsignificant for $d'$ ($p = .11$; females performed slightly better than males.) Also, there was no BDNF × sex interaction ($F = .95, p = .33$) for $d'$. These results indicate that BDNF had similar effects on discriminability within each sex.

Fourth, in stepwise multiple regression analyses, we analyzed the contributions of BDNF genotype, gender, and IQ to $d'$, total hits (across all conditions), beta, and total correct rejections in the combined group as listed in Table 1. BDNF genotype accounted for 15% of the variance in $d'$ and 9.7% of the variance in hits. IQ was a weak and independent predictor of $d'$. The overall model was significant ($p = .01$). Sex was not a predictor of hits or $d'$. In contrast, for beta and correct rejections, neither BDNF genotype, gender, nor IQ significantly predicted performance.

### 4. Discussion

Consistent with our predictions, val/val subjects performed better than met carriers in identifying previously encoded stimuli, i.e., in detecting targets. Indeed, BDNF accounted for

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variables$^a$</th>
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<tbody>
<tr>
<td>$d'$ ($F(2.45) = 5.56, p = .01$)</td>
<td>BDNF genotype</td>
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<td></td>
<td>Step</td>
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<tr>
<td>Hits ($F(1.43) = 4.12, p = .05$)</td>
<td>IQ</td>
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<td>Step</td>
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<tr>
<td>Beta ($F &lt; .20, p &gt; .20$)</td>
<td>Sex</td>
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<tr>
<td>Correct rejections ($F &lt; .10, p &gt; .50$)</td>
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$^a R^2$ is cumulative.
approximately 10% of the variance in this measure. These results are consistent with our previous finding of BDNF genotype effects on verbal episodic memory, though the variance “captured” by BDNF is greater in the present study as well as recent work (Ho et al., 2006; Dempster et al., 2005).

The results related to delay are compatible with recent findings about the molecular biology of BDNF. In effect BDNF val homozygotes demonstrated an advantage at the earliest delay and maintained it over subsequent delays. BDNF has a role in early phase LTP, an important cellular model of learning and consolidation. Critically, it is also presumed that BDNF impacts late phase LTP, perhaps through interaction with TPA, and is thought to be dependent on protein synthesis. Ultimately, this type of plasticity related change should improve or refine connectivity in regions critical for memory processing. Alternatively, differences in BDNF genotype might also result in medial temporal lobe volumetric differences and may be related to BDNF’s neurotrophic properties present from early development onward (Pezawas et al., 2004; Szszko et al., 2005). They might also play a role in genotype based differential engagement of the MTL, and ultimately, behavioral memory differences.

On mnemonic processes thought to engage non-MTL cortical regions (e.g., prefrontal cortex), BDNF genotype did not have significant effects. Thus, BDNF genotype did not interact with level of encoding. The effect of the polymorphism was similar irrespective of whether encoding was deep or shallow. While semantic encoding is thought to engage prefrontal cortex to a greater degree than shallow nonsemantic encoding, resulting in a behavioral advantage, such processing may not depend on BDNF expression, or at least not to the same extent as does medial temporal memory processing. BDNF genotype also did not impact correct rejections. This may be because a decision about new word (i.e., a foil) is based upon factors other than recollection or familiarity in regions not highly dependent upon BDNF expression. For instance, correct rejections of foils may occur through processes that engage prefrontal and occipital cortices and parahippocampal cortex (Cabeza et al., 2001; Kahn et al., 2004; Okado and Stark, 2005; Schacter and Stolnick, 2004; Slotnick and Schacter, 2004). Critically, BDNF appears to be expressed at relatively higher levels in the MTL as opposed to neocortex during memory tasks.

It is interesting to note that $d'$ was predicted more strongly than beta by BDNF genotype. While this was not unexpected given the greater presumptive role of BDNF in the MTL rather than neocortex and concomitant engagement of these structures in encoding of memoranda versus monitoring, results must be accepted with caution. This is because beta may be a relative measure that can shift based on task demands (Wixted and Stretch, 2000).

There are several limitations to this study. The sample was rather small by genetic association standards and its imbalance in sex ratio was less than optimal. Also, our sample was ethnically heterogeneous. Nevertheless, when we excluded African-Americans from the analyses, $d'$ and beta changed to a very small degree and results remained significant for $d'$ (though there was some loss in power) and nonsignificant for beta (analyses not shown).

In short, these results, obtained through the use of a verbal recognition memory paradigm, usefully refine and extend our previous findings. Consistent with the neurobiology of BDNF effects on both early and late LTP, the effects of the val66met polymorphism on intracellular trafficking of the pro-form of the protein, and regional expression patterns of BDNF during memory tasks, we have shown that BDNF val/met genotypes impact “hits” in a recognition memory paradigm across a wide range of delays (and account for nearly 10% of the variance in this measure). These results also highlight BDNF’s potential as a target for pharmacogenetic studies of memory enhancement.

References


