Variant BDNF (Val66Met) impact on brain structure and function

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Neurotrophins, such as brain-derived neurotrophic factor (BDNF), are a unique family of polypeptide growth factors that influence differentiation and survival of neurons in the developing nervous system. In adults, BDNF is important in regulating synaptic plasticity and connectivity in the brain. Recently, a common single-nucleotide polymorphism in the human BDNF gene, resulting in a valine to methionine substitution in the prodomain (Val66Met), has been shown to lead to memory impairment and susceptibility to neuropsychiatric disorders. An understanding of how this naturally occurring polymorphism affects behavior, anatomy, and cognition in adults is an important first step in linking genetic alterations in the neurotrophin system to definable biological outcomes in humans. We review the recent literature linking this BDNF polymorphism to cognitive impairment in the context of in vitro and transgenic animal studies that have established BDNF's central role in neuronal functioning in the adult brain.

Neurotrophins, which include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4), are a unique family of polypeptide growth factors, present in all vertebrate species, that influence the proliferation, differentiation, and survival of neuronal cells. These proteins are evolutionarily young and do not exist in invertebrate species such as *Drosophila melanogaster* or *Caenorhabditis elegans*. The late evolutionary appearance of neurotrophins implies that these signaling molecules may be necessary for both the development and functioning of a more complex nervous system (Beck et al., 2004; Chao, 2000). The efficacy of neurotrophins is dependent on their level of availability, appropriate sorting and release, and their binding affinity to transmembrane receptors.

Neurotrophins were initially identified as signaling molecules that are critical in the development of the nervous system. Transgenic mice deficient in any of the neurotrophins (with the exception of NT-4) exhibit early postnatal death. Recently, advances have been made—through the generation of transgenic conditional knockout animals—showing that neurotrophins continue to be important in the functioning of the adult central nervous system (CNS). These genetic manipulations, which allow the gene to remain active in the developing animal and be deleted in adulthood, have shown that these proteins have a potent effect on higher order processes, such as learning and memory, later in life.

Recently, a naturally occurring polymorphism in the human BDNF gene leading to a valine to methionine substitution at position 66 in the prodomain (Val66Met) has been identified and shown to lead to impairments in discrete forms of hippocampal-dependent memory (Egan et al., 2003; Hariri et al., 2003). This polymorphism in the BDNF gene represents an important initial example of a role for neurotrophins in behavioral processes in humans, and provides an important link to the prior behavioral studies in transgenic animals. This review will focus on the behavioral consequences of this variant form of BDNF in humans, and compare and contrast these findings with the established behavioral phenotypes of transgenic mice with altered levels of BDNF or its cognate receptor, tropomyosin-related kinase B (TrkB).

Neurotrophin Ligands

Neurotrophins are a recent addition to the previously known polypeptide factors that affect the survival, growth, and differentiation of cells in the nervous system. The neurotrophins comprise NGF, BDNF, NT-3, and NT-4, each of which has been shown to serve multiple functions in the development and maintenance of the nervous system (Figure 1). NGF is predominantly expressed in the peripheral nervous system in sensory and sympathetic neurons, with discrete expression in the CNS in cholinergic neurons in the basal forebrain. BDNF and NT-3 are highly expressed in CNS structures, including the cortex, the hippocampus, the limbic structures, the cerebellum, and the olfactory bulb (Huang & Reichardt, 2001; Vigers, Baquet, & Jones, 2000; Yan et al., 1997). NT-4 is also widely expressed in the CNS but is found in significantly lower levels than are BDNF and NT-3. The neurotrophins are synthesized from a 32 kDa precursor protein known as proneurotrophin, which binds with high affinity to the p75^{NTR} neurotrophin recep-

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Figure 1. Functions of neurotrophins and Trk receptors. The neurotrophins nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4) bind selectively to specific Trk receptors. Neurotrophin-mediated activation of Trk receptors leads to a variety of biological responses, which include cell survival, axonal and dendritic growth, and synaptic plasticity.

tor (Lee, Kermani, Teng, & Hempstead, 2001; Teng et al., 2005). Upon cleavage to the mature 13–14 kDa forms, they bind to an alternative set of neurotrophin receptors, the Trk family of receptor tyrosine kinases (Chao, 2003).

Neurotrophin Receptors

Neurotrophins are unique in exerting their cellular effects through the actions of two different receptors, the Trk receptor tyrosine kinase and the p75 neurotrophin receptor (p75^{NTR}), a member of the tumor necrosis factor receptor superfamily. Trk receptors consist of an extracellular ligand binding region, a single transmembrane domain, and a highly conserved intracellular tyrosine kinase domain. p75^{NTR} receptors consist of an extracellular ligand binding region, a single transmembrane domain, and an intracellular portion containing a protein association region termed the death domain (Figure 1). All neurotrophins bind to the p75 receptor and appear to elicit similar biological activities, such as mediating cell death, myelination, and neuronal plasticity (Chao, 2003). There are three vertebrate Trk receptor genes (TrkA, TrkB, and TrkC). All Trk receptors exhibit high conservation in their intracellular domains, including the catalytic tyrosine kinase domain. NGF binds specifically to TrkA; BDNF and NT-4 bind to TrkB; and NT-3 binds to TrkC receptors (Figure 1). There are no sequence similarities between Trk and p75 receptors in either ligand binding or their cytoplasmic domains. Neurotrophins bind as dimers to Trk family members, leading to the dimerization of the receptors and subsequent activation of their catalytic tyrosine kinase domains. The dimerized Trk receptors autophosphorylate several key intracellular tyrosine residues and rapidly initiate intracellular signaling cascades.

Neurotrophic factors regulate numerous critical, basic neuronal functions, such as neuronal survival and axonal

outgrowth. As a result, neurotrophins have been implicated in the pathophysiology of a wide variety of neurodegenerative disorders and have been considered as a therapeutic strategy (Thoenen & Sendtner, 2002). In addition, the high levels of expression in the adult CNS of BDNF and TrkB in cortical and hippocampal structures (Yan et al., 1997), as well as their critical roles in maintaining synaptic connections (Huang & Reichardt, 2001), synaptic plasticity (McAllister, Lo, & Katz, 1995), and neurotransmission (Poo, 2001) in these regions, has been the basis for their association with the regulation of cognitive processes, such as learning and memory.

Human Variant BDNF (Val66Met)

Until recently, no genetic associations had been identified linking neurotrophin genes to human cognitive functioning. A recent series of studies, however, has linked a single nucleotide polymorphism in the BDNF gene, leading to a valine (Val) to methionine (Met) substitution at codon 66 in the prodomain (BDNF_{Met}), with memory impairments, as well as altered susceptibility to neuropsychiatric disorders, such as Alzheimer's disease (Ventriglia et al., 2002), Parkinson's disease (Momose et al., 2002), depression (Sen et al., 2003), eating disorders (Ribases et al., 2003; Ribases et al., 2004), and bipolar disorder (Neves-Pereira et al., 2002; Sklar et al., 2002). This BDNF polymorphism represents the first alteration in a neurotrophin gene that has been linked to clinical pathology. In addition, to date, this BDNF polymorphism has not been identified in any vertebrate species other than humans. A common clinical symptom of these disorders is a varying degree of impairment in higher cognitive abilities. Given BDNF's established role in mediating processes related to learning and memory (Desai, Rutherford, & Turrigiano, 1999; Korte et al., 1995; Patterson et al., 1996), this in-

creased susceptibility to cognitive impairment suggests that the variation in BDNF may play a role in the development of neuropsychiatric disorders as well as affect nervous system functioning.

In any study attempting to associate a gene with pathology or behavioral variation, it is often unclear how the change in genotype results in the phenotypic change. This is especially difficult when attempting to link a change in genotype with a discrete change in cognitive functioning. It is possible that the identified genetic variant has a direct effect on cognition, but it is also plausible that the genetic variation mediates an effect through some other downstream functional change, or through the regulation of some other gene. These caveats must be kept in mind during this discussion.

Recently, researchers have begun to describe the distribution of the Met substitution in the population. Approximately 30%–50% of people worldwide are either heterozygous (Val/Met) or homozygous (Met/Met) for the methionine substitution (Shimizu, Hashimoto, & Iyo, 2004). It should be noted that this percentage varies, depending on both region and ethnicity. Carriers of the Met variant form of BDNF make up approximately 30% of the U.S. population (Shimizu et al., 2004), with the most commonly affected individuals being of Indo-European descent. In Japan, 51% of the population are carriers of the Met allele (Shimizu et al., 2004). Cognitive and behavioral effects associated with the Met genotype have been shown to produce a much more robust effect in Caucasians. Thus, the majority of studies that have been conducted to date have either excluded subjects not of European descent, or have removed other ethnic groups prior to data analysis. It is possible that in these other populations, a compensatory mechanism may be in place that supports or eliminates the negative effects of the Met mutation. Such a change may explain the large difference in allele frequency between Caucasian and Asian populations.

One of the most reliable effects observed in carriers of the Met allele (Val/Met) is a difference in cortical and hippocampal morphology. In studies of brain morphology using structural MRI scans, Val/Met individuals have repeatedly been shown to have a smaller hippocampal volume relative to controls who are homozygous for the Val allele (Val/Val) (Pezawas et al., 2004; Szeszko et al., 2005). This difference may be related to the role BDNF and its receptors play in the development as well as continued plasticity of the brain (Huang & Reichardt, 2001; Lu, Pang, & Woo, 2005). The differences in hippocampal volume found across genotypes could arise through any combination of changes, including: (1) decreased dendritic complexity, (2) fewer neuronal and supporting cells, and (3) increased cell death or decreased neurogenesis during embryological development or over the lifespan in this brain area. BDNF and its receptors have been shown to be important in mediating all of these processes.

Although there is a wealth of information about individuals heterozygous for the Met polymorphism, little information exists about individuals who are homozygous for the Met allele (Met/Met), because this genotype

is rare in the general population, comprising only 4% of people in the United States (Shimizu et al., 2004). Studies have shown, in addition to effects on the hippocampus, decreased volume in the dorsolateral prefrontal cortex, an area associated with planning and higher order cognitive functioning, as well as decreased volume in subcortical regions such as the caudate nucleus in heterozygous carriers of the Met allele (Pezawas et al., 2004).

Individuals with the Val/Met genotype have also been shown to perform more poorly than control subjects (Val/Val) on memory tasks that rely heavily on the hippocampus (Egan et al., 2003; Hariri et al., 2003). Using batteries of neuropsychological tests, carriers of the Met allele (Val/Met and Met/Met) were shown to perform worse on tasks that involved recalling places and events, but did not differ from Val/Val individuals on tasks that have been classically shown to be less hippocampal dependent, such as word learning and planning tasks (Egan et al., 2003; Hariri et al., 2003). Using functional magnetic resonance imaging (fMRI), Hariri et al. showed that during a place recognition task, a task that has been shown to result in strong hippocampal activation (Gabrieli, Brewer, & Poldrack, 1998; Schacter & Wagner, 1999), individuals with the Val/Met genotype had significantly lower levels of activation compared with Val/Val individuals during both the encoding and retrieval portions of the task. In a similar functional imaging study, Egan et al. employed a task that required individuals to remember a set of stimuli and recall the stimulus that had been presented *n* stimuli previous from the current stimulus (*N*-back task), a working memory task that relies heavily on dorsolateral prefrontal cortex, and typically results in a disengagement of the hippocampus. They found that individuals with the Val/Met genotype failed to disengage the hippocampus to the same degree as Val/Val subjects. These findings, taken together, suggest that carriers of the Met allele have a selective impairment in hippocampal-dependent memory.

It is unclear whether or not other memory systems are affected by this polymorphism in the BDNF gene or if the tasks currently employed are merely not sensitive enough to detect a behavioral effect. Studies of patient populations have shown an effect of genotype on other forms of higher order functioning. Rybakowski, Borkowska, Skibinska, and Hauser (2006) found that Val/Met individuals who were recently diagnosed with bipolar disorder performed significantly worse than Val/Val patients on the Wisconsin card sort task, a task that has classically been used to diagnose patients with disorders of the frontal lobes and to test executive functioning (such as planning and rule learning).

It is still unclear what these findings mean for a broader population. As mentioned previously, the Met allele is not uniformly distributed across all ethnicities or all regions of the world. As has been previously described in several studies investigating the association between BDNF genotype and neuropsychiatric disorders, northern Europeans appear to be much more affected than Asian populations, despite the fact that a higher proportion of the Asian population carries this allele. This suggests that some ethnicities may compensate for the variation in the BDNF gene

through some as yet unidentified mechanism and are thus less affected by the presence of this polymorphism.

Molecular Mechanism

The molecular mechanisms underlying altered BDNF_{Met} function have begun to be studied primarily in in vitro cell culture systems. The Met substitution in the prodomain is in an area of the BDNF protein that is later cleaved, but is thought to be critical for the trafficking of BDNF to the secretory pathway. Studies in neurosecretory cells and primary cultured neurons have shown that the Met substitution leads to three trafficking defects: (1) decreased variant BDNF distribution into neuronal dendrites, (2) decreased variant BDNF targeting to secretory granules, and (3) subsequent impairment in regulated secretion (Chen et al., 2005; Chen et al., 2004; Egan et al., 2003). In addition, when the two are expressed together in the same cell, BDNF_{Met} alters the trafficking of wild-type BDNF (BDNF_{Val}) through the formation of heterodimers that are less efficiently sorted into the regulated secretory pathway (Chen et al., 2004). These initial findings are consistent with previous studies indicating that the prodomain of neurotrophins plays an important role in regulating their intracellular trafficking to secretory pathways (Suter, Heymach, & Shooter, 1991). Together, these in vitro studies with BDNF_{Met} point to the presence of a specific trafficking signal in the BDNF prodomain region encompassing the Met substitution that is required for efficient BDNF sorting.

Recently, a trafficking protein, sortilin, was shown to be necessary for the efficient sorting of BDNF to the regulated secretory pathway. Sortilin interacts specifically with BDNF in a region encompassing the Met substitution (Chen et al., 2005). Replacement of Met at this position led to decreased interaction of BDNF with this trafficking protein and suggests that decreased protein–protein interaction between BDNF and the trafficking machinery may be one plausible molecular model for the secretion defect observed with the variant BDNF. This variant BDNF provides an example of how appropriate trafficking of BDNF may have significant impact on the physiological responses to neurotrophins.

Neurotrophins and Synaptic Plasticity

Recently, neurotrophic factors have been shown to play a significant role in mediating synaptic plasticity in the adult brain. The majority of evidence has been obtained via a well-established in vitro methodology of hippocampal slice preparation. Exposing hippocampal slice preparations to exogenous BDNF promoted the induction of a rapid and long-lasting enhancement of synaptic strength, termed *long-term potentiation* (LTP) (Kang & Schuman, 1995; Levine, Dreyfus, Black, & Plummer, 1995). In addition, preparations from transgenic mice deficient in BDNF or its cognate receptor, TrkB (Patterson et al., 1996), showed the same reduction in LTP, which was restored following the addition of BDNF. These data are suggestive of a role for BDNF in the modulation of LTP. Neurotrophins have also been shown to be involved in other forms of synaptic

remodeling. The application of exogenous BDNF or NT-3 induced enhanced evoked responses in both hippocampal preparations as well as at the neuromuscular junction (Lohof, Ip, & Poo, 1993). Thus, while the molecular and signaling mechanisms are not completely understood, there is increasing evidence that neurotrophins can modulate synaptic strengthening and neurotransmission.

BDNF—in addition to its role in modulating synaptic transmission—has been shown to have significant impact on neuronal connectivity, especially in terms of maintenance of proper anatomical structures. In conditional transgenic mice lacking postnatal BDNF or TrkB, both cortical and hippocampal volumes were reduced (Baquet, Gorski, & Jones, 2004; Gorski, Zeiler, Tamowski, & Jones, 2003; Xu et al., 2000). Although the actual number of neurons in these regions showed no significant decrease, there were significant alterations in cell morphology. Both cell soma size and dendritic complexity were reduced by deficits in BDNF or TrkB (Baquet et al., 2004; Gorski, Zeiler, et al., 2003; Xu et al., 2000). These results point to an essential role of BDNF in the development and maintenance of proper neuronal morphology in the adult CNS.

Neurotrophins and Behavior

Initial attempts to study the functional or behavioral consequences of the removal of BDNF or its receptor (TrkB) from the CNS met with limited success, because total deletion of these genes led to early postnatal death (Snider, 1994). In order to study the role of BDNF and TrkB in the functioning adult CNS, it was necessary to generate lines of transgenic conditional knockout mice. In these mice, it was possible to turn off BDNF or TrkB production later in life or remove the genes that code for these proteins from targeted areas of the brain. By testing mice with alterations in BDNF or its receptor, TrkB, on tasks that probe different memory systems, it is possible to gain a better understanding of how alterations in BDNF may affect both the structure and function of the CNS. Here, we review the current animal literature; an overview of the major findings can be found in Table 1.

The targeted deletion of BDNF from the forebrain of mice (Emx-BDNF^{KO}) led to a significant impairment of spatial learning in the Morris water maze; performance of this task is known to rely heavily on the hippocampus (Gorski, Balogh, Wehner, & Jones, 2003). Mice lacking BDNF in the forebrain took longer to locate the platform and exhibited a significantly altered search pattern, typically swimming near the wall and failing to swim over the previous location of the platform, a behavior known as thigmotaxis. In the same study, the Emx-BDNF^{KO} mice were tested in a fear-conditioning task, which was designed to probe the hippocampal memory system by testing for anxious behavior (freezing) when a subject is returned to a context (a distinct place) in which the subject previously encountered an aversive stimulus. In these studies, a cue, such as a tone, is typically paired with the aversive stimulus so that experimenters may also assess in a novel context whether alterations in freezing behavior are observed in response to the tone, which is dependent

Table 1
Behavioral Results Following Genetic Manipulation of BDNF
or TrkB in Mice

Genetic Manipulation	Hippocampal Learning	Locomotor Activity	Anxiety	Depression Indices
BDNF ^{-/-} (forebrain) ^a	+/-	+	+	n.a.
BDNF ^{+/-} (full brain) ^{b,c,g}	-b,c	+b/-g	-b,c	+b
BDNF ^{-/-} (juv. whole brain) ^f	+	+	-	n.a.
BDNF ^{-/-} (adlt. whole brain) ^f	+	-	-	+
TrkB ^{-/-} (forebrain) ^{d,e}	+c	+d	+d	+d

Note—"+" significant effect; "-" no effect; n.a., no test done. ^aGorski, Balogh, et al. (2003). ^bChourbaji et al. (2004). ^cMontkowski & Holsboer (1997). ^dZörner et al. (2003). ^eMinichiello et al. (1999). ^fMonteggia et al. (2004). ^gMacQueen et al. (2001).

upon the amygdala. By adding this control condition, one can dissociate a hippocampal defect (failure to recognize context) from an amygdala defect (failure to associate an aversive stimulus with a cue). By doing this, experimenters gain insight into whether the defect is due to a hippocampal memory impairment or is an impairment in associative learning. Emx-BDNF^{KO} mice did not differ from wild-type controls on contextual fear conditioning; however, it should be noted that these mice engaged in freezing behavior in all contexts, suggestive of an increase in general anxiety. The high level of anxiety exhibited by these mice could also explain the altered search pattern in the Morris water maze task, because thigmotaxis is indicative of an anxiety phenotype. However, when tested on other tasks that can indicate high levels of anxiety, such as the elevated plus maze or the open field task, these mice did not differ from controls.

In a similar study, using an inducible knockout line of mice (Monteggia et al., 2004), removal of BDNF from the entire brain, later in life, resulted in a significant effect on contextual but not cue-dependent fear conditioning. Knockout mice were significantly less fearful—that is, froze less—in the context in which they were shocked. It should also be noted that the early removal of BDNF from the brains of these animals resulted in a hyperactive phenotype, which may have contributed to the decreased freezing behavior. No comparisons can be made between these studies, because inducible knockout animals were not tested for spatial learning abilities.

The differences observed between these two experiments are difficult to interpret, because there are several factors that could explain the differences in results. First, the methodologies result in differences in the degree of BDNF removal. Forebrain knockout animals continue to produce BDNF in the amygdala, another area shown to be important in fear-conditioning paradigms. In addition, forebrain structures have been shown to be important in suppression of the functioning of the amygdala; therefore, changes in this forebrain response may lead to a heightened response in the amygdala. Secondly, there are significant differences in the time course of BDNF removal. Forebrain knockout animals lacked BDNF throughout their development (due to deletion of BDNF coding sequences prior to the onset of BDNF expression; Gorski,

Zeiler, et al., 2003), whereas inducible deletion allowed for normal BDNF levels until later in life. As Monteggia et al. (2004) demonstrated, deletion of BDNF at different time points leads to significant differences in the phenotypes of the mice. Finally, there are also slight differences in the genetic background of these animals. It has been shown that strain variation has a significant impact on freezing behavior in contextual fear-conditioning tasks (Nguyen, Abel, Kandel, & Bourtochouladze, 2000).

Studies of transgenic mice lacking a single BDNF allele (BDNF^{+/-}) have also led to mixed results. Using a contextual fear-conditioning task, BDNF^{+/-} mice have been shown to differ (Liu, Lyons, Mamounas, & Thompson, 2004) and not differ (Chourbaji et al., 2004) from wild-type controls. Again, these results are difficult to interpret, because the mice used in these studies differ both in their genetic background and in the sex of the mice. Mice from the Liu et al. study were derived from an SV129 line and backcrossed 15–18 generations onto a C57BL/6J line of mice, whereas those in the Chourbaji et al. study were derived from an SV129 line and backcrossed for significantly fewer generations onto a C57BL/6J line. In addition, Chourbaji et al. used only female mice, whereas Liu et al. used exclusively males. We are unaware of any studies demonstrating sex differences in contextual fear conditioning, but the differences in sexes used in these studies may contribute to the differing results.

Assessment of hippocampal function via the Morris water maze task using BDNF^{+/-} mice also produced inconsistent findings. BDNF^{+/-} mice derived from an SV129 line and backcrossed onto a BALB/c line of mice demonstrated an impairment in this learning task (Linnarsson, Björklund, & Ernfors, 1997). BDNF^{+/-} mice derived from an SV129 line and backcrossed onto a C57BL/6J line of mice (Montkowski & Holsboer, 1997) displayed no impairment in spatial learning as assessed by performance on the Morris water maze. Thus, strain differences could also explain the differences in these findings.

Conditional transgenic mice lacking TrkB, the receptor for BDNF, in specific brain regions (such as the hippocampus and the cortex) were shown to have deficits in performance of memory tasks consistent with deficits in hippocampal LTP. Mice lacking TrkB have been shown to have impairments of performance in the Morris water

maze and the eight-arm radial maze (Minichiello et al., 1999). Interestingly, in a naturalistic study used to assess learning deficits, conditional TrkB mice displayed deficits in behavioral flexibility in a complex foraging task (Vyssotski et al., 2002).

In light of these studies, care must always be taken when drawing conclusions from genetically modified lines of animals, beyond consideration of strain and sex differences. There may be significant differences in sensory and motor function that are related to the mutation in genes, such as neurotrophins, that have multiple functions. Care should also be taken when selecting tasks that depend heavily on certain sensory systems to perform, such as tasks that rely on visual referencing. In these cases, it may be necessary to perform appropriate control tests, such as tests of visual acuity or motor function, to eliminate these variables as potential explanations for observed group differences.

Conclusions

The human genetic variant BDNF (Val66Met) represents the first example of how one neurotrophin family member, BDNF, may have an impact on higher cognitive processes in humans. It is striking that the major reproducible phenotypes observed in humans with the BDNF_{Met} allele (hippocampal-dependent memory impairment and decreased hippocampal volume) are consistent with previous studies of transgenic animals with reduced BDNF or TrkB levels. These transgenic animals have impairments in cellular hallmarks of learning and memory, such as LTP, as well as anatomical and behavioral alterations associated with the hippocampus.

Future studies focused on further understanding the underlying basis of this BDNF variant's effect on cognitive processes will require a multidisciplinary approach that will likely focus on the following questions. First, what is the molecular and cellular basis of altered BDNF_{Met} function? In vitro studies have provided evidence that the Met substitution leads to impaired regulated secretion of the variant BDNF due to altered engagement of the trafficking machinery in neurons (Chen et al., 2004; Egan et al., 2003). Although one potential trafficking protein, sortilin, has been identified as being involved in impaired BDNF_{Met} trafficking (Chen et al., 2005), other components of this trafficking operation are not yet known. Further basic cell biological studies on the regulation of BDNF secretion from neurons will be needed to understand further the molecular basis of the BDNF_{Met} defect.

Second, what is the extent and nature of the BDNF_{Met} allele's effects on cognitive processes? Whereas impairments in hippocampal-dependent memory have been shown, with concomitant anatomical alteration in the hippocampi of humans with the BDNF_{Met} allele, given BDNF's wide distribution in the CNS, it is likely that other cognitive processes will also be affected. It has already been demonstrated that in humans with the BDNF_{Met} allele, cortical regions, such as the dorsolateral prefrontal cortex, as well as subcortical structures, such as the caudate nucleus, have decreased volumes (Pezawas et al., 2004).

Assessment of further alterations in cognitive function due to the BDNF_{Met} allele will probably require extensive examination of humans who are homozygous for the BDNF_{Met} allele, who represent only 4% of the population. Even in the assessment of hippocampal-mediated deficits, humans heterozygous for the BDNF_{Met} allele did not show significant differences compared with BDNF_{Val/Val} subjects in some studies (Egan et al., 2003).

In a parallel future approach, generation of transgenic mice in which the BDNF gene is replaced by the BDNF_{Met} allele will allow for assessment of the in vivo consequences, not only for behavioral, but also for anatomical and electrophysiological measures. Such analyses would provide a detailed cellular and anatomical basis for the behavioral consequences being measured. Most importantly, studies of transgenic mice with the BDNF_{Met} allele would allow not only for a complementary series of behavioral, anatomical, and molecular analyses, but also for detailed assessment of homozygotes with the BDNF_{Met} allele, which would inform the direction and scope of studies focused on humans homozygous for the BDNF_{Met} allele.

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