Epistasis of the DRD2/ANKK1 Taq Ia and the BDNF Val66Met polymorphism impacts Novelty Seeking and Harm Avoidance

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Abstract

Mounting evidence from animal studies show that the mesolimbic dopaminergic pathways are modulated by the brain derived neurotrophic factor (BDNF). This study investigates in N = 768 healthy Caucasian participants the influence of two prominent functional single nucleotide polymorphisms (SNPs) on the BDNF gene (BDNF Val66Met SNP) and the ANKK1 gene (DRD2 Taq Ia / ANKK1 SNP) on the personality traits of *Novelty Seeking* and *Harm Avoidance*, which are mediated, in part, through dopaminergic mesolimbic circuitry. Carriers of the 66Met+/A1+ variant scored lowest on *Novelty Seeking* and highest on *Harm Avoidance*, compared to all other genotype groups. These participants are characterized by a relatively low D2 receptor density in the striatum and an impaired activity-dependent secretion of BDNF. This is one of the first genetic association studies to demonstrate a modulatory role for BDNF genetic variation on genetically mediated differences in the mesolimbic dopaminergic system in the context of human personality.

Introduction

There is a long tradition in personality research suggesting that personality traits comprising approach behavior towards appetitive stimuli may reflect underlying individual differences in the functioning of the dopaminergic mesolimbic system. This idea was put forward in the *Behavioral Activation System (BAS)* in Gray`s reinforcement sensitivity theory (Gray & McNaughton, 2000, Pickering & Smillie, 2008), the *SEEK* dimension in a broader theory of affective neuroscience by Panksepp (1998; see also Davis et al., 2003), and the dimension of *Novelty Seeking* in Cloninger's biosocial personality theory (Cloninger et al., 1993).

Recent advances in molecular genetics have begun to identify common genetic variations (polymorphisms) that may be associated with individual differences in heritable personality traits, including traits associated with the dopaminergic mesolimbic system. For example, due to its impact on the dopaminergic neurotransmission via the influence on D_2 receptor density in the striatum, one of the most prominent gene loci in molecular genetic dopamine research is the DRD2/ANKK1 Taq Ia polymorphism (rs1800497) on chromosome 11q23.1. This polymorphism is located 10 kb downstream of DRD2 (exon 8) on the adjacent ankyrin repeat and kinase domain containing 1 (ANKK1) gene within a protein–coding region (Neville, Johnstone, & Walton, 2004). This SNP is formerly known under "DRD2 Taq Ia", and is here used with the double name DRD2/ANKK1 Taq Ia, because it became apparent that this SNP is located on the ANKK1 and not the DRD2 gene but nevertheless influences the D_2 receptor density (see below). This influence on the D_2 receptor density might be due to the closeness of the ANKK1 and DRD2 gene resulting in potential linkage effects of the DRD2/ANKK1 Taq Ia polymorphism with other genetic variants on the DRD2 gene. Studies investigating the influence of the DRD2/ANKK1 Taq Ia polymorphism on diverse phenotypes usually group subjects into A1 carriers (A1+) vs. A1 non-carriers (A1-), as the homozygous A1/A1 genotype is quite seldom in the Caucasian population (2-3%). Several studies revealed that carriers of the A1+ variant (A1/A1 and A1/A2 genotypes) of this single nucleotid polymorphism (SNP) have a 30 to 40 percent reduced D_2 receptor density (Jonsson et al., 1999; Pohjalainen et al., 1998; Ritchie & Noble, 2003) In the field of personality research, Lee et al. (2007) reported that the A1+ variant was associated with higher scores on the subscale *Reward Responsiveness* of the BAS dimension in the BIS/BAS scales by Carver & White (1994). Smillie et al. (2010) reported similar effects on Extraversion measured with Eysenck`s Personality Questionnaire (Eysenck & Eysenck, 1991). Examining the molecular genetic underpinnings of Gray`s BAS, Reuter et al. (2006) reported that it is not only the

DRD2/ANKK1 Taq Ia polymorphism but a special configuration of the DRD2/ANKK1 Taq Ia polymorphism and the COMT Val158Met polymorphism which influences the BAS. By combining a genetic association study approach with an endocrinological approach, Reuter et al. (2006) showed that the genetic configurations being associated with low activity of the dopamine degrading enzyme catechol-o-methyltransferase (COMT) and high D_2 receptor density but also high enzyme activity of COMT going along with low D_2 receptor density were associated with highest BAS scores and potentially also highest dopamine levels (derived indirectly through low prolactin levels in these subjects). Thus, the currently available evidence provides a link between the DRD2/ANKK1 Taq Ia polymorphism and approach-related personality traits.

Recently, several animal studies suggest that the mesolimbic dopaminergic neurotransmission is crucially modulated by the brain derived neurotrophic factor (BDNF) (Goggi et al., 2003, Berton et al., 2006, Vargaz-Perez et al., 2009). For example Goggi et al. (2003) reported in an *in vitro* study using rat brain slices, that BDNF potentiates release of dopamine on dopaminergic nerve terminals in the nucleus accumbens after triggering the TrkB receptor. The protein BDNF from the neurotrophin family influences synaptic plasticity and plays a critical role in a variety of processes, ranging from cell growth to apoptosis (Groves, 2007; Martinowitch et al, 2007). The study by Berton et al. (2006) linked BDNF in a mice study to stress resilience, which shows the importance of BDNF in the context of psychopathological disorders such as depression. Besides the impact of BDNF on the mesolimbic dopaminergic system, BDNF also influences the neuroplasticity in several areas of the temporal lobe (Montag et al., 2009), which has been discussed to play an important role in depression and anxiety research (Groves, 2007). On the molecular genetic level the prominent BDNF Val66Met polymorphism (rs6265) on the BDNF gene (MIM 113505) on human chromosome 11p14.1 gained a lot of interest in the investigation of negative but not positive emotionality. The BDNF Val66Met polymorphism leads to an exchange of amino acids from valine to methionine and therefore yields three genotypes Val66Val, Val66Met and Met66Met. The more rare 66Met allele occurs between 20-30 percent in the Caucasian population, the homozygous Met66Met genotype is rare (2-3%). Egan et al. (2003) reported a diminished activity-dependet secretion of BDNF being associated with the 66Met allele. In a knock-in-mice model the homozygous Met66Met variant has been associated with the most pronounced anxious behavior (Chen et al., 2006). In a study of 610 participants our group had obtained analogous results to the knock-in-mice study with respect to trait anxiety in humans (Montag et al., in press). Using the self-report scale Temperament and Character Inventory

(TCI) by Cloninger et al. (1993), we found that homozygous carriers of the 66Met allele had higher scores in two trait subscales of *Harm Avoidance* called *Anticipatory Worry* and *Fear of Uncertainty*. The same trend - but not significant - was also visible for the anxiety related construct *Neuroticism* measured with the EPQ. Similar results - although for the 66Met+ variant (Val66Met and Met66Met genotypes grouped together) and not for the homozygous Met66Met genotype - have been reported by Jiang et al. (2005) based on the same questionnaire. Because only three of their 153 participants were carriers of the Met66Met variant, the study by Jiang et al. could not evaluate any effects of the homozygous 66Met group. Distinct contradictory findings also exist. For example, Sen et al. (2003) reported that carriers of the Met66+ variant showed lowest *Neuroticism* scores in the EPQ. Hünnerkopf et al. (2007) investigated the interaction between the BDNF Val66Met polymorphism and two other gene polymorphisms, and reported reduced scores for *Neuroticism* and *Harm Avoidance* in individuals who carried at least one copy of the Met66 allele of the BDNF Val66Met polymorphism and one 9 allele of the prominent 40-base-pair VNTR dopamine transporter (DAT) polymorphism in the 3′ translated region of the DAT gene (SLC6A3). The DAT polymorphism influences the DAT gene expression, although it is still not clear if the 9 repeat allele of the DAT polymorphism variant is associated with higher (Fuke et al., 2001) or lower DAT expression (Michelhaugh et al., 2001). Given these contradictory data, the association of the Met allele with traits such as *Neuroticism* or *Harm Avoidance* awaits further evaluation.

Based on the above discussion the aim of this study was to assess a possible interaction between the DRD2/ANKK1 Taq Ia polymorphism and the BDNF Val66Met polymorphism on the temperament scales *Harm Avoidance* and *Novelty Seeking*. *Novelty Seeking* has been related to the dopaminergic mesolimbic system (Cloninger et al., 1993), which has found support in related concepts such as the personality dimension SEEK by Panksepp (1998). Nevertheless, the anchoring of the temperament *Novelty Seeking* in the pathway of the mesolimbic dopaminergic system is still an ongoing debate with only partial empirical support (Zald et al., 2008; Cohen et al., 2009, Krebs et al., 2009). Considering the mentioned growing body of animal research showing the modulation of the mesolimbic dopaminergic system by BDNF, the question arises if BDNF also modulates the neurotransmission of dopamine in the human brain. Such an influence of BDNF on the dopaminergic neurotransmission could then be linked to individual differences in temperament dimensions such as *Novelty Seeking*. Moreover, recent own findings from genetic imaging provide evidence that the epistasis effect of BDNF Val66Met and DRD2/ANKK1 Taq Ia is also of relevance for the modulation of the structure of the brain

(Montag et al., in press). Carriers of the A1+/Met66+ variant showed the lowest gray matter volume in parts of the anterior cingulate cortex (ACC). As the ACC represents an integral part of the limbic system and itsself seems to have an important role for personality demonstrated by personality changes in patients who underwent cingulotomy (Cohen et al., 2001), the mentioned epistasis effect of BDNF Val66Met and DRD2 / ANKK1 Taq Ia on the structure of the brain makes it even more plausible that an epistasis effect of both SNPs on personality traits such as *Novelty Seeking* exists. In this context especially the rostral-ventral part of the ACC might be of importance for personality, because here it comes to the resolving of emotional conflict accompanied by a downregulation of amygdala activity demonstrated by the use of emotional stroop tasks in fMRI experiments (see the review by Bush et al., 2000 and Etkin et al., 2006).

Due to the more traditional role of BDNF in the investigation of negative emotionality and also encouraged by the epistatic effect between the DAT VNTR polymorphism and BDNF Val66Met on *Neuroticism* (which is positively correlated with *Harm Avoidance*, Hünnerkopf et al., 2007), we were interested to determine whether a potential epistatic effect between DRD2/ANKK1 Taq Ia and BDNF Val66Met would impact the anxiety related temperament dimension *Harm Avoidance*, too. In our own structural imaging study we reported that especially the carriers of the A1+/66Met+ variant were of interest, because they showed the smallest gray matter volume of the anterior cingulate cortex (Montag et al., in press). In accordance to these findings we expected that especially this allelic configuration as compared to the other allelic constellations would be associated with altered personality scores: Mounting evidence shows that smaller gray matter volumes of areas such as the amygdala (Reuter et al., 2009) or the hippocampus in the temporal lobe (Yamasue et al., 2008) are associated with higher scores in negative emotionality. Although it is highly speculative if these results are transferable to the ACC, we predict that the A1+/66Met+ group is associated with the highest *Harm Avoidance* and lowest *Novelty Seeking* scores. From a psychometric point of view both temperament dimensions are negatively correlated (Maitland et al., 2009), and thus share common variance. Positive evidence for our interaction hypothesis – an epistatic effect of both polymorphisms on *Harm Avoidance* and *Novelty Seeking* - would provide additional evidence from a molecular genetic perspective that negative and positive emotionality share a common biological substrate. This would partly account for the psychometrically obtained correlation between both constructs.

Materials & Methods

Participants

We administered the TCI in N = 768 healthy Caucasian participants (mainly students), who were recruited in Bonn and Heidelberg, Germany. All participants are part of the Bonn Brain Behavior Gene Project (BBBGP), a large gene data bank, making it possible to recruite participants also for further experimental studies. The current sample consisted of 267 males and 501 females and has in part been investigated before in the study by Montag et al. (in press) examining the influence only of the BDNF Val66Met polymorphism on anxiety-related traits in a smaller n = 610. The mean age of the current sample was 25.97 (SD = 8.75). Exclusion criteria in this study were psychopathological/neurological disorders (such as anxiety disorders, depression or alcohol addiction). We asked for a life time occurrence of these disorders with a separate self-constructed questionnaire. Besides that we asked for smoking status (which was not an exclusion criterium). In N = 390 participants we also have data from the Beck`s Depression Inventory yielding unobtrusive scores (M = 6.67, SD = 6.37). Nevertheless, we are aware that our screening of psychopathological disorders is a shortcoming of the study, because structural interviews would be much more appropriate. Unfortunately, the N of the study does not allow to conduct such an interview with every participant. The study was approved by the ethics committee of the German Psychologist Association (Bonn) and the local ethics committee of the Medical School of the University of Heidelberg.

Self report questionnaire

The TCI consists of 240 dichotomous items measuring four temperament and three character dimensions. Cloninger hypothesizes that temperaments have a stronger genetic basis than characters. The latter should develop mainly under environmental influences. The four temperaments are *Novelty Seeking*, *Harm Avoidance*, *Reward Dependence,* and *Persistence*. The three character dimensions are called *Self-Directedness*, *Cooperativeness,* and *Self-Transcendence*. With respect to this study, *Novelty Seeking* and *Harm Avoidance* were of interest. *Novelty Seeking* refers to being enthusiastic, impulsive, and explorative in response to (or maybe "anticipation of") rewarding situations. *Harm Avoidance* is associated with high trait anxiety, ruminating about future outcomes, shyness, moodiness, and being careful in uncertain situations.

Genotyping

DNA was extracted from buccal cells. Automated purification of genomic DNA was conducted by means of the MagNA Pure® LC system using a commercial extraction kit (MagNA Pure LC DNA isolation kit; Roche Diagnostics, Mannheim, Germany). Genotyping of the BDNF Val66Met polymorphism was performed by real time PCR using fluorescence melting curve detection analysis by means of the Light Cycler System 1.5 (Roche Diagnostics, Mannheim, Germany). The primers and hybridization probes (TIB MOLBIOL, Berlin, Germany) for BDNF Val66Met are as follows:

forward primer: 5'-ACTCTGGAGAGCGTGAATGG-3';
reverse primer: 5'-CCAAAGGCACTTGACTACTGA-3';
anchor hybridization probe: 5'-LC640-CGAACACATGATAGAAGAGCTGTT-phosphate-3';
sensor hybridization probe: 5'-AAGAGGCTTGACATCATTGGCTGACACT-fluorescein-3'.

The primers and hybridization probes (TIB MOLBIOL, Berlin, Germany) for DRD2/ANKK1 Taq Ia are as follows:

forward primer: 5'-CGGCTGGCCAAGTTGTCTAA-3';
reverse primer: 5'-AGCACCTTCCTGAGTGTCATCA-3';
anchor hybridization probe: 5'-LCRed640-TGAGGATGGCTGTGTTGCCCTT-phosphate-3';
sensor hybridization probe: 5'-CTGCCTCGACCAGCACT-fluorescein-3'.

Statistical Analyses

In order to detect differences in the temperament dimensions *Harm Avoidance* and *Novelty Seeking* depending on the BDNF Val66Met and DRD2/ANKK1 Taq Ia polymorphism, MANOVAs were computed with the traits *Harm Avoidance* and *Novelty Seeking* as the dependent and the allelic variants as the independent variables. Statistical analyses on the genotype level were not possible due to low cell frequencies in extreme genotype configurations (e. g. A1/A1 by Met66Met: n = 1). In the literature analyses on the allele instead of on the genotype level were common if the genotype frequencies for a given gene locus are skewed in the population. This is the case for the two SNPs under investigation in the present study: As the homozygous A1/A1 variant of the DRD2/ANKK1 Taq Ia polymorphism and the homozygous Met66Met variant of the BDNF Val66Met polymorphism are quite rare in the Caucasian population (each 2-3%), we exclusively investigated the effects of the allelic variants A1+ (A1/A1 and A1/A2) vs. A1- (A2/A2) of the DRD2/ANKK1 Taq Ia polymorphism and Met66+ (Val66Met and Met66Met) vs. Met66- of the BDNF Val66Met polymorphism on these temperaments. MANOVAs were calculated with SPSS 11.5. The

advantage of using MANOVAs instead of univariate analyses is that these models control for multiple testing. This phenomenon is known as familywise alpha correction (Huberty & Morris, 1989). Another advantage of calculating a MANOVA is to obtain a composite effect on the two highly negatively correlated personality traits novelty seeking and harm avoidance (-.37; $p < .0001$). Furthermore, the MANOVA routine in SPSS also indicates results of inbetween subject effects (ANOVAs), separately for both dependent variables.

Results

Genotype Frequencies

Both the BDNF Val66Met and the DRD2/ANKK1 polymorphism were in Hardy Weinberg Equilibrium (BDNF: $Chi^2 = 0.85$, $df = 1$, n. s.; DRD2/ANKK1: $Chi^2 = 0.33$, $df = 1$, n. s.). The genotype frequencies for the total sample and for each gender separately can be found in Tables 1 and 2. The genotype distributions did not differ between genders (BDNF: $Chi^2 = 0.86$, $df = 2$, n. s.; DRD2: $Chi^2 = 0.91$, $df = 2$, n. s.). Most important for the subsequent analyses, information on participant numbers in the allelic configurations of interest are shown in Table 3.

Allelic effects of the BDNF Val66Met and the DRD2/ANKK1 Taq Ia polymorphism on Harm Avoidance and Novelty Seeking

The MANOVA results indicated that there were no significant main effects of the allelic variants of the DRD2/ANKK1 Taq Ia and of the BDNF Val66Met polymorphisms on personality (the composite effect on both TCI dimensions *Harm Avoidance* and *Novelty Seeking*). However, the interaction DRD2/ANKK1 Taq Ia by BDNF Val66Met on personality was significant ($F_{(2,\ 763)} = 5.49$), $p = .004$). The direction of the interaction effect was elucidated by the ensuing ANOVA models and are depicted in Figures 1 and 2. Participants with at least one 66Met allele and one A1 allele show the highest mean in *Harm Avoidance* score and the lowest mean in *Novelty Seeking*.

(Please insert Figure 1 about here)

Figure 1: Interaction effect of the allelic variants A1+ and 66Met+ on the dimension *Novelty Seeking*. Carriers with at least one 66Met allele and one A1 allele show the lowest mean in *Novelty Seeking*.

(Please insert Figure 2 about here)

Figure 2: Interaction effect of the allelic variants A1+ and 66Met+ on the dimension *Harm Avoidance*. Carriers with at least one 66Met allele and one A1 allele show the highest mean in *Harm Avoidance*.

In detail the ANOVA effects are as follows: The BDNF 66Met+ variant showed a significant main effect on the temperament dimension *Harm Avoidance* ($F_{(1,764)} = 4.52$, $p = .03$), such that the Met66+ showed higher *Harm Avoidance* scores. However, this effect does not hold correction for multiple testing. Furthermore, we could observe two strong interaction effects on *Novelty Seeking* ($F_{(1,764)}=7.10$, $p =.008$, $eta^2 = .01$) and *Harm Avoidance* ($F_{(1,764)}=7.93$, $p=.005$, $eta^2 = .01$). The mean values and standard errors of the mean (SEM) can be found in Table 4. It has to be stressed that both ANOVA interaction effects are stable even after Bonferroni correction. The convention in molecular genetics for correcting for multiple testing was among others described by Hünnerkopf et al. (2007): The p-value must be divided by the number of statistical tests performed. In our study this results in 6 tests (2 gene loci (with two main effects and one interaction effect) by 2 dependent variables) and therefore a critical p-value of 0.0083.

As both personality dimensions *Harm Avoidance* and *Novelty Seeking* were significantly negatively intercorrelated (-.37; $p < .0001$) we expected an underlying trait representing the shared variance of *Novelty Seeking* and *Harm Avoidance* that is afflicted by the described gene by gene interaction effect. This hypothesis is corroborated by the fact that the BDNF x DRD2/ANKK1 Taq Ia epistasis influences both temperaments simultaneously as indicated by the overall MANOVA effect. To further clarify this aspect we conducted a principal component analysis (PCA) to be able to analyze the mentioned epistasis effect on a potential underlying composite trait. The PCA yielded one factor with an eigenvalue of $\lambda = 1.37$ explaining 68.63% of the variance of both personality constructs. A second factor with an eigenvalue of $\lambda = .63$ could be neglected according the Kaiser Guttmann criterion (i.e. eigenvalue of $\lambda < 1$).. The gene epistasis effect (inserting the allelic variants A1+/- and 66Met+/- as independent variables) on the composite trait underlying *Novelty Seeking* and *Harm Avoidance* was even stronger compared to the MANOVA effects mentioned above (($F_{(1,764)}=10.98$, $p=.001$; descriptive statistics are presented in Table 5).

Further evidence for the hypothesis that the mentioned epistasis effect falls into the shared variance of both constructs can be obtained by an analyses of covariance (ANCOVA): ANCOVAs with either *Novelty Seeking* or *Harm Avoidance* as dependent variable controlling for the other temperament (inserted as a covariate in the model) should yield non significant gene effects. Here, the ANCOVA with *Novelty Seeking* as dependent variable and *Harm Avoidance* as covariate yields a barely non significant effect ($F_{(1,763)}$=3.86, p=.050). The same was true for the ANCOVA with *Harm Avoidance* as a dependent variable controlling for *Novelty Seeking* as a covariate (((($F_{(1,763)}$=3.03, p=.082). In line with our hypothesis these marginally significant results do not hold correction for multiple testing indicating that the epistasis effect is related to the shared variance of both temperaments.

Although not in the focus of interest in this study, it is important to mention that no other significant effects of BDNF Val66Met and DRD2/ANKK1 Taq Ia on the remaining scales of the TCI could be observed.

Discussion

The neurotransmitter dopamine has long been associated with positive emotionality in personality research (Depue & Collins, 1999, Reuter & Hennig, 2005; Reuter et al., 2006). Especially the mesolimbic dopaminergic system seems to be of special interest here (Cloninger et al. 1993; Panksepp, 1998). Not until recently it became apparent that the protein BDNF, a neurotrophic growth factor, has important functions for the neuroplasticity of the dopaminergic mesolimbic system (Goggi et al., 2003, Berton et al., 2006, Vargaz-Perez et al., 2009). As of now, only one molecular genetic association study searched for a non-independent influence of BDNF and dopamine on personality traits. Hünnerkopf et al. (2007) reported that an interaction of the BDNF Val66Met polymorphism and the dopamine transporter polymorphism influences trait anxiety as measured with the *Harm Avoidance* dimension by Cloninger et al. (1993) and *Neuroticism* by Eysenck (1991). The present study extends these findings by demonstrating an interaction between the BDNF Val66Met polymorphism and the DRD2/ANKK1 Taq Ia polymorphism on two personality traits – namely *Harm Avoidance* and *Novelty Seeking*.

In line with previous studies (Jiang et al., 2005, Montag et al., 2008), the 66Met allele was associated with higher trait anxiety. Adding to our recent finding that individuals with two copies of the 66Met allele exhibited significantly elevated scores on the subdimensions *Anticipatory Worry* and *Fear of Uncertainty* of the temperament *Harm Avoidance* (Montag et al., in press), we now find that individuals who carry at least one copy of the 66Met allele

report highest trait anxiety measured with the total *Harm Avoidance* score, but only in the context of a special allelic configuration – namely with the A1+ allele of the DRD2/ANKK1 Taq Ia polymorphism. Contrasting the effects of both studies, it is apparent, that the main effects of the homozygous 66Met variant on *Harm Avoidance* are much more narrow (influencing subscales) compared to the epistatic effect of the BDNF and DRD2 genes (influencing the total scales of *Harm Avoidance* and *Novelty Seeking*) on personality traits.

In the present study, the epistatic gene effects are strong for both the total *Harm Avoidance* scale (trait anxiety in general) and interestingly also equally strong for *Novelty Seeking*. This finding is remarkable in the manner that the interaction effect of BDNF Val66Met and DRD2/ANKK1 Taq Ia affects both negative and positive personality traits in opposite directions, whereas commonly, genetic association studies report associations with only one or the other valenced trait (see e. g. above Montag et al., in press). Moreover, our current data suggest that - besides the high negative correlation between *Harm Avoidance* and *Novelty Seeking* - the constructs *Novelty Seeking* and *Harm Avoidance* are not orthogonal due to these epistatic genetic effects. From a psychometric point of view this is not suprising, because *Novelty Seeking* and *Harm Avoidance* correlate in our sample inversely with -.37, and thus share approximately 14% of variance. This has been also shown in the study by Maitland et al. (2009) with an inverse correlation of -.41. A molecular genetic finding like ours, influencing both positive and negative emotionality at the same time, aims at the shared variance of *Novelty Seeking* and *Harm Avoidance* then. In contrast, genetic effects usually influence either one of the scales - in other words, the non-shared variance. Concluding, we speculate that the epistastic gene effect demonstrated here yields evidence that both positive and negative emotionality traits may share, at least in part, some of the same neuronal circuitry and this not only from a psychometric but also from a molecular genetic perspective. As one or two genetic variants can only explain for a small amount of variance of complex phenotypes like personality (in our case 1%), obviously several other gene loci have an influence on both shared and non-shared variance of the constructs *Harm Avoidance* and *Novelty Seeking*. In the light of the anxiety conceptualization of Gray & McNaughton (2000), the anxiety-related Behavioral Inhibition System (BIS) circuitry is activated in situations of high uncertainty and followed by careful exploration behavior to reach clarification about the potential dangers of the environment. Therefore, both in positive and negative emotionality approach tendencies play an important role although in reaction to different stimuli, namely pleasant vs. uncertain stimuli. These different approach tendencies could partly be mediated by the mesolimbic dopaminergic system.

If indeed temperament dimensions like *Novelty Seeking* are associated with the mesolimbic dopaminergic system, the observed interaction between the DRD2 and BDNF gene polymorphisms in the present study hints at a transferability of results from rodent research to human beings. Both BDNF and the D_2 receptors are accepted to be essential players acting upon the dopaminergic mesolimbic pathways. With respect to the function of both gene polymorphisms under investigation, the highest *Harm Avoidance* and lowest *Novelty Seeking* scores are associated with the smallest D_2 receptor density (Johnsson et al., 1999) and a diminished activity-dependent secretion of BDNF (Egan et al., 2003). Thus, highly anxious individuals would be expected to be characterized by reduced BDNF secretion (compared to less anxious individuals). Given that BDNF can trigger dopamine release in the nucleus accumbens (Goggi et al., 2003), we predict that highly anxious individuals also exhibit reduced levels of dopamine in the mesolimbic area of the brain. While there is no direct evidence for low levels of dopamine in individuals with negatively valenced traits, there is evidence for high levels of dopamine in individuals with positively valenced traits: In this context positive emotionality was measured with *Extraversion* in Depue & Collins` study (1999) and with BAS in Reuter et al.`s study (2006). Given the the lack of further empirical support for our here presented argumentation line, these assumptions remain highly speculative.

As both DRD2/ANKK1 Taq Ia and BDNF Val66Met exert effects on a vast range of brain areas, it is only possible to conjecture on the neuroanatomical targets being involved by the mentioned genetic variants. This also holds true for our speculation of the here reported epistasis effect potentially to be located in the mesolimbic dopaminergic system – a hypothesis derived from a large body of personality research. Interestingly in this context is the recent finding by Krebs et al. (2009) showing that *Novelty Seeking* is associated with a positive activation of the substantia nigra and the ventral tegmental area in response to novel but not rewarding cues. This indirectly supports the idea that our epistasis effect targets the dopaminergic mesolimbic pathway. Empirical evidence from genetic structural imaging shows that the epistasis of BDNF Val66Met and DRD2/ANKK1 Taq Ia modulates parts of the ACC. Interestingly, the allelic configuration A1+/Met66+ being associated with lowest *Novelty Seeking* and highest *Harm Avoidance* scores in the present study was also associated with the lowest gray matter volume in the ACC, therefore making it likely that the ACC is also involved in both personality traits (Montag et al., in press). A next step should now test the functionality of the ACC and other dopaminergic limbic structures in the light of the present described personality traits under the use of a genetic functional MRI design.

Summarizing, we provide evidence from a molecular genetic association study that two personality traits, *Novelty Seeking* and *Harm Avoidance*, are differentially associated in humans with genes that regulate, in part, the mesolimbic system through BDNF and dopamine receptor (D_2) density. Furthermore, the data presented here suggest that positively and negatively valenced personality traits may share, at least in part, a common genetic mechanism.

Conflict of Interest

None.

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Tables

Table 1: Genotype frequencies for the BDNF Val66Met polymorphism in the total sample and seperately for males and females

	Val66Val	Val66Met	Met66Met
Total Sample	499	245	24
Male Subsample	170	90	7
Female Subsample	329	155	17

Table 2: Genotype frequencies for the DRD2/ANKK1 Taq Ia polymorphism in the total sample and splitted for gender

	A1/A1	A1/A2	A2/A2
Total Sample	32	237	499
Male Subsample	9	80	178
Female Subsample	23	157	321

Table 3: Number of participants in the allelic configuations of interest (number of subjects in percent are presented in parentheses)

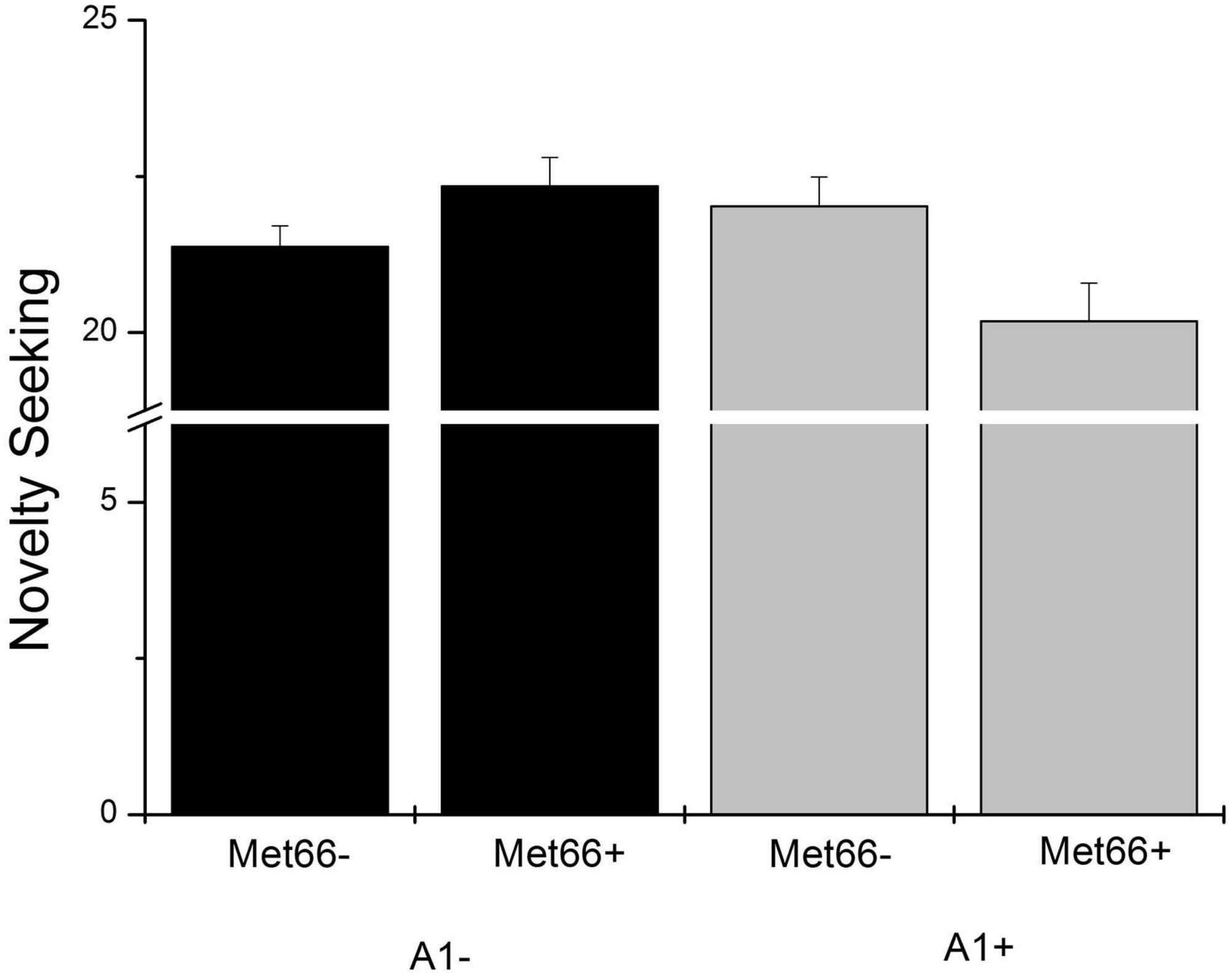
	DRD2 A1+	DRD2 A1-
BDNF Met66+	97 (12,63 %)	172 (22,40 %)
BDNF Met66-	172 (22,40 %)	327 (42,57 %)

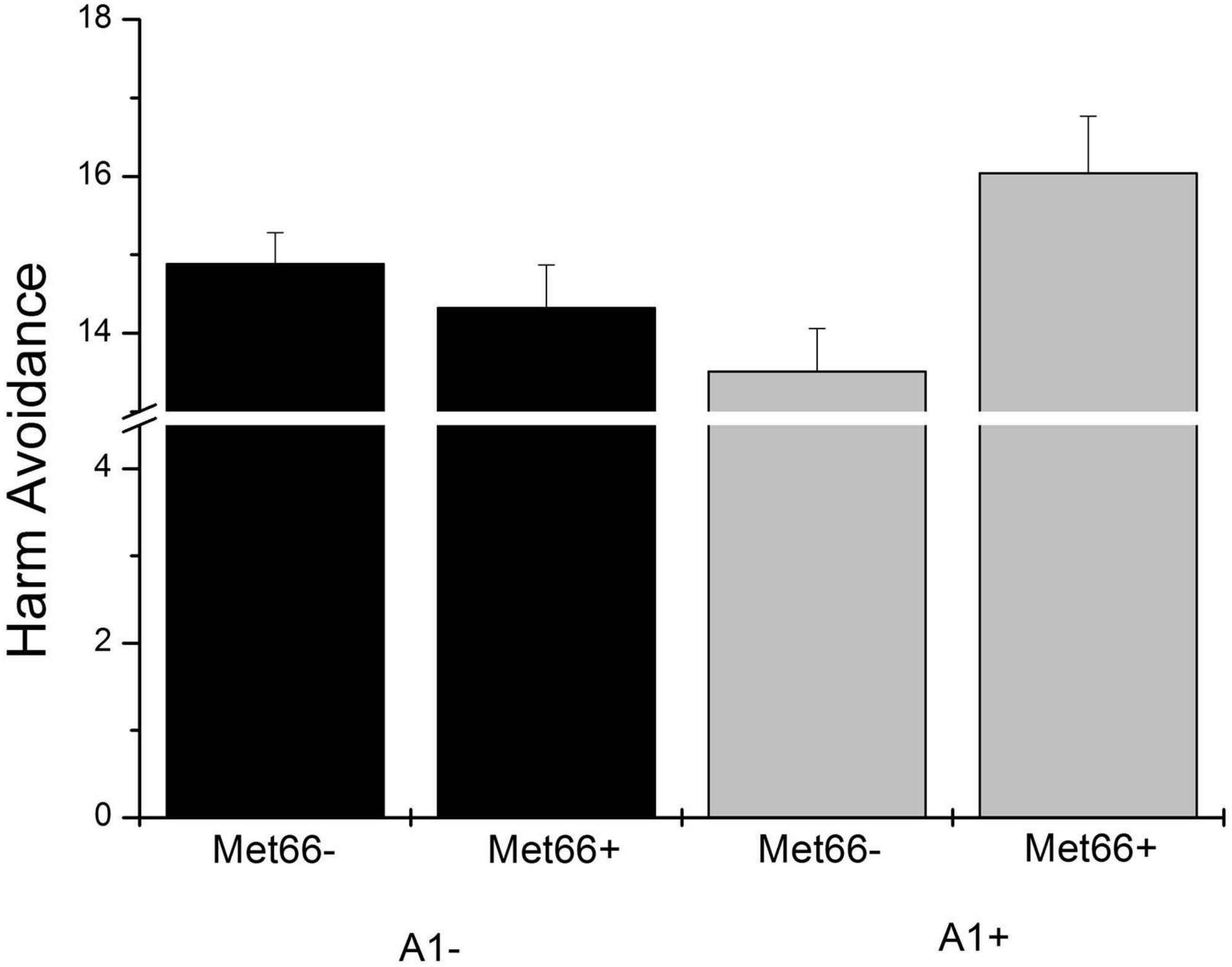
Table 4: Mean (SEM) scores of *Novelty Seeking* and *Harm Avoidance* in the four allelic configurations of interest

	Novelty Seeking	Harm Avoidance
DRD2 A1+ / BDNF Met66+	20.29 (.61)	16.04 (.72)
DRD2 A1+ / BDNF Met66-	21.98 (.46)	13.28 (.54)
DRD2 A1- / BDNF Met66+	22.24 (.46)	14.47 (.54)
DRD2 A1- / BDNF Met 66-	21.40 (.33)	14.86 (.39)

Table 5: Mean factor scores and standard errors of means (SEM) of the PCA factor based on *Novelty Seeking* and *Harm Avoidance*

	Z-Score (SEM)
DRD2 A1+ / BDNF Met66+	-0.26 (.10)
DRD2 A1+ / BDNF Met66-	0.15 (.08)
DRD2 A1- / BDNF Met66+	0.07 (.08)
DRD2 A1- / BDNF Met 66-	-0.04 (.06)


Novelty Seeking
25
20
5
0
Met66-
Met66+
Met66-
Met66+
A1-
A1+


Harm Avoidance
18
16
14
4
2
0
Met66-
Met66+
Met66-
Met66+
A1-
A1+