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Two Types of Aggression Are Differentially Related to Serotonergic Activity and the A779C *TPH* Polymorphism

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The authors investigated whether different types of aggression relate to the A779C tryptophan hydroxylase (*TPH)* polymorphism and to serotonergic activity in volunteers. A factor analysis of the Buss–Durkee Hostility Inventory yielded 2 factors representing *Neurotic Hostility* (NH) and *Aggressive Hostility* (AH). The authors used a neuroendocrine challenge with Citalopram in 48 volunteers and increased cortisol concentrations only in those with high levels of AH. Finally, an association study with 58 volunteers revealed that the A779C *TPH* polymorphism significantly relates to AH, with the highest aggression levels for the genotype AA and the lowest aggression levels for the genotype CC, but not to NH. Results are discussed with respect to inconsistent findings in the literature, which may be explained by this distinction of types of aggression.

Aggression is one of the most extensively studied areas in human behavior. Despite a large number of aggression theories, numerous findings have indicated that aggression is a heterogeneous construct resulting in different phenotypes. Several studies have shown, through the use of the Buss–Durkee Hostility Inventory (BDHI), at least two factors representing different aspects of aggression. However, the nomenclature of these two components has differed from study to study. For example, the components of *experiential* and *expressive* hostility have been distinguished (Meesters, Muris, & Backus, 1996) and the terms *covert* and *overt* aggression have been used as well (Lange, Dehghani, & de Beurs, 1995). The original article by Buss and Durkee (1957) favored the terms *aggression* and *hostility*. Although several articles used different nomenclatures and factors were not quite congruent with respect to subscale loadings, a considerable amount of semantic overlap between them cannot be questioned.

In addition to psychoanalytical explanations, learning theories, and the well-known frustration–aggression theory, recent psychobiological approaches have become more important. Whereas a central causal role of androgens (i.e., testosterone) has recently been questioned (Albert, Walsh, & Jonik, 1993), it has been established that the central nervous serotonin system 5-hydroxytryptamine (5-HT) relates to specific components of aggressive behavior associated with a lack of impulse control (Brown & Linnoila, 1990; Lesch & Merschdorf, 2000; Reist, Helmeste, Albers, Chhay, & Tang, 1996). Because direct indicators of serotonin activity in the central nervous system are difficult to obtain, the use of the neuroendocrine challenge test has been established in this field. Coccaro and colleagues (1987) demonstrated that impulsive aggression is accompanied by blunted prolactin (PRL) responses to the treatment of the 5-HT-releaser d-Fenfluramine (d-Fen). This approach led to the assumption that this form of aggression is characterized by low serotonin activity. Moreover, aggression and blunted PRL responses to d-Fen have been reported for other groups of patients (Cherek & Lane, 1999; Davis, Clark, Kramer, Moeller, & Petty, 1999; Dolan, Anderson, & Deakin, 2001; Roy, Virkkunen, & Linnoila, 1987) and for healthy volunteers with respect to impulsive–aggressive personality (Netter, Hennig, & Rohrmann, 1999; Roy, Virkkunen, & Linnoila, 1988). However, interactions with gender cannot be ruled out because the inverse correlation between PRL responses to d-Fen and aggression or impulsivity were not found in women (Cleare & Bond, 1997). It seems likely that serotonin and testosterone may interact (Higley et al., 1996).

On the basis of findings that personality traits are heritable to a certain extent (30%–60%), as shown by many studies in the field of behavioral genetics (e.g., Bouchard, 1994; Bouchard & Loehlin, 2001; Bouchard, Lykken, McGue, Segal, & Tellegen, 1990; Bouchard, Segal, & Lykken, 1990; Plomin & Nesselroade, 1990), molecular genetic association studies have tried to relate candidate genes coding for transporters, receptors, and enzymes involved in neurotransmitter systems with personality (including aggressiveness and impulsivity) as well (for review see Reif & Lesch, 2003).

One of the candidate genes that turned out to be associated with aggression, anger, and impulsivity is the tryptophan hydroxylase (*TPH*) gene. *TPH* is a rate-limiting biosynthetic enzyme in the serotonin pathway and regulates levels of 5-HT by converting tryptophan into 5-hydroxytryptophan, which is the direct precursor of 5-HT. It is conceivable that variations in the *TPH* gene could contribute to low activity of the 5-HT system. Two single nucleotide polymorphisms (SNPs) that show associations to aggression and anger-related traits have been detected on the short arm of chromosome 11 in intron 7. Both polymorphisms have been shown to be in strong linkage disequilibrium (Nielsen et al., 1998). Of course, this does not mean that both polymorphisms must be

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identical with respect to their functions. But if genotypes for the A218C and the A779C are identical in nearly 100% of a sample as suggested by Kunugi and colleagues (1999), one cannot expect different associations between the one and the other with behavior or other outcome variables. The A779C polymorphism is marked by an adenine to cytosine transversion resulting in two alleles referred to in the literature as *upper* (U) and *lower* (L). Several studies found associations between the A779C polymorphism and lowered cerebrospinal fluid (CSF) 5-HIAA levels. Lower CSF 5-HIAA levels in healthy men, but not in healthy women, carrying the *TPH* U allele (779A corresponds to the U allele) have been reported (Jonsson et al., 1997), whereas the lowest CSF 5-HIAA levels in LL allele carriers were found in a sample of impulsive alcoholic violent offenders (Nielsen et al., 1994). Further findings suggest an association between variations of the *TPH* gene with suicide-related behavior. In a group of subjects who had impulsively attempted suicide, the 779C allele (L allele) was found to be represented excessively, whereas this association was inversed in the nonimpulsive group (Nielsen et al., 1994, 1998). However, no or weak associations between suicide-related behavior and the 779C allele were observed (Bennett et al., 2000; New et al., 1998). Results concerning an association between the A218C SNP of the *TPH* gene and suicide-related behavior are also inconsistent. Among violent offenders and arsonists, the 218C allele was more common in persons who had attempted suicide than it was among those who had not (Nielsen et al., 1994). This finding was corroborated by several researchers who also reported that the more prevalent 218C allele was more common in Caucasians who attempted suicide (Abbar et al., 2001; Souery et al., 2001). However, this finding could not be replicated in other studies (Furlong et al., 1998). Nevertheless, a meta-analytic approach published very recently (Rujescu, Giegling, Sato, Hartmann, & Moller, 2003) established a relationship between suicide attempts and the A218 SNP *TPH* polymorphism.

It is interesting to note that associations of both *TPH* polymorphisms, A779C and A218C, with temperament measures of anger in a sample of healthy volunteers as well as in a sample of subjects who had attempted suicide were described as well (Rujescu et al., 2002). U carriers in both groups had significantly higher scores in anger than L carriers, but there were no differences in allele frequencies between healthy volunteers and subjects who had attempted suicide.

Simple association studies relating genotypes or allele frequencies to personality traits that are supposed to have a serotonergic basis are the first step in the process of detecting the biological origins of personality. The next step would be to find associations between polymorphisms of genes coding for a substrate involved in the metabolism of 5-HT and altered sensitivity in the respective neurotransmitter system. If the sensitivity of the neurotransmitter system is also related to personality, then the cascade from genes to behavior could be much better described. Even if the candidate genes coding *TPH* are not believed to influence serotonin biosynthesis directly, any significant association with behavior or neurotransmitter metabolism would most likely indicate linkage disequilibrium (LD) between the polymorphisms and functional variations in a coding or regulatory region of the *TPH* or a nearby gene (Manuck et al., 1999). This explanation is plausible but somewhat speculative as well. Because *TPH1* is restricted to mostly nonneuronal tissue (except of the pineal gland) and is obviously involved in peripheral cardiovascular reactions (Cote et al., 2003), it would be very helpful to identify these candidate genes which are in LD with the *TPH1* gene. However, no reports addressing this question are available so far.

Recently, Walther et al. (2003) have identified a second *TPH* isoform, referred to as *TPH2*, in mice which is predominantly expressed in the brain stem, whereas the classical *TPH* gene, now called *TPH1*, is expressed in the gut, pineal gland, spleen, and thymus. The authors also identified a *TPH2* homolog on chromosome 12 (GenBank: AY098914). Historical evidence for two *TPH* isoforms, predicted characteristics of *TPH2,* and clinical implications have been discussed by Walther and Bader (2003). However, no polymorphisms on *TPH2* have been detected until now. Therefore, *TPH2* is a promising candidate gene for future research but could not be considered in the present study.

The aims of the present studies were (a) to investigate the underlying two-factor structure of the BDHI more closely with respect to convergent and discriminant validity, (b) to investigate whether different types of aggression as obtained by questionnaire data (BDHI) relate differently to hormone responses in a neuroendocrine challenge paradigm, and (c) to investigate whether these different aggression factors relate to the A779C polymorphism of the *TPH* gene.

Method

Subjects

A total of 48 healthy male volunteers between 20 and 34 years of age participated in the challenge study (Study 1). Volunteers were carefully screened and were rejected on the basis of current disease or past neurologic, endocrinological, or immunological diseases as well as frequent alcohol consumption, smoking, or present drug intake. All subjects were fully informed about the study objectives and the requirement for blood sampling as well as the possible side effects of the challenge substance used. Subjects gave informed consent and were paid for their participation. The study was approved by the ethics committee of the German Association of Psychology.

A total of 58 nonsmoking healthy male volunteers participated in the other experiment (Study 2). These subjects were drawn from a much larger sample that included smokers and women. However, to warrant similar samples in the two studies we included only male nonsmokers. They met the following inclusion criteria: male sex, age between 18 and 35 years, and graduation from high school. As ethical concerns require, participants were informed about objectives and procedures. Subjects could quit the experiments at any time without giving their reasons.

Study Protocol

The experiments in Study 1 were conducted in two adjacent rooms of the Psychology Department building of the University of Giessen. Subjects were instructed to refrain from eating chocolate (or chocolate-containing food), nuts, or bananas; to have lunch before 1 p.m. on each day of the experiment; and to go to bed before midnight on each day prior to the experiment. All experiments started at 3 p.m. to control for circadian rhythm of blood parameters and psychological vigilance. After arrival, subjects were asked to go to the restrooms. Afterward, they were seated in a comfortable armchair and their blood pressure and heart rate were measured. First, subjects were asked to fill in a German translation of the BDHI and the Freiburg Personality Inventory (FPI; Fahrenberg, Hampel, &

Selg, 1984), which contains the dimensions (secondary factors) of neuroticism and extraversion. However, both secondary factors consist of the following primary factors: Satisfaction with Life (which is a kind of inverted depression scale), Altruism, Ambitiousness, Inhibition, Irritability, Aggression, Stress Experience, Psychosomatic Complaints, Worries About Health, and Openness (an inverted lie scale).

Following the completion of the questionnaires, an indwelling catheter was inserted into the antecubital vein. Blood was drawn invisibly from the subject in an adjacent room that was connected to the experimental room ("through-the-wall technique"). Further samples were collected at 50, 80, 110, 140, 170, and 200 min after catheterization. At 3:30 p.m., a single oral dose of either 20 mg Citalopram (Cipramil, Lundbeck, Denmark) or placebo was administered in identical-looking capsules in a randomized balanced crossover design under double-blind conditions. The selective serotonin reuptake inhibitor (SSRI) Citalopram was used with a single oral dose of 20 mg because of its high specificity for the serotonin system (for more details, see Hennig & Netter, 2002).

After every test day, blood samples were centrifugated (10 min at 4,000 g). Plasma was taken and stored at −30 °C until cortisol levels were measured with a commercial enzyme immunoassay (EIA; DRG, Marburg, Germany). All analyses were performed fully automatically by use of the Labotech II (Biochem, Freiburg, Germany), yielding very low intra- and interassay variations (both CV $<$ 5%).

For Study 2, subjects were asked to collect buccal cells, which can easily be obtained by mouth washings with specific cotton wool swabs. After collecting cells, subjects were asked to fill in the BDHI.

Genotyping (Study 2)

Genomic DNA was extracted from whole blood by the use of a standard extraction kit (High Pure Polymerase Chain Reaction [PCR] Template Preparation Kit; Roche Diagnostics). Genotyping of the A779C *TPH* SNP was performed with real-time PCR and by fluorescence melting-curve detection analysis by means of the Light Cycler System (Roche Diagnostics; Mannheim, Germany). The primers and hybridization probes used (TIB MOLBIOL, Berlin, Germany) were as follows: forward primer, 5′-CTTATATGTGTGAGTCTGAGTGG-3′; reverse primer, 5′-GGACATGACCTAAGAGTTCATGGCA-3′; acceptor hybridization probe, 5′-LCRed640-CACGCTGCAGTGCTTAACATACGTTTATAA-phosphate-3′; and donor hybridization probe, 5′-CTGAAAGAGAGGTACAAGTT-fluorescein-3′. The PCR run constituted 55 cycles of denaturation (95 °C, 0 s, ramp rate 20 °C $^{s-1}$), annealing (60 °C, 10 s, ramp rate 20 °C $^{s-1}$) and extension (72 °C, 10 s, ramp rate 20 °C $^{s-1}$), which followed an incubation period of 10 min to activate the FastStart Taq DNA Polymerase of the reaction mix (Light Cycler FastStart DNA Master Hybridization Probes, Roche Diagnostics). After amplification, a melting curve was generated by keeping the reaction time at 40 °C for 2 min and then heating slowly to 95 °C with a ramp rate of 0.2 °C $^{s-1}$. The fluorescence signal was plotted against temperature to yield the respective melting points (Tm) of the two alleles. Tm was 51.47 °C (*SEM* = 0.07) for the A allele and 56.99 °C (*SEM* = 0.06) for the C allele. The melting points markedly differed from those reported by Rujescu et al. (2002), who also performed the PCR by means of the Light Cycler System (Tm-A allele: 53 °C; Tm-C allele: 48 °C). Evidently, Rujescu et al. (2002) reported a higher Tm for the A allele, whereas our analysis yielded a higher one for the C allele. To avoid confusion, it should be noted that the sequence of the acceptor hybridization probe differs between both reports. The base on Position 5 and 6 should be C-T instead of T-C in the article by Rujescu et al. (2003). However, with respect to the different allocation of Tms to alleles, the following is crucial: The C allele must have a higher Tm than the A allele because the donor hybridization probe was designed in a way that it perfectly binds to the sequence containing the C allele. Therefore, the hybridization probe is already displaced at a lower temperature from the DNA strand when the fit is not perfectly complementary, as it is in the case of the sequence containing the A allele. Because the frequencies of all genotypes are comparable across both studies, it can be assumed that a misspelling in the report by Rujescu et al. (2003) is more likely the source of differences than is genotyping per se.

To corroborate our genotyping (i.e., the correctness of our melting temperatures), we also conducted a conventional PCR followed by gel electrophoresis to verify the size of the *TPH* fragments. Initial incubation time for this PCR was 3 min at 95° followed by 30 cycles with a denaturation period of 30 s at 94°, annealing for 30 s at 60°, elongation for 45 s at 72°, and the final extension step was 72° for 3 min. Taq-polymerase and the same primers were used as in the Light Cycler run. The PCR product was analyzed by gel electrophoresis in a 1% agarose gel containing ethidium bromide and visualized under ultraviolet light. A 1 kb DNA marker was used (MBI Fermentas, St. Leon-Rot, Germany). This was done for two reference samples: a homozygote A779 sample and a homozygote 779C sample. For both samples, a single band of 210 bp length could be found (data not shown). To further validate our results, we sequenced the PCR products to detect the base conversion C to A which discriminates the genotypes unequivocally.

Statistical Analyses

First of all, a factor analysis (simple structure) with varimax rotation was performed for the subscales of the BDHI in Study 2, which revealed the higher sample size. On the basis of the factor solution, the most important subscales of the BDHI (with respect to factor loadings) were then summed up, yielding a score for the underlying aggression dimension. This definition was taken to compute dimensions of aggression for Study 1 as well. With this procedure, the same aggression factors were present for both studies.

An analysis of variance (ANOVA) for repeated measures with two within-factors (substance [2 levels] and time point of blood sampling [3 levels]) was calculated to investigate whether Citalopram increased levels of cortisol significantly. Convergent and discriminant validity of aggression dimensions was determined by Pearson correlations with subscales of the FPI (Study 1). Finally, associations between genotype of the A779C polymorphism and aggression was determined with univariate ANOVAs with the genotype as the independent factor consisting of 3 levels (AA, AC, CC).

Results

Factor Structure of BDHI

It should be noted that a two-factor solution of the BDHI scales emerged in our sample as well. Table 1 gives the factor loadings of the rotated factor solution.

The factor analysis confirmed that the BDHI scales represent different aspects of aggression. Two factors were extracted by use of the extraction criteria of an eigenvalue $>$1. These two factors explain more than 60% of the total variance, which can be considered as sufficient. Following the rotated factor solution, two factors can be clearly distinguished. The first factor (eigenvalue = 3.77, 38.4% of variance) is characterized by high factor loadings on Irritability, Resentment, Verbal Hostility, and Guilt. This can be classified to represent a neurotic dimension of aggression and is named *Neurotic Hostility* (NH). The second factor (eigenvalue = 1.6, 24.1% of variance) represents a different pattern of aggression with high loadings on Assault and Negativism, and, most interestingly, with a negative loading on Guilt, which, however, is low. This kind of aggression represents a more "cold" aspect of aggres-

Table 1
Factor Loadings of the BDHI Scales, Eigenvalues, and Amount of Explained Variance for a Rotated (Varimax) Factor Solution

BDHI scale	Factor 1	Factor 2
Irritability	**.78**	.31
Resentment	**.72**	.32
Verbal Hostility	**.75**	.26
Guilt	**.77**	−.21
Assault	.21	**.64**
Negativism	.06	**.83**
Suspicion	.61	.55
Indirect Hostility	.57	.33
Eigenvalue	3.77	1.6
Explained Variance	38.4%	24.1%

Note. BDHI = Buss–Durkee Hostility Inventory. Bold values in Factor 1 were used to compute Neurotic Hostility. Bold values in Factor 2 were used to compute Aggressive Hostility.

sion and is named *Aggressive Hostility* (AH). Indirect hostility and suspicion share common variance between both factors and are not considered for any further result. NH was computed as the mean of those BDHI scales with factor loadings on Factor 1 (see Table 1); AH was computed as the mean of those BDHI scales with factor loadings on Factor 2. However, inverted scale values of guilt were added to AH as well. This decision is based on the fact that guilt discriminates in an optimal way between both factors. Conceptually, aggression without guilt seems to be the core of AH. In addition, scores for NH and AH were correlated with other dimensions of personality (for further information concerning validity, see Table 2).

As depicted in Table 2, it became evident that NH relates to neuroticism and, consequently, to the primary factors of that trait (Irritability, Low Satisfaction With Life, Psychosomatic Complaints). Subjects high on NH scored highly on Openness as well. This is somewhat trivial because social desirability should cause low levels of aggression obtained by questionnaires. The interesting point is that AH is completely independent from neuroticism except for the shared variance with the subscale Aggression. Both aspects of aggression are not related to extraversion. The relationship between altruism and AH (which barely failed significance) makes clear that AH is accompanied by a low social bonding and, therefore, is probably associated with the personality trait of psychoticism.

Challenge Test

It should be noted that Citalopram leads to the expected increase in cortisol levels (Hennig & Netter, 2002). As depicted in Figure 1, the increase in cortisol follows the pharmacokinetics of the drug.

Although the amount of increases in cortisol is not substantial, the repeated measures ANOVA indicated a significant Time Course × Drug interaction, $F(6, 276) = 2.7$, $p < .05$. To investigate whether subjects differ in responsivity according to the level of NH or AH, we dichotomized these variables by median split to obtain groups of high versus low NH and AH, respectively. Furthermore, responses were calculated as difference scores between placebo and Citalopram at 30 min, 60 min, and 120 min.

As depicted in Figure 2, cortisol responses after treatment with a single oral dose of 20 mg of Citalopram did not differ between subjects with low or high scores on NH. There seems to be a tendency of higher responses in high scorers; however, this is far beyond statistical significance: NH × Drug × Time interaction, $F(2, 92) = 0.53$, $p = .6$. On the basis of these results, it can be concluded that serotonergic function as indicated by cortisol responses to Citalopram does not relate to NH. A very different picture occurs for subjects differing in AH (see Figure 3).

The results as depicted in Figure 3 clearly reveal that high scorers in AH are high responders to Citalopram. This effect becomes highly significant with respect to the Drug × AH × Time interaction, $F(2, 92) = 5.71$, $p < .01$. At this point, serotonergic activity is differently involved in these types of aggression.

Molecular Genetics

The final question to be answered is whether the A779C polymorphism of the *TPH* gene discriminates both aspects of aggression. First, some general results are presented.

The distribution of genotype and allele frequencies of the A779C SNP in the *TPH* gene is shown in Table 3. The genotype distribution was in Hardy–Weinberg equilibrium, $\chi^2(1) = 0.108$, $p = .743$. The proportion of the less common (A or U) alleles was 33.6%, and the proportion of the more common (C or L) alleles was 66.4%. Sequencing confirmed that the intended DNA fragment had been amplified and that the sample with the higher Tm in the Light Cycler run had the C allele and that the sample with the lower Tm had the A allele (see Figure 4).

It is interesting to note that the genotype significantly related to AH (see Figure 5). Subjects homozygotic for the A allele exhibited higher levels of AH compared with the other genotypes, $F(2,$

Table 2
Correlations Between Neurotic Hostility (NH) and Aggressive Hostility (AH) With Other Personality Traits

Trait	NH	AH
Primary factors		
Satisfaction with life	−.56*	.12
Altruism	.02	−.22
Ambitiousness	.03	.13
Inhibition	.06	−.14
Irritability	.70*	−.11
Aggression	.54*	.46*
Stress experience	.54*	−.21
Psychosomatic complaints	.52*	−.22
Worries about health	.12	.09
Openness (inverted lie scale)	.54*	.13
Secondary factors		
Extraversion	.04	.05
Neuroticism	.72*	−.06

Note. $N = 48$.
* $p < .01$.

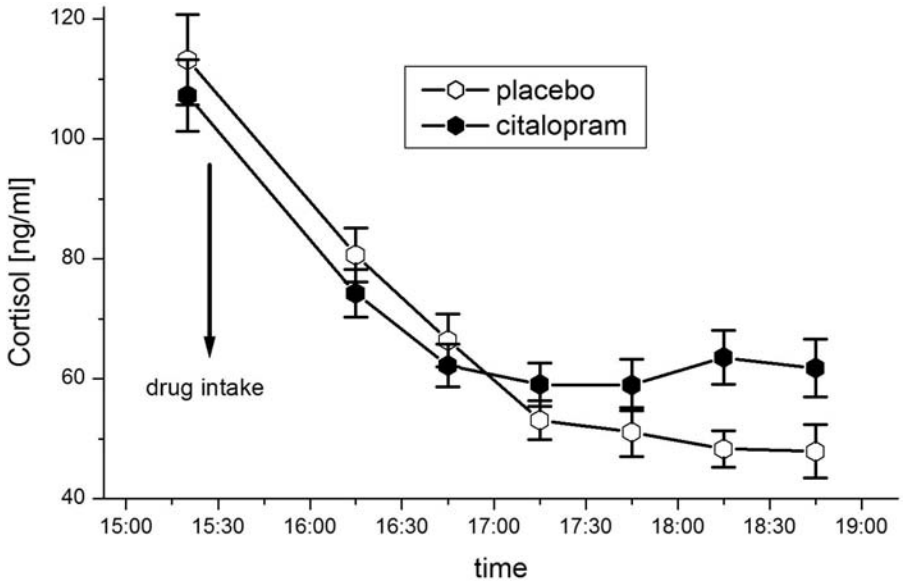


Figure 1. Means (± *SEM*) of changes in cortisol according to treatment with Citalopram and placebo.

55) = 3.76, $p < .05$. In contrast, NH did not relate significantly to the A779C polymorphism of the *TPH* gene, $F(2, 55) = 1.48$, *ns*.

Discussion

The present study clearly indicates that at least two types of aggression have to be identified when the BDHI is used (see Table 1). This is in line with several other investigations that yielded similar results. Although the nomenclature for these factors differs across the studies, there is evidence that the personality dimension of neuroticism especially helps to differentiate both aspects. As depicted in Table 2, both aspects of aggression share common variance as indicated by a significant correlation with the person-

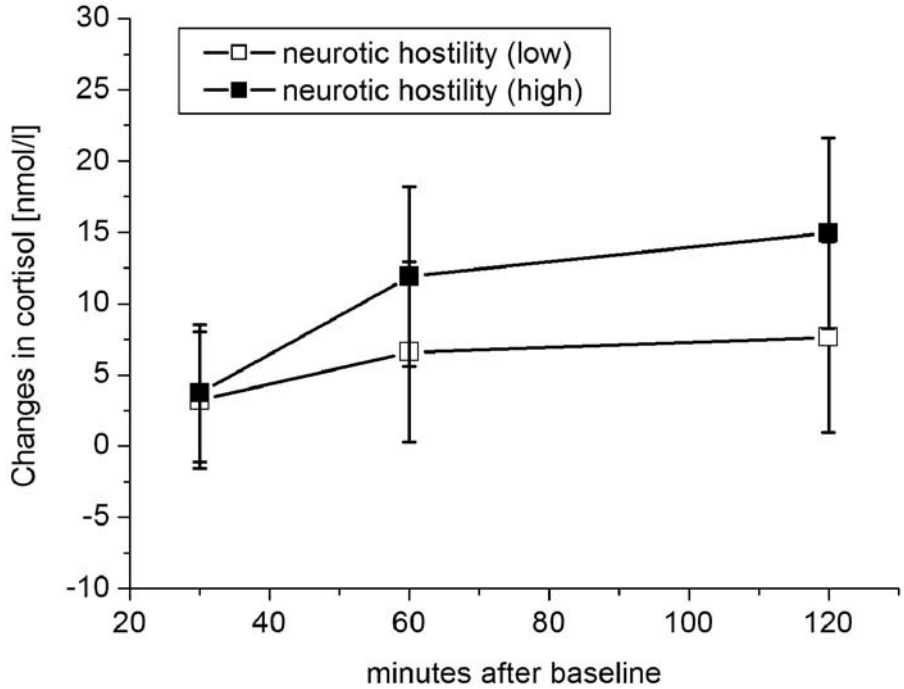


Figure 2. Means (± *SEM*) of changes in cortisol 30, 60, and 120 min after baseline for high versus low scorers on neurotic hostility.

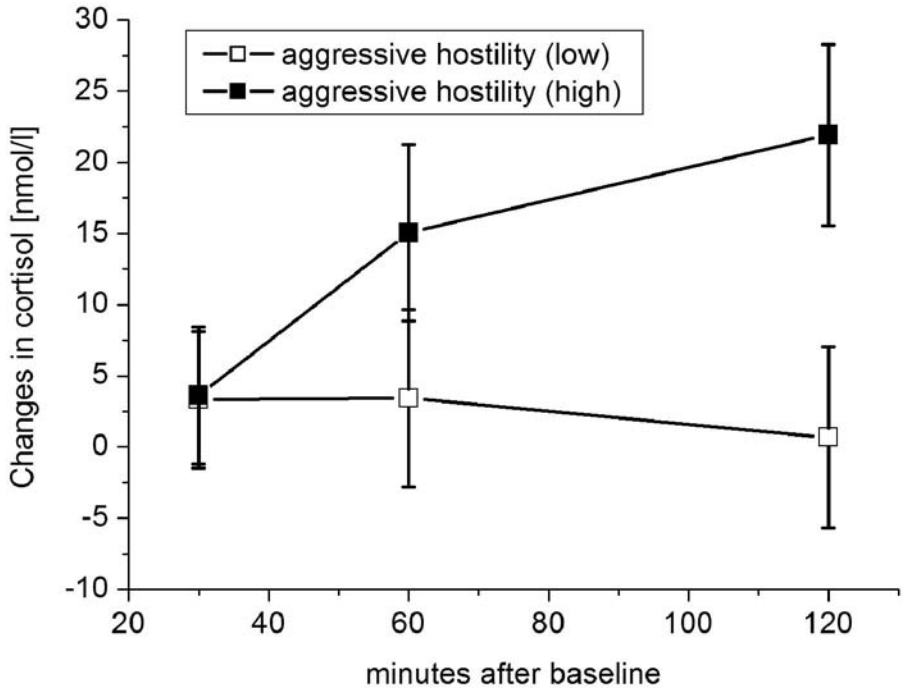


Figure 3. Means (± *SEM*) of changes in cortisol 30, 60, and 120 min after baseline for high versus low scorers on aggressive hostility.

ality dimension of aggression and by their common loadings on suspicion. However, it is interesting to note that only one component of aggression (NH) correlated with Low Satisfaction With Life, Irritability, Stress Experiences, and Psychosomatic Complaints. All of these traits are subscales of the major personality dimension of neuroticism, which can be described as a tendency toward overall emotional instability. In contrast, the other component (AH) did not relate to neuroticism at all. The interesting point is that the BDHI Guilt subscale relates to the neurotic component of aggression but not to the aspect of AH (see Table 1). This finding, in addition to the slightly negative correlation between AH and altruism, justifies the notion that AH relates much clearer to the personality dimension of psychoticism.

Table 3
Genotype Distribution and Allele Frequencies of the A779C Polymorphism

	Observed		Expected	
Genotype/allele	*n*	%	*n*	%
Genotype				
AA	7	12.1	6.4	11.1
AC	25	43.1	26.1	45.0
CC	26	44.8	25.4	43.9
Allele				
A	32	33.6		
C	51	66.4		

Note. Hardy–Weinberg equilibrium: $\chi^2(1, N = 58) = 0.108$, $p = .743$.

With respect to neuroendocrine challenge tests, oral applications of Citalopram were only reported occasionally. The 20-mg dose did not lead to cortisol, prolactin, or thyrotropin responses in a group of 10 depressed women compared with the responses in a placebo group (Papakostas et al., 2000). However, because that study lacks an appropriate (healthy) control group, it may be that the missing hormone responses characterized blunted responses in these patients. In samples of healthy volunteers, two independent studies of our group with relatively large sample sizes revealed that the oral dose of 20 mg increases cortisol concentrations in 48 male nonsmokers (Hennig & Netter, 2002) and that 30 mg are sufficient to induce a comparable increase in 36 male smokers (Netter, Toll, Lujic, Reuter, & Hennig, 2002). However, in both studies prolactin and growth hormone levels were not changed. The intravenous administration of Citalopram has resulted in a different pattern of hormone responses showing additional increases in prolactin concentrations (Attenburrow, Mitter, Whale, Terao, & Cowen, 2001; Bhagwagar, Whale, & Cowen, 2002; Kapitany et al., 1999; Seifritz et al., 1996). Moreover, with respect to psychopathology, blunted PRL responses have been described for major depression (no differences noted for cortisol; Kapitany et al., 1999) whereas others could demonstrate that blunted cortisol responses are present only in acute but not in recovered patients. PRL responses were blunted in both cases (Bhagwagar et al., 2002). Sound differences in cortisol responses after Citalopram according to diagnosis are missing in current research.

Our results can be interpreted by two lines of argument. First, a blunted cortisol response was detected in high-NH subjects. This is comparable with findings in clinical samples (e.g., Kapitany et al.,

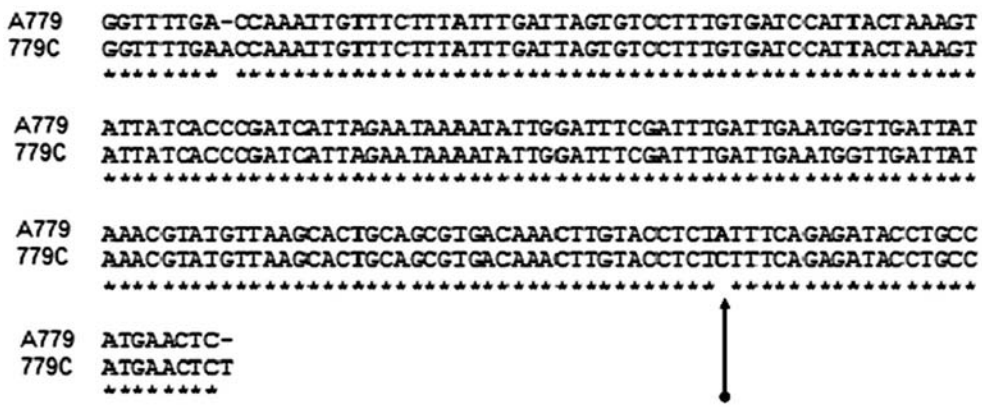


Figure 4. Results of sequencing the amplification product (arrow = single nucleotide polymorphisms).

1999) because this type of aggression relates to subclinical depression (low satisfaction with life, see Table 2).

However, a second, more attractive perspective relates to high-AH subjects. They exhibited high increases in cortisol concentrations after treatment with Citalopram, which confirms findings with the 5-HT1a partial agonist Ipsapirone in subjects scoring high on aggression in a previous study (Netter, Hennig, & Rohrmann, 1999). Given that high responses after treatment with the reuptake inhibitor Citalopram may indicate elevated postsynaptic sensitivity (as a consequence of low serotonin availability), AH may be characterized by low 5-HT activity. With respect to the possible underlying mechanisms of increased levels of cortisol after stimulation with Citalopram, it should be noted that previous studies that have used SSRIs (Paroxetine) have demonstrated that the drug induced increases in cortisol seem to be mediated (at least partly) by the postsynaptic 5-HT2a/c-receptor as indicated by attenuated responses after pretreatment with Cyproheptadine (5-HT2-receptor antagonist; Kojima et al., 2003). It seems likely that low central availability of 5-HT results in an upregulation of postsynaptic receptors (including 5-HT2a/c), leading to more pronounced cortisol increases in subjects with moderate 5-HT deficiency (AH). From this point of view, genetic differences in *TPH* may relate (probably as a result of linkage with other regulating genes) to a reduced 5-HT synthesis, which itself could be respon-

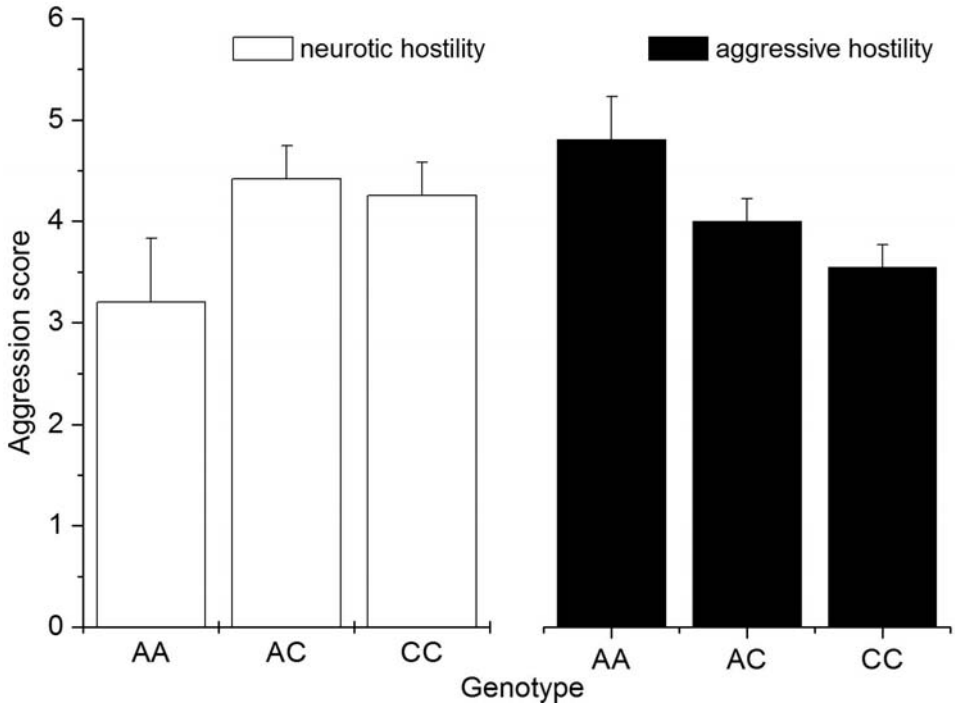


Figure 5. Means (+ *SEM*) of levels of neurotic (left) and aggressive (right) hostility depending on the genotype (AA, AC, or CC).

sible for postsynaptic receptor sensitization. A short-term prolongation of 5-HT availability in the synaptic cleft due to acute effects of SSRIs would then lead to increased cortisol responses compared with subjects with other *TPH* genotypes. With respect to Citalopram, we can exclude peripheral effects due to paracrine stimulation of cortisol secretion by mast-like cells located in the adrenal cortex. These cells initiate their effects after stimulation of the 5-HT4 receptor in frogs and humans (5-HT7-receptor in rats; Contesse et al., 2000) and are, in principle, candidates for peripherally induced increases in cortisol after treatment with certain serotonergic substances. However, Citalopram has no effects on these receptors after a single administration, and 5-HT4 antagonists do not diminish the effects of Citalopram (Stenfors, Yu, & Ross, 2001). Therefore, synaptic plasticity as discussed above on a peripheral level is very unlikely to explain our results.

Moreover, AH was mainly defined by high scores on assault and low feelings of guilt. This combination comes very close to the sample characteristics in the early study of Coccaro (1989) who detected blunted PRL responses after treatment with d-Fen in violent offenders with high levels of assault. There is evidence that one aspect of aggression (the one associated with emotional stability and lack of depression) more clearly relates to 5-HT compared with the more neurotic component.

It may be possible that the overall conflicting results concerning associations between polymorphisms of the gene coding for the *TPH* are linked to different aspects of aggression as well. It was found that aggressive subjects, especially those who had experienced unprovoked anger and outward expression of anger, were significantly more frequently carriers of the A218C U allele (Manuck et al., 1999). Moreover, these subjects (i.e., the subgroup of men) are characterized by blunted PRL responses to d-Fen as well. Again, this finding confirms the result of the present study. As shown in Figure 5, the highest AH scores were found in subjects homozygotic for the U allele, whereas subjects homozygotic for the L allele had the lowest AH scores, with heterozygotic subjects falling between the two extremes. The study of Rujescu and colleagues (2002) is in line with these findings, and it also demonstrates that several aspects of anger relate to U-allele carriers in a sample of German volunteers whereas irritability (related to the neurotic component of aggression) does not.

The necessity to distinguish different aspects of aggression may become evident in another study demonstrating that carriers of the LL allele exhibit higher levels of impulsive aggression as determined by BDHI sum scores (New et al., 1998). Because most of the BDHI subscales load on the NH factor (see Table 1), this finding must not be contradictory to the above mentioned ones. Moreover, the genotype was not related to d-Fen-induced changes in PRL concentrations. These results are partly confirmed in another study demonstrating that violent schizophrenic patients more often carry L-alleles in contrast with less violent patients, however, this effect was not very pronounced (Nolan, Volavka, Lachman, & Saito, 2000). It becomes clear that the disposition rather than the diagnosis seems to be the relevant factor (Paik, Toh, Kim, & Lee, 2000). The same line of argument may be valid for the literature concerning suicide. It is noteworthy that even meta-analytic approaches have yielded different conclusions (Lalovic & Turecki, 2002; Rujescu et al., 2003). It is most likely that specific types of aggression may modulate suicidal behavior and that violent suicide relates to the *TPH* gene differently compared with less violent attempts which have been associated with CSF 5-HIAA for many years (Asberg, Traskman, & Thoren, 1976). Although the present study reveals that specific components of aggression relate to the U allele of the *TPH* gene and to the outcome of a neuroendocrine challenge test indicating that AH is associated with low 5-HT activity, some limitations should be mentioned. On the one hand, the sample size for the association study is small. This, however, is due to the restriction that only male nonsmoking subjects were included. Both criteria are important and have been demonstrated to modulate the relationship between serotonin and personality. Moreover, the polymorphism of the *TPH* gene has been related to smoking behavior in a population-based control study with nearly 800 subjects (Sullivan, Jiang, Neale, Kendler, & Straub, 2001). Because many psychiatric patients are heavy smokers, one cannot exclude the possibility that the slightly more homogeneous results obtained in studies with healthy nonsmoking volunteers may be, in part, a consequence of eliminating this confounding factor.

Another point should be stressed as well. We performed two independent studies not for theoretical reasons but for technical ones. We performed the study examining the endocrine responses to Citalopram prior to the association study, and it was completed prior to the acquisition of facilities enabling us to run genotyping in our own laboratory. Therefore, when conducting the challenge study we did not ask our subjects for informed consent concerning genotyping and, thus, we were forced to run the second study as described above. It would have been better to obtain genotypes and responses to specific drugs like Citalopram in the same subjects. In this case, the polymorphism of the 5-HT-transporter (5-HTTLPR) could be examined as well, as this protein is the target for SSRIs. We are currently running such a study. Moreover, a functional discrimination between *TPH1* and *TPH2* polymorphisms would be worthwhile as well. However, because *TPH2* polymorphisms are not included in GenBank and a protocol for RT-PCR is not available, we could not contribute to this exciting area of research. In fact, the identification of a new SNP on the *TPH2* gene requires a genetic screening of hundreds of samples and a job specialization in the field of molecular genetics. However, a multidimensional approach including behavior, genotyping, and neurochemical indicators of neurotransmitter activity would be promising for the future.

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Correction to Chambers and Wang (2004)

The article "Role of the Lateral Parabrachial Nucleus in Apomorphine-Induced Conditioned Consumption Reduction: Cooling Lesions and Relationship of c-Fos-Like Immunoreactivity to Strength of Conditioning," by Kathleen C. Chambers and Yuan Wang (*Behavioral Neuroscience,* 2004, Vol. 118, No. 1, pp. 199–213), contained an error.

In Table 1, the first line entry under the heading "Amount of sucrose consumed," subheading "E1–E8," now reads Apo1 <. It should read Apo10 <.