Additive Effects of Serotonin Transporter and Tryptophan Hydroxylase-2 Gene Variation on Emotional Processing

Prior studies reported that functional variants of both the serotonin transporter (5-HTT) and tryptophan hydroxylase-2 genes (TPH2), 2 key regulators of the serotonergic signaling pathway, modulate amygdala activity during emotional processing. We addressed the question whether these 2 gene variants modulate each other, using an emotional picture-processing task. Specifically, we measured event-related potentials (ERPs) during a passive emotional picture perception task, focusing on ERPs for the early posterior negativity (EPN) around 240 ms and for the slow wave starting at 315 ms. We found evidence for increased neural activity at 240 ms in individuals who carried 1 or 2 copies of the low-expression short variant of the 5-HTT. Carriers of T variant of the TPH2 also showed a tendency toward increased neural activity at 240 ms. Moreover, we observed an additive effect of both genotypes for EPN, with highest neural activity to emotional stimuli in individuals carrying combination of both short variant of 5-HTT and T variant of TPH2. Our results indicate that both the 5-HTT and the TPH2 genotypes modulate the sensory encoding of affective stimuli during early steps of visual processing and reveal additive effects of 2 genes in the serotonergic control of emotion regulation.

**Keywords:** emotion, ERP, genetics, 5-HTT, TPH2

**Introduction**

Length variation of a repetitive sequence in the transcriptional control region of the serotonin (5-HTT) transporter gene (SCL6A4, 5-HTT) regulates 5-HTT expression and function, modulates brain activity associated with affective processing, and is associated with anxiety- and depression-related behaviors (Lesh and others 1996; Canli, Omura, and others 2005; Lesch and Gutknecht 2005). Individuals with the short variants of the 5-HTT exhibit greater amygdala activity during processing of emotional stimuli (Hariri and others 2002, 2005). Extending these results, recent functional magnetic resonance imaging (fMRI) studies showed that the 5-HTT genotype modulates the functional connectivity between the amygdala and prefrontal cortex (Heinz and others 2005; Pezawas and others 2005). Likewise, a single-nucleotide polymorphism (SNP) in the upstream regulatory region of the gene for tryptophan hydroxylase-2 (TPH2, rs4570625), the rate-limiting enzyme of 5-HT synthesis in the brain, was also found to influence processing of emotional stimuli (Brown and others 2005; Canli, Congdon, and others 2005) with T allele carriers showing greater amygdala reactivity in 2 recent studies.

Because both the 5-HTT and TPH2 regulate the synaptic availability of 5-HT and because variations of the encoding genes influence the amygdala reactivity during emotional processing, an additive or otherwise interactive effect of both gene variations may be expected. Based on previous studies cited above, we expected that carriers of the T allele of the TPH2 and carriers of either 1 or 2 copies of the 5-HTT short allele should exhibit increased activity to emotional stimuli. To acquire detailed information at which stage of emotional processing the influence of 5-HTT and TPH2 variations is present, we measured event-related potentials (ERPs) enabling a temporal resolution of milliseconds.

The effect of emotional arousal on the amplitudes of ERPs can be detected within 2 time windows. An early posterior negativity (EPN) potential for emotional compared with neutral visual stimuli was described as early as 240 ms after stimulus presentation (Schupp and others 2003). The term EPN might be somewhat misleading, as the emotional modulated component has a positive deflection. Emotional stimuli diminish the positive amplitudes of this component, and consequently the difference between emotional minus neutral condition leads to a negative wave. This negative wave leads to the label of the EPN (see also Fig. 2). Schupp and others argued that this EPN reflects facilitated sensory encoding of affective stimuli by naturally occurring selective attention. Additionally, a second process is captured by ERP around 300 ms after stimulus presentation over the parietal cortex, which is characterized by greater positive amplitudes to emotional compared with neutral stimuli (slow wave [SW]) (Guthbert and others 2000; Schupp and others 2000). This effect is considered to index postsensory (higher order) stages of stimulus evaluation.

In the present study, we investigated if and how the 5-HTT and TPH2 genotypes modulate neuronal activity during the processing of visual emotional stimuli during the 2 time windows described above. Based on previously reported functional evidence, we hypothesized that neural activity is greater in carriers of 5-HTTs variant and in carriers of the TPH2 T variant. In addition, recent evidence for a subdivision of the 5-HTTLPR allele by SNP rs25531 (lA/lG) (Wendland and others 2006) was also considered and evaluated. Finally, we expected an additive or otherwise interactive effect of both genotypes on neural activity.

**Materials and Methods**

Forty-seven healthy right-handed adults (mean age = 24.9 years; standard deviation [SD] = 3.5; 25 males) participated in the study after they gave written informed consent. Exclusion criteria were a history of diagnosed psychiatric disorder, current use of mood-altering medication, and history of severe head trauma, neurosurgery, or neurological condition. Participants were genotyped for functional 5-HTT and TPH2 variations according to previously published protocols (5-HTT linked polymorphic region [5-HTTLPR]; Lesch and others 1996, 5-HTTLPR lA/lG; Wendland and others 2006, TPH2 G-703T; Walitza and others 2005).

Electroencephalogram (EEG) was recorded from 21 electrodes according to the extended international 10–20 system (Fp1, Fp2, F3, F4, F7, F8, T3, T4, C3, C4, T5, T6, P3, P4, O1, O2, Fpz, Fz, Cz, Pz, and Oz).
Three additional electrodes were placed at the outer canthi of both eyes and below the right eye to register horizontal and vertical eye movements. The recording reference was placed at FCz; ground electrode was placed at A2Fz. Electrode impedances were kept below 5 kΩ. EEG was sampled continuously at a rate of 1000 Hz with a band-pass filter from 0.1–70 Hz.

During EEG measurement, the subjects passively viewed 200 pictures from the international affective picture system (IAPS) (Lang and others 1999). The pictures were presented for 1000 ms with a variable interstimulus interval between 1000–1500 ms in a randomized order. The sample of IAPS pictures used in this study consisted of the 100 lowest arousing (mean arousal = 2.87, mean valence = 5.47) and the 100 highest arousing (arousing = 6.87, valence = 4.06) pictures, based on norm ratings of arousal and valence (values ranging between 1 and 9; in the arousing dimension 1 = low arousing; in the valence dimension 1 = negative). Time epochs lasting from −200 before to 800 ms after stimulus presentation were extracted and analyzed for artifacts (588 µV) after average reference recalibration. All subjects had sufficient (at least 20 artifact free) numbers of trials, which were averaged to the ERPs. ERPs were filtered using a band-pass filter from 1–40 Hz. Baseline was corrected using the 200 ms before stimulus presentation. For the EPN, we calculated the mean amplitudes around the grand-mean peak at 60 ms for every subject and condition. For the SW, we calculated the mean amplitudes for the time period between 315 and 745 ms due to missing comparable peaks in both conditions. For statistical analysis, we dichotomized the 5-HTT genotype by presence (L/s and s/s, S group; 29 subjects) versus absence (L/L, L group; 18 subjects) of the s variant, based on previously reported functional evidence (Lesch and others 1996; Lesch and Gurknecht 2005).

We dichotomized the TPH2 genotype by presence (G/T and T/T, T group; 16 subjects) versus absence (G/G, G group; 31 subjects) of the T variant, based on previously reported functional evidence (Brown and others 1999). The pictures were presented for 1000 ms with a variable interstimulus interval between 1000–1500 ms in a randomized order. The subjects with a combined 5-HTT/TPH2 genotype were then divided into three genotypic subgroups: subjects with a combined 5-HTT/TPH2 genotype with the S/T genotype had the most negative EPN values (m = −1.27; SD = 1.06; t15 = 1.41, P = 0.1) (see Table 1).

Examination of the effects of the gene variations revealed that the combination of the 5-HTT and TPH2 genotypes significantly influenced the neural activity of emotional processing for the EPN (R² = 0.13; F1 = 6.65, P < 0.05). The additive effect of the combined 5-HTT/TPH2 genotype was in the predicted direction (b = −0.57; standard error = 0.22; t = −2.6, P = 0.013): subjects with the L/G genotype had the least negative EPN values (m = −0.82; SD = 1.13), subjects with the L/T or S/G genotype had intermediated EPN values (m = −1.52; SD = 1.02), and subjects with the S/T genotype had the most negative EPN values (m = −1.94; SD = 0.95) (see Fig. 1). The remaining variables (age, sex) were not included in the stepwise linear regression model due to missing additional significant effects (all P > 0.20).

For the SW, no interaction effect with genotype was found. None of the variables were included in the stepwise linear regression model due to missing significant effects. Comparing the genotype subgroups alone did not reveal any significant effects. A post hoc correlation analysis between the EPN and the SW (the difference values between high- and low-arousing pictures) showed no significant correlation between these 2 measures of emotional processing (r = −0.21, P = 0.14).

**Results**

When examining the electrophysiological response toward emotional visual stimuli, the hypothesized increased amplitudes for high-arousing compared with low-arousing pictures were found for the EPN as well as for the SW. For the EPN, high-arousing pictures lead to significantly lower amplitudes (m = 5.8; SD = 3.1) compared with the low-arousing pictures (m = 7.2; SD = 3.1; t46 = 9.0, P < 0.0001), reflecting the increased negative amplitudes of the EPN in the difference wave. For the SW, the high-arousing pictures (m = 2.1; SD = 1.3) showed significantly higher amplitudes compared with the low-arousing pictures (m = 0.9; SD = 1.0; t46 = 11.0, P < 0.0001).

Comparing the genotype subgroups revealed significantly more negative EPN for the S group (m = −1.69; SD = 0.95) compared with the L group (m = −1.02; SD = 1.20; t46 = −2.13, P < 0.05, see Fig. 2) and a trend toward more negative EPN for TPH2 T group (m = −1.74; SD = 1.10) compared with the G group (m = −1.27; SD = 1.06; t15 = 1.41, P < 0.1) (see Table 1).

Examination of the effects of the gene variations revealed that the combination of the 5-HTT and TPH2 genotypes significantly influenced the neural activity of emotional processing for the EPN (R² = 0.13; F1 = 6.65, P < 0.05). The additive effect of the combined 5-HTT/TPH2 genotype was in the predicted direction (b = −0.57; standard error = 0.22; t = −2.6, P = 0.013): subjects with the L/G genotype had the least negative EPN values (m = −0.82; SD = 1.13), subjects with the L/T or S/G genotype had intermediated EPN values (m = −1.52; SD = 1.02), and subjects with the S/T genotype had the most negative EPN values (m = −1.94; SD = 0.95) (see Fig. 1). The remaining variables (age, sex) were not included in the stepwise linear regression model due to missing additional significant effects (all P > 0.20).

For the SW, no interaction effect with genotype was found. None of the variables were included in the stepwise linear regression model due to missing significant effects. Comparing the genotype subgroups alone did not reveal any significant effects. A post hoc correlation analysis between the EPN and the SW (the difference values between high- and low-arousing pictures) showed no significant correlation between these 2 measures of emotional processing (r = −0.21, P = 0.14).

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Examination of the effects of the gene variations revealed that the combination of the 5-HTT and TPH2 genotypes significantly influenced the neural activity of emotional processing for the EPN (R² = 0.13; F1 = 6.65, P < 0.05). The additive effect of the combined 5-HTT/TPH2 genotype was in the predicted direction (b = −0.57; standard error = 0.22; t = −2.6, P = 0.013): subjects with the L/G genotype had the least negative EPN values (m = −0.82; SD = 1.13), subjects with the L/T or S/G genotype had intermediated EPN values (m = −1.52; SD = 1.02), and subjects with the S/T genotype had the most negative EPN values (m = −1.94; SD = 0.95) (see Fig. 1). The remaining variables (age, sex) were not included in the stepwise linear regression model due to missing additional significant effects (all P > 0.20).

For the SW, no interaction effect with genotype was found. None of the variables were included in the stepwise linear regression model due to missing significant effects. Comparing the genotype subgroups alone did not reveal any significant effects. A post hoc correlation analysis between the EPN and the SW (the difference values between high- and low-arousing pictures) showed no significant correlation between these 2 measures of emotional processing (r = −0.21, P = 0.14).
Recalculation of the data with respect to the effect of 5-HTT lG allele did not enhance but reduced the main effect of the biallelic 5-HTT genotypes on EPN and was therefore not further considered.

**Discussion**

This is the first demonstration of an additive gene effect on neural correlates of emotional processing. Specifically, we had predicted that both 5-HTT and TPH2 genotypes should modulate neuronal activity during the processing of visual emotional stimuli. Our results confirmed this prediction for the EPN around 240 ms. With respect to each gene’s individual contribution, we found that neural activity to emotionally arousing pictures was significantly greater for individuals of the 5-HTT S group than the L group and tendentially greater for individuals of the TPH2 T group than the G group. This is consistent with previous studies showing greater amygdala activity to emotional (relative to a neutral) stimuli for individuals of the 5-HTT S group (Hariri and others 2002, 2005) and for individuals of the TPH2 T group (Brown and others 2005; Canli, Congdon, and others 2005). Furthermore, the analysis of the 2 combined genotypes revealed an additive effect of both genes that confirmed our directional hypothesis. Individuals whose genotype assigned them to both the 5-HTT S group and the TPH2 T group showed the highest neural activity during the processing of high-arousing emotional pictures. In contrast, individuals whose genotype assigned them to both the 5-HTT L group and the TPH2 G group showed the lowest neural activity during this task. All other individuals showed intermediate neural activity. Our results clearly underscore the relevance of both the 5-HTT and TPH2 genotypes for individual differences in the neural responses to emotional stimuli and show for the first time an additive effect when both genotypes are considered in conjunction.

Recent evidence indicates that subdivision of 5-HTT into s and l alleles may require reconsideration. SNP rs25531 within 5-HTTLPR results in further differentiation of the high-expressing l allele into lA and lG, with lG apparently representing an equivalent to the low-expressing s allele (Nakamura and others 2000; Hu and others 2005). Inclusion of the presumed effect of 5-HTT lG allele in the analysis of our data reduced the main effect of the biallelic 5-HTT genotypes on EPN, suggesting that a potential impact of SNP rs25531 on brain activation requires further evaluation.

In contrast to the previously mentioned fMRI studies (Hariri and others 2002, 2005; Brown and others 2005; Canli, Congdon, and others 2005; Heinz and others 2005; Pezawas and others 2005), we cannot determine the neuroanatomical origin of the measured brain potentials. Therefore, we can only speculate which brain areas might be influenced by the investigated genotypes. Schupp and others (Schupp and others 2003) argue that the distribution of the EPN over the occipital cortex suggests the primary visual cortex as the origin of the EPN. This seems plausible, as fMRI studies have also reported increased activation of the occipital cortex in response to emotional compared with neutral stimuli (Sabatinelli and others 2004). Although source localizations of the EPN have not been published, the sources of the EPN can be derived from 2 published fMRI studies, using a similar paradigm as previous EEG studies that employed rapid presentation of emotional pictures (Junghofer and others 2005, 2006). In both studies, increased activation to high emotional stimuli was found in the extended visual cortex (in addition frontal, temporal, parietal, and subcortical structures). The authors (Sabatinelli and others 2004) suggested that the emotional modulation of the level of visual cortical activation happens via its dense interconnections with subcortical structures in emotional processing, such as the amygdala (Shi and Davis 2001). This interpretation was further supported by the fact that amygdala activity during emotional processing was modulated by the 5-HTT genotype (Hariri and others 2002, 2005) and the TPH2 genotype (Brown and others 2005; Canli, Congdon, and others 2005). Both the increased activation of the primary visual cortex found in fMRI studies, as well as the EPN, were interpreted as facilitated sensory encoding of affectively arousing stimuli. In lieu of its lack of spatial resolution, ERPs offer the advantage of a very high temporal resolution. Thus, our data show gene effect that is temporally limited to a period between 180 and 300 ms poststimulus, which is a resolution that is not possible using fMRI. In addition, we did not find any genotype effects for the SW between 315 and 745 ms. Therefore, the genotype effects seem to be specific for the EPN. Although this has not been investigated explicitly, we suggest that both the EPN and the SW reflect different aspects of emotional processing, which was underscored by the lack of a significant correlation between both variables. In general, ERPs seem to be well suited to measure effects of genetic variants on fast cognitive or emotional processes. This principle of imaging genomics with ERPs has already been published fMRI studies, using a similar paradigm as previous EEG studies that employed rapid presentation of emotional pictures (Junghofer and others 2005, 2006). In both studies, increased activation to high emotional stimuli was found in the extended visual cortex (in addition frontal, temporal, parietal, and subcortical structures). The authors (Sabatinelli and others 2004) suggested that the emotional modulation of the level of visual cortical activation happens via its dense interconnections with subcortical structures in emotional processing, such as the amygdala (Shi and Davis 2001). This interpretation was further supported by the fact that amygdala activity during emotional processing was modulated by the 5-HTT genotype (Hariri and others 2002, 2005) and the TPH2 genotype (Brown and others 2005; Canli, Congdon, and others 2005). Both the increased activation of the primary visual cortex found in fMRI studies, as well as the EPN, were interpreted as facilitated sensory encoding of affectively arousing stimuli. In lieu of its lack of spatial resolution, ERPs offer the advantage of a very high temporal resolution. Thus, our data show gene effect that is temporally limited to a period between 180 and 300 ms poststimulus, which is a resolution that is not possible using fMRI. In addition, we did not find any genotype effects for the SW between 315 and 745 ms. Therefore, the genotype effects seem to be specific for the EPN. Although this has not been investigated explicitly, we suggest that both the EPN and the SW reflect different aspects of emotional processing, which was underscored by the lack of a significant correlation between both variables. In general, ERPs seem to be well suited to measure effects of genetic variants on fast cognitive or emotional processes. This principle of imaging genomics with ERPs has already been proven for 5-HTT (Fallgatter and others 1999, 2004).

One recent study (Canli, Omura, and others 2005) challenged the conventional view that presence of the 5-HTT’s variant is associated with increased amygdala reactivity to emotional stimuli. These authors replicated earlier studies of greater amygdala activation to emotional, relative to neutral, stimuli in the 5-HTT S group. However, by using a different baseline condition (fixation rest), they then showed that this effect was...
due to decreased activation in the neutral condition, rather than increased activation in the emotion condition. Indeed, the 5-HTT S group was not characterized by greater responsiveness to an emotional stimulus (in the emotion-fixation contrast), but rather by greater activation during the rest condition (in the neutral-fixation contrast). Because this increased activation of the right amygdala during a passive condition compared with an active task is consistent with a meta-analysis of 9 positron emission tomography studies (Shulman and others 1997), Canli and others argued that individuals of the S group may be characterized by tonic amygdala activation during a passive rest condition, reflecting ongoing processes such as constant vigilance (Canli, Omura, and others 2005). The present study cannot directly comment on this possibility because the signal from the amygdala may not have been captured with our measures or may not have been isolated from signals from other regions of the brain. Furthermore, the present study compared 2 active conditions (low- versus high-arousing pictures) and did not include measures of EEG activity during a passive rest condition.

In conclusion, we found that both 5-HTT and the THP2 genotypes modulate brain response to emotional stimuli at 240 ms poststimulus. Furthermore, our results indicate that these effects are additive. It appears that both genotypes modulate the facilitated sensory encoding of affective stimuli, which might be relevant for anxiety-related psychopathology that has been linked, for example, with the short variants of the 5-HTT (Furmark and others 2004).

Notes
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