

Further Evidence for an Association of 5-HTTLPR with Intensity Dependence of Auditory-Evoked Potentials

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Intensity dependence of auditory-evoked potentials (IAEP) has been suggested as an indicator of central serotonergic neurotransmission. Two recent studies investigated a possible association of IAEP with a functional polymorphism in the transcriptional control region of the serotonin transporter gene (5-HTTLPR) that has a short (s) and a long (l) variant. Although both studies found an association between 5-HTTLPR and IAEP, Gallinat *et al* found *ll* individuals to exhibit lower IAEP, whereas Strobel *et al* observed stronger IAEP in *ll* individuals. These conflicting results require further evaluation and more attention needs to be paid to variables that are known to be confounded with the effects of IAEP and 5-HTTLPR. Using a paradigm comparable to Strobel *et al*, the present study analyzes the effect of 5-HTTLPR on IAEP in a healthy male student sample ($N = 91$; age = 23 years, $SD = 1.9$) that was homogenous for most significant confounding variables. A stronger IAEP was shown in *ll* individuals, irrespective of the method of IAEP parametrization. This also held at retest after 3 weeks in a subsample ($N = 18$). Given the successful replication of Strobel *et al*, several possible reasons for conflicting results with regard to Gallinat *et al* are discussed. It is argued that the most significant difference between Gallinat *et al* on the one hand, and Strobel *et al* and this study on the other, is that different intensity ranges are used which impact IAEP. Therefore, this study encourages further analysis of dose dependence of results.

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INTRODUCTION

Serotonergic neurotransmission is involved in several psychiatric disorders and is an important target of pharmacotherapeutic interventions. Therefore, valid and reliable indicators of central serotonergic function are of great importance.

Intensity dependence of auditory-evoked potentials (IAEP) has been suggested as a noninvasive indicator of central serotonergic neurotransmission (Hegerl and Juckel, 1993). A strong increase in amplitudes of the auditory N1/P2 component, that is, a strong N1/P2 IAEP, is thought to reflect low serotonergic activity and vice versa.

Animal studies have revealed that reducing or increasing serotonergic activity increases or decreases N1/P2 IAEP in the primary auditory cortex (Juckel *et al*, 1999, 1997).

Moreover, several studies on clinical samples have lent indirect support to the hypothesis that N1/P2 IAEP is related to serotonergic neurotransmission: Higher N1/P2 IAEP was observed in patients with a better treatment response to selective serotonin reuptake inhibitors (SSRI) or lithium (Gallinat *et al*, 2000; Hegerl *et al*, 2001; Juckel *et al*, 2004; Lee *et al*, 2005) and the severity of drug-induced serotonin syndrome has been shown to be related to lower N1/P2 IAEP (Hegerl *et al*, 1998). Furthermore, IAEP has emerged to be positively correlated with personality traits like impulsivity and sensation seeking, which are thought to be characterized by low serotonergic neurotransmission (Brocke *et al*, 1999; Hegerl *et al*, 1995; Zuckerman, 1994). In patients with supposed serotonergic dysfunction (migraine patients, bipolar affective disorder patients, and Ecstasy users), high IAEP has been observed (Brocke *et al*, 2000; Croft *et al*, 2001; Siniatchkin *et al*, 2000; Tuchtenhagen *et al*, 2000).

Serotonergic medications altered IAEP in some studies in humans (Proietti-Cecchini *et al*, 1997; Roon *et al*, 1999). To date, however, most attempts to directly test the hypothesis that serotonin modulates IAEP *in humans* by manipulating serotonergic neurotransmission with acute tryptophan depletion (ATD) have failed or their results have been

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contrary to expectations (Debener *et al*, 2002; Dierks *et al*, 1999; Kähkönen *et al*, 2002; Massey *et al*, 2004). These findings support the notion that it is difficult to predict the effects of ATD on IAEP. On the one hand, ATD causes reduced serotonin availability in the cortex (Nishizawa *et al*, 1997); on the other hand, this is immediately followed by adaptive receptor and transporter changes (eg Milak *et al*, 2005; Yatham *et al*, 2001). Considering these problems, naturally occurring (eg genetically driven) interindividual differences in serotonergic function may provide a promising context for indirect validation of IAEP.

The serotonin transporter plays a pivotal role in serotonergic function (Lesch and Mössner, 1998). In recent years, much research has been conducted on the serotonin transporter gene-linked polymorphic region (5-HTTLPR), a functional length variation in the transcriptional control region of the serotonin transporter gene that has a short (s) and a long (l) variant. The 5-HTTLPR s allele has proved to be associated with traits assumed to be modulated by serotonin, such as anxiety-related traits, in several studies (Schinka *et al*, 2004; Sen *et al*, 2004). It is also related to stress susceptibility (Caspi *et al*, 2003) and psychiatric disorders that involve serotonergic dysfunction; for example, affective disorders (Lasky-Su *et al*, 2005; Lotrich and Pollock, 2004) and alcohol dependence (Feinn *et al*, 2005; Nilsson *et al*, 2005). Furthermore, the s allele is associated with lower response to serotonergic agents (Moreno *et al*, 2002; Perna *et al*, in press; Smits *et al*, 2004). However, complex behavior is modulated by multiple genes interacting with one another and with diverse environmental influences across the lifespan. Therefore, the effect of a single polymorphism on complex behavior will usually be small (Reif and Lesch, 2003) and difficult to detect. A promising approach is thus to assess the effects of genes on more direct biological indices of serotonergic function (Hariri and Weinberger, 2003). To date, the 5-HTTLPR s allele has been successfully related to lower CSF-serotonin metabolites (Williams *et al*, 2003), to lower prolactin response in serotonergic drug challenge studies (Reist *et al*, 2001; Smith *et al*, 2004; Whale *et al*, 2000), to lower brain-derived neurotrophic factor (Lang *et al*, 2005), to stronger amygdala response in brain-imaging studies (Furmark *et al*, 2004; Hariri *et al*, 2002, 2005; Heinz *et al*, 2005; Pezawas *et al*, 2005), to lower hippocampal N-acetyl-aspartate (Gallinat *et al*, 2005), to better acquisition of fear conditioning as measured by skin conductance response (Garpenstrand *et al*, 2001), and to electrophysiological indices of motor control and error processing (Fallgatter *et al*, 1999, 2004).

So far, only two studies have related 5-HTTLPR to IAEP. Although both found an association between 5-HTTLPR and IAEP, supporting the hypothesis that serotonergic function is involved in the modulation of IAEP, Gallinat *et al* (2003) found l/l individuals to exhibit lower IAEP, whereas Strobel *et al* (2003) observed stronger IAEP in l/l individuals.

At present, it is difficult to make a clear prediction of which genotype will be associated with higher IAEP. *In vitro* studies of non-neural cells showed higher transcriptional activity and higher serotonin reuptake of the l allele (Greenberg *et al*, 1999; Lesch *et al*, 1996). Postmortem-binding studies and three recent SPECT studies produced

conflicting results, however (Willeit *et al*, 2001; for other studies see an overview in van Dyck *et al*, 2004). Assuming that s allele carriers have a less efficient serotonin transporter, more serotonin should be left in the synaptic cleft, as has been shown in a mouse model (Holmes *et al*, 2003). This high serotonin availability in s allele carriers could result in low IAEP (Manjarrez *et al*, 2001) in line with the findings of Strobel *et al* (2003). Yet there are also reasons for predicting the opposite: Given the impaired ability of the s allele for rapid reuptake of serotonin from the synaptic cleft, it has been suggested that a 5-HT_{1A} receptor-mediated negative feedback mechanism ultimately results in a decreased serotonergic firing rate (Lesch and Mössner, 1998). This decrease in firing rate in s allele carriers could lead to high IAEP. On the other hand, changes in neurotransmission are normally accompanied by up- or downregulation of receptors, such as the postsynaptic 5-HT_{1A} and 5-HT₂ receptors, both of which may be involved in IAEP (Juckel *et al*, 1997, 2003). In conclusion, then, it is difficult to predict the net effect of serotonin levels in the synaptic cleft, the raphe firing rate, and adaptive processes in pre- and postsynaptic receptors on IAEP.

Moreover, the influence of 5-HTTLPR on IAEP could simply be the result of genotype-driven differential development, for example, of the auditory system, and might not necessarily reflect acute monoamine status. There is evidence that serotonin transporter alterations act in early developmental phases (Ansoorge *et al*, 2004; Maciag *et al*, in press; Pezawas *et al*, 2005).

Thus, as long as it remains difficult to make directional bottom-up predictions from gene (5-HTTLPR) to endophenotype (IAEP), replications are needed to clarify which genotype is in fact related to high IAEP. Such studies with nondirectional hypotheses should primarily consider variables that are known to be confounded with the effects of IAEP and 5-HTTLPR. To this end, the present study analyzes the effect of 5-HTTLPR on IAEP in a sample that was homogenous for most significant confounding variables. A further objective of this study is to test the stability of results against several different IAEP parametrizations used in literature. This is done to rule out the possibility of different methods of IAEP parametrization producing conflicting results.

METHODS

Subjects

A total of 91 healthy right-handed Caucasian nonsmoking male students between 19 and 27 years of age (mean = 23.3; SD = 1.9) met the following inclusion criteria and participated in the study. The inclusion criteria were applied to control or keep constant major confounding factors that are related to either 5-HTTLPR genotype or serotonergic neurotransmission, or that have a strong impact on auditory-evoked potentials (AEPs). As gender influences serotonergic neurotransmission, as with gender-specific effects of serotonin transporter alterations, for example (Amin *et al*, 2005; Barr *et al*, 2004b; Bouali *et al*, 2003; Currie *et al*, 2004; Dominguez *et al*, 2003; Halbreich *et al*, 1995; Nishizawa *et al*, 1997; Rubinow *et al*, 1998; Williams *et al*, 2003), and because gender also impacts IAEP

(Bruneau *et al*, 1986; Camposano and Lolas, 1992; Schwerdtfeger and Baltissen, 1999; Silverman *et al*, 1973), only male subjects were included in the sample. Sampling a female population would have increased the sample size unreasonably, as it would have then been necessary to control the effects of the menstrual cycle and contraceptives on AEPs (Walpurger *et al*, 2004). As age is also correlated with serotonergic function (Tauscher *et al*, 2001) and with IAEP (Hegerl *et al*, 1994; Siniatchkin *et al*, 2000), only a narrow age range was sampled. In addition, because smoking alters serotonergic neurotransmission (Seth *et al*, 2002) and because smoking behavior is related to 5-HTTLPR (Gerra *et al*, 2005; Kremer *et al*, 2005), only nonsmokers were included. According to written self-reports, 75 of the subjects had never smoked and 16 had stopped smoking, 10 of them more than 1 year ago and the remaining six at least 2 months ago. Furthermore, subjects had to be free of any psychiatric or neurological disorder thought to be influenced by serotonergic neurotransmission (especially migraine and affective or anxiety disorders). Centrally acting medications or casual use of drugs (Ecstasy in particular) were further exclusion criteria. Subjects were asked about these exclusion criteria twice: At an initial telephone interview and again via a questionnaire in the laboratory. Normal hearing was verified by means of audiometry.

Participants were paid and gave written informed consent. The study was conducted in accordance with the Ethics Commission of the University of Dresden and the Declaration of Helsinki.

EEG Recording

EEG recording was performed with eyes open in an acoustically and electrically shielded room (IAC, Niederrüchten, Germany). No attention or vigilance instruction was given. The subjects were told only to avoid body movements and to lightly fixate a point in front of them in order to reduce alpha artifacts and eye movements. EEG was recorded with Ag/AgCl electrodes at Cz, C3, and C4, with the nose tip as the reference point and AFz as ground. Additionally, a vertical and a horizontal electro-oculogram was recorded to monitor ocular artifacts. Impedances were kept below 8 k Ω with less than 0.5 k Ω difference between single electrodes. EEGs were continuously recorded with a Synamps 4.1 amplifier (NeuroScan Inc., El Paso, TX, USA) at a digitization rate of 1000 Hz. Calibrated sinus tones (1000 Hz, 30 ms duration with 10 ms rise and 10 ms fall time) of six intensities (66, 72, 78, 84, 90, 96 dB SPL) were presented binaurally via Eartone 3A Audiometric Insert Earphones (Aearo Company, Indianapolis, IN, USA) in pseudo-randomized order. Presentation 0.80 (Neurobehavioral Systems Inc., Albany, CA, USA) was used to present the sounds. Simultaneity of trigger and sound was verified using SCLA (Neurobehavioral Systems). Each intensity level was repeated 108 times and the interstimulus interval varied pseudo-randomly between 1600 and 2100 ms.

EEG Analysis

AEP analysis was performed with Brain Vision Analyzer 1.04 (Brain Products GmbH, Munich, Germany). EEGs were

epoched off-line into sweeps of 600 ms, starting 200 ms prior to stimulus onset. In line with previous studies (Brocke *et al*, 2000), a digital offline bandpass filter from 0.1 to 30 Hz was used. All sweeps with a voltage exceeding $\pm 50 \mu\text{V}$ in any of the channels were excluded from averaging. This resulted in a mean of 77 accepted sweeps per intensity level. The number of accepted sweeps did not differ across the six intensity levels.

Baseline to peak amplitudes of AEPs were automatically derived for N1 (55–160 ms) and P2 (110–260 ms), and N1/P2 peak-to-peak amplitudes were then calculated. To test the assumption of a linear increase in amplitudes over intensity levels, a repeated measurement analysis of variance was performed with intensity level (six intensities) and position (Cz, C3, C4) as repeated measures and age as covariate. Since a significant linear trend was observed ($p = 0.001$), the individual intensity dependence was calculated as the slope of the linear regression of amplitudes against stimulus intensities (known as the amplitude-stimulus function slope or ASF slope).

In order to further evaluate the results, alternative parametrizations of IAEP were calculated. Following Strobel *et al* (2003), a nonlinear calculation of IAEP was performed based on the median of the 15 slopes between all possible pairs of the six amplitude values. This slope (called median slope) does not require linearity of IAEP. Calculating ASF and median slopes based on peaks of AEP components still entails the problem of multiple peaks within a single AEP component, however. ASF and median slopes were therefore also calculated using the whole area under the EEG curve within a window of the AEP component drawn from grand averages (N1: 65–145 ms; P2: 130–230 ms). Finally, because calculating a slope only partly reflects individual variability in amplitudes at the six intensity levels, all six amplitudes were entered directly in a repeated measures analysis of variance with intensity level (six intensities) and position (Cz, C3, C4) as repeated measures and age as covariate.

Genotyping

DNA was extracted from venous blood, and with regard to the 5-HTTLPR polymorphism, PCR-based genotyping was performed according to a modified protocol by Collier *et al* (1996). In order to confirm that the correct region of the serotonin transporter gene was amplified, PCR products representing all genotypes were sequenced using BigDye[®] Terminator chemistry (Applied Biosystems) and analyzed by an automated ABI PRISM[™] (Perkin Elmer, Foster City, CA, USA). The DNA fragments were analyzed using the Sequencer[™] 3.1.1 software.

RESULTS

At 40.7% for the s allele and 59.3% for the l allele, the allele frequencies of 5-HTTLPR were comparable with other studies on European subjects. The genotype distribution of study subjects (l/l = 29 (32%), s/l = 50 (55%), s/s = 12 (13%)) is in Hardy-Weinberg equilibrium. The IAEP function of each genotype is plotted in Figure 1.

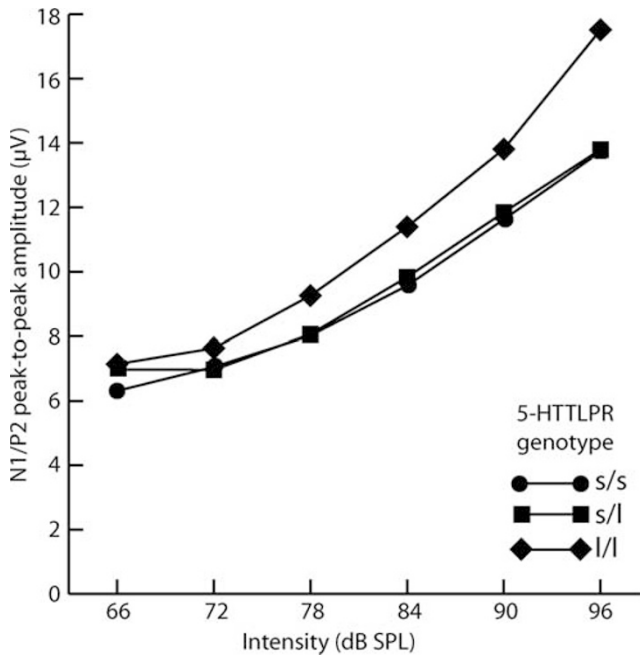


Figure 1 IAEP function at central sites (Cz, C3, C4) for each 5-HTTLPR genotype group (s/s, s/l, l/l).

The association of 5-HTTLPR genotype with IAEP slope was analyzed in a repeated measures analysis of variance with electrode position (C3, C4, Cz) as within-subjects factor, 5-HTTLPR genotype (l/l vs s/l vs s/s) as between-subjects factor, and age as covariate. There was a significant main effect of 5-HTTLPR on ASF slope ($F_{2,87} = 4.796$; $p = 0.011$), accounting for 10% of the variance ($\eta^2 = 0.099$). Bonferroni-corrected *post hoc* tests revealed that the 5-HTTLPR l/l genotype was associated with higher ASF slopes than the s/l genotype ($p = 0.013$; Figure 2). The main effects for age and position did not reach significance level, but a significant interaction of 5-HTTLPR and position emerged, with Cz having stronger IAEP ($F_{3,4,149.1} = 3.346$; $p = 0.016$).

Further analyses were performed to confirm these results in greater detail: Median slopes correlated highly with ASF slopes (Cz: $r = 0.94$; C3 $r = 0.96$; C4: $r = 0.93$; all $p < 0.0001$). Entering median instead of ASF slopes in repeated measures analysis of variance corroborated the results (main effect of 5-HTTLPR: $F_{2,87} = 5.456$; $p = 0.006$).

In order to avoid the problem of multiple peaks in AEP components, ASF and median slopes were also calculated using the whole area under the EEG curve instead of a single peak. Again, this revealed a significant main effect of 5-HTTLPR on IAEP (area of ASF slope: $F_{2,87} = 5.326$; $p = 0.007$; area of median slope: $F_{2,87} = 6.410$; $p = 0.003$).

Apart from calculating ASF and median slopes, individual amplitude values were directly entered in a repeated measures analysis of variance with intensity level (six intensities) and position (Cz, C3, C4) as repeated measures and age as covariate. These analyses also revealed significant interactions of 5-HTTLPR with intensity level (peaks: $F_{4.5,196.3} = 2.932$; $p = 0.017$; areas: $F_{4.5,193.7} = 3.584$; $p = 0.006$).

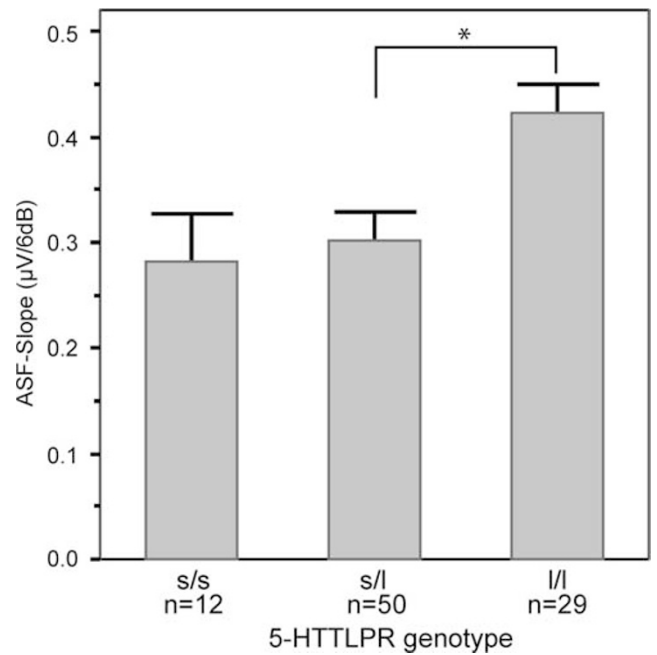


Figure 2 Mean values and SE of the ASF slope at central sites (Cz, C3, C4) for the three 5-HTTLPR genotype groups (s/s, s/l, l/l). The l/l group shows a significantly (*) higher IAEP than the s/l group ($p = 0.013$; l/l vs s/s: $p = 0.099$; s/s vs s/l: $p = 1.000$).

Additionally, all analyses were repeated with 5-HTTLPR grouped in terms of functional dominance (s/s and s/l vs l/l; Lesch *et al*, 1996). This did not change the overall pattern of results, but improved the significance levels; for example, 5-HTTLPR main effect with functional dominance: ASF slope: $F_{1,88} = 9.663$; $p = 0.003$; median slope: $F_{1,88} = 10.952$; $p = 0.001$.

It was possible to retest 18 subjects using the same paradigm 3 weeks later. Although the sample size at t2 is quite small, it is noteworthy that results were replicated. The main effect for 5-HTTLPR became significant in analyses using ASF slopes ($F_{2,14} = 5.379$; $p = 0.018$), median slopes ($F_{2,14} = 3.852$; $p = 0.046$), and area-based slopes (area of ASF slope: $F_{2,14} = 4.639$; $p = 0.028$; area of median slope: $F_{2,14} = 6.187$; $p = 0.012$). Using repeated measurement over the six tone intensities instead of slopes, only dominant genotype classification reached significance level at t2 (peaks: $F_{5,75} = 3.046$; $p = 0.015$; areas: $F_{2.5,38.2} = 4.631$; $p = 0.010$).

DISCUSSION

The present study confirms the association between 5-HTTLPR and N1/P2 IAEP, with l/l subjects being found to have stronger IAEP in line with the results of Strobel *et al* (2003). The findings remained significant irrespective of the method of IAEP parametrization (ASF and median slopes based on peaks or on the area under the curve; repeated measurement with six intensity levels rather than slope calculations). This also held at retest after 3 weeks in a small subsample. The effect size of about 10% explained variance (11% for median slope) is similar to the effect size obtained by Strobel *et al* (15%) and higher than that found by Gallinat *et al* (4%).

The results are consistent with the dominant short allele effect that has been shown in other studies (eg Gallinat *et al*, 2003; Lesch *et al*, 1996). The finding that l/l genotype is associated with stronger IAEP is also consistent with other data. Some studies found an association of l/l genotype with increased serotonin transporter level (van Dyck *et al*, 2004), and Pogarell *et al* (2004) found a positive correlation between IAEP and serotonin transporter density in obsessive compulsive disorder. Young age and female gender are also related to higher serotonin transporter levels (Hesse *et al*, 2003; McQueen *et al*, 1997; Staley *et al*, 2001) as well as to higher IAEP (Bruneau *et al*, 1986; Hegerl *et al*, 1994; Pogarell *et al*, 2004). High IAEP and l/l genotype are both predictors of better SSRI treatment response. Since these results are correlational in nature, however, caution is warranted in making causal interpretations.

The conflicting results reported by Gallinat and associates, on the one hand, and Strobel *et al* and the present study, on the other, may reflect marked methodological differences, for example, in terms of the intensity ranges used. Very high sound intensities are known to change the IAEP. The highest intensity used in Strobel *et al* (2003) and in the present study was 96 dB SPL with a tone duration of 30 ms. Gallinat *et al* (2003) used a longer tone (40 ms) with much higher intensities (up to 113 dB SPL). Thus, the highest intensity in the study by Gallinat and associates is physically about seven times higher than the maximum intensity in the Strobel *et al* study or in the present study. Intensity levels of about 100 dB SPL have been shown to lead to paradoxical reductions in evoked potentials and to impact on the linear regression slopes of IAEP (Brocke *et al*, 1999; Orlebeke *et al*, 1989; Prescott *et al*, 1984). This phenomenon has been interpreted as a protection mechanism against overstimulation. Therefore, one can assume that subjects with a steep IAEP within moderate intensity ranges could reach the point of paradoxical reductions in evoked potentials at high intensities earlier, while subjects with a low IAEP within moderate intensities could still have a linear increase in potential within high intensities. Consequently, it cannot be ruled out that differences between subjects are totally reversed: high IAEP subjects at moderate intensities could become low IAEP subjects at high intensities and vice versa. Davis *et al* (1983) have theoretically discussed this possibility, namely that high IAEP subjects could become low ones depending on intensity used. Similar conclusions were also drawn by Prescott *et al* (1984) from an experiment with an intensity range from 70 to 100 dB SPL: 'In fact if 90 dB had been the maximum intensity, the classification of augmenters (ie high IAEP) and reducers (ie low IAEP) would have been reversed!' (p 38). As Prescott *et al* (1984) did not calculate slopes but did dichotomous classifications ('augmenter' vs 'reducer'), their results cannot be directly compared with this study, but they underline the possible importance of intensity range.

Differences in the populations tested might also explain the conflicting results. In Strobel *et al*, a young population of university students and staff, including more women than men, was examined (mean age = 21 years; SD = 3.0; 35 women, 25 men). In the study by Gallinat and associates, there were slightly more men than women (96:89) and subjects were much older (mean age = 39; SD = 13). IAEP

values were only residualized for age and gender influences in Strobel *et al*. Since age and gender correlate with IAEP, serotonergic function, and serotonin transporter function (see Methods) and because gender can even invert 5-HTTLPR effects (Sjöberg *et al*, 2005; Williams *et al*, 2003), the results of these two studies cannot easily be compared. Regarding gender differences, the successful replication of Strobel *et al* could mean either that statistical control of gender effects carried out in Strobel *et al* is sufficient, or that there are no significant gender effects on the results.

Further known moderators of the effects of allelic variation of serotonin transporter function are environmental influences and socioeconomic status. The student samples in Strobel *et al* and the present study might differ in terms of these moderators from a sample of, for instance, paid nonacademic participants recruited via newspaper advertisements (as was the case in Gallinat and associates). Mostly nonacademic paid subjects recruited via newspaper advertisements might not only differ in test anxiety (due to unfamiliar university and laboratory surroundings) but might also differ in socioeconomic status. Belonging to a lower socioeconomic group, they will probably also vary in health, nutrition, early adversity and early environmental enrichment. These factors could contribute to systematic differences in serotonergic neurotransmission, 5-HTTLPR effects and EEG parameters. Early adversity affects the influence of 5-HTTLPR on serotonergic function, psychological vulnerability, and stress reactivity (Barr *et al*, 2004 a, b; Bennett *et al*, 2002; Caspi *et al*, 2003; Kendler *et al*, 2005; Suomi, 2003). Furthermore, in a study by Kaufman *et al* (2004), the interaction between maltreatment and 5-HTTLPR s/s genotype had little impact on depression scores if social support was available. These interactions with environmental variables may be one reason for the socio-economic influences observed on 5-HTTLPR effects (Manuck *et al*, 2004, 2005; Williams *et al*, 2003).

Taking these modulating factors into account may well explain some of the contradictory findings from 5-HTTLPR research. The aim of this study was two-fold: First, we wanted to show if it is possible to replicate *any* effect of 5-HTTLPR on IAEP when all the confounding variables which could produce artificial findings are controlled. Secondly, we wondered whether these results were stable over different parametrizations. Both questions have been answered successfully. The noteworthy effect sizes found in the present study, the stability of results over 3 weeks and the replication of the Strobel *et al* findings irrespective of the method of IAEP parametrization are encouraging. Now further studies should systematically analyze the effects of gender, age, socioeconomic status, and most of all, intensity range (dose) on results. Such a research program will show if IAEP results are dose dependent and if results are generalizable to other populations. The generalizability of the current study is limited due to the sample homogeneity.

Since a broad range of behavioral correlates of both IAEP and 5-HTTLPR have been identified in recent years, with implications for both basic research and pharmacological interventions, further investigation of the inter-relations between these two fields of research is likely to improve our understanding of IAEP and 5-HTTLPR.

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