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The 5-HTTLPR Polymorphism is Associated with Altered Hemodynamic Responses During Appetitive Conditioning

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Abstract: *Background:* Current models suggest that a variation in the promoter region of the serotonin transporter gene (5-HTTLPR) is associated with altered amygdala reactivity not only towards negative but also towards positive stimuli, which has been neglected in the past. This association may possibly convey an elevated vulnerability for psychopathology like abuse, craving, and relapses. Since appetitive conditioning is a crucial mechanism in the pathogenesis of these psychiatric disorders, the identification of specific factors contributing to interindividual variation is important. *Methods:* In the present study ($N = 86$), an appetitive conditioning paradigm was conducted, in which a neutral stimulus (CS+) was associated with appetitive stimuli, while a second stimulus (CS−) predicted their absence. Subjects were genotyped according to the 5-HTTLPR genotype. *Results:* As the main result, we report a significant association between the 5-HTTLPR genotype and hemodynamic responses. Individuals with the s-allele displayed elevated conditioned bilateral amygdala activity in contrast to l/l-allele carriers. Further, increased hemodynamic responses in s-allele carriers were also found in the extended emotional network including the orbitofrontal cortex, the thalamus, and the ventral striatum. *Conclusion:* The present findings indicate an association of the 5-HTTLPR and altered conditioned responses in appetitive conditioning. Further, the findings contribute to the ongoing debate on 5-HTTLPR dependent hemodynamic response patterns by emphasizing that s-allele carriers are not exclusively biased towards fearful, but also towards positive stimuli. In conclusion, our results imply that s-allele carriers might be better described as hyper-reactive towards salient stimuli, which may convey vulnerability for the development of psychiatric disorders. *Hum Brain Mapp* 34:2549–2560, 2013.

Key words: 5-HTTLPR; amygdala; classical conditioning; imaging genetics; fMRI; positive emotion

INTRODUCTION

Recently, research on the association of candidate genetic variations and emotional processing has gained increased interest due to the ongoing debate regarding their role as vulnerability factors for the development of psychiatric disorders [Caspi et al., 2010]. One of the best

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investigated genetic variations in context of psychiatric disorders is the functional genetic variation within the promoter region of the serotonin transporter gene (5-HTTLPR). The 43bp insertion/deletion polymorphism alters the function of the serotonin transporter (5-HTT) gene (SLC6A4), more specifically its transcription rate. This polymorphism comprises a low-expressing short (s-allele), which is associated with reduced 5-HTT protein availability and reduced functioning, as well as a high-expressing long (l-allele) variant [Lesch et al., 1996; Stoltenberg et al., 2002].

Findings have repeatedly shown a relationship between the 5-HTTLPR genotype and altered emotional processing. For instance, recent meta-analyses reported an association between the s-allele and the risk for major depression probably mediated by stressful life events [Karg et al., 2011] but see [Risch et al., 2009]. In addition, the s-allele has been associated with exaggerated stress reactivity [Alexander et al., 2009; Way and Taylor, 2010]. One explanation for these effects is the idea that s-allele carriers are characterized by a hyper-reactive subcortical and cortical network towards fearful stimuli [Canli and Lesch, 2007; Caspi et al., 2010; Munafò et al., 2008]. In particular, amygdala responses to fearful stimuli were found to be exaggerated in s-allele as compared to l-allele carriers in healthy humans, psychiatric patients, and animal studies with different fear stimuli and neurophysiological methods [Brown and Hariri, 2006; Furmark et al., 2004; Hariri et al., 2005; Morey et al., 2011]. Altogether, the association between the 5-HTTLPR and exaggerated fear processing could be assumed to be a potential mechanism for the development of psychiatric disorders.

Recently, studies have begun to extend the view of elevated stress sensitivity associated with the 5-HTTLPR genotype by highlighting that s-allele carriers are not only characterized by an increased reactivity to negative but also to positive emotional stimuli [Beevers et al., 2011; Belsky et al., 2009; Pérez-Edgar et al., 2010; Uher, 2008; for review see Homberg and Lesch, 2011]. With regard to hemodynamic responses, only few neuroimaging studies have investigated the association between the 5-HTTLPR genotype and positive stimuli. Overall, these studies showed increased hemodynamic responses not only towards negative but also towards positive stimuli in s-allele as compared to l-allele carriers [Beevers et al., 2010; Lemogne et al., 2011]. Notably, although such a positivity bias may have advantages, it may also be a risk factor for substance abuse and other addiction-related disorders [Homberg and Lesch, 2011].

Appetitive conditioning is considered to be a crucial mechanism in the etiology of many psychiatric disorders like substance abuse, and also for craving and relapses [Martin-Soelch et al., 2007]. In appetitive conditioning paradigms, a neutral stimulus (CS+) is paired with salient positive stimuli (UCS), while another stimulus (CS−) predicts the absence of these stimuli. After a few trials, the CS+ elicits conditioned responses (CRs) like increased skin conductance responses (SCRs), changes in preference ratings, and changes in brain activity [Kirsch et al., 2003; Klucken et al., 2009b; for review see: Martin-Soelch et al., 2007]. Whereas the hemodynamic correlates of conditioned fear responses are already understood in considerable detail [Büchel and Dolan, 2000; Hamm and Weike, 2005; Mechias et al., 2010], relatively few studies have investigated classical CRs in human appetitive conditioning [Martin-Soelch et al., 2007]. Converging evidence indicates a network of several cortical and subcortical structures: the amygdala, the ventral striatum, the orbitofrontal cortex (OFC), the anterior cingulate cortex (ACC), the insula, and the thalamus. One of the most important structures for classical conditioning is the amygdala, which is crucially involved in the formation of the CS–UCS associations [Kirsch et al., 2003; Martin-Soelch et al., 2007]. Besides the amygdala, the ventral striatum is considered an important structure for appetitive conditioning due to its central role in reward anticipation and, from a psychiatric point of view, in craving and addiction learning processes [Day and Carelli, 2007; Kirsch et al., 2003; Peciña, 2008]. OFC, insula, and ACC activations might reflect conscious evaluation processes of the current CS value and are also important for the awareness of bodily cues [Craig, 2009; Etkin et al., 2011; Kalisch, 2009; Mechias et al., 2010].

Twin studies suggest that genetic influences account for up to 30% of the variance in CRs [Hettema et al., 2003]. So far, only few studies have reported a significant association between 5-HTTLPR and classical (fear) conditioning [Crişan et al., 2009; Garpenstrand et al., 2001; Klucken et al. (in press); Lonsdorf et al., 2009]. Consistently, all studies observed higher CRs in s-allele carriers. However, a potential relationship between the 5-HTTLPR genotype and appetitive conditioning has been neglected so far and might contribute to a better understanding of vulnerability factors for certain disorders.

Based on the aforementioned literature, the present study aimed to investigate the following topics: First, the study was designed to investigate the hemodynamic responses of appetitive conditioning in a large human sample. Second, the study aimed to explore the hemodynamic responses of appetitive conditioning associated with the 5-HTTLPR genotype. In order to validate the current model of the 5-HTTLPR genotype dependent bias towards positive stimuli on the level of hemodynamic response [Homberg and Lesch, 2011], we expected increased hemodynamic responses within the s-allele carriers in contrast to individuals with the l/l genotype.

MATERIAL AND METHODS

Participants

For the present study, 100 subjects (mean age: 25.3; SD: 4.7, 49 males) were recruited. To avoid potential confounds due to stratification strategy, we included only

Caucasian participants, with European background, who were native German speakers. Current or past mental, sexual or chronic problems and consumption of psychotropic drugs were defined as exclusion criteria. All 100 participants were right-handed, had normal or corrected-to-normal vision, and received 30 Euro for their participation. Participants signed an informed consent. The study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of the German Psychological Society. Five subjects had to be excluded due to technical problems leaving 95 participants in the final sample.

Genotyping

DNA was extracted from buccal cells, using a standard commercial extraction kit (High Pure PCR Template Preparation Kit; Roche, Mannheim, Germany) in a MagNA Pure1 LC System (Roche). Subjects were genotyped for the 5-HTTLPR (rs25531) by means of polymerase chain reaction (PCR) and gel electrophoresis. A detailed protocol is provided elsewhere [Alexander et al., 2009]. In the current sample, genotype frequencies were as followed: 24 s/s-allele carriers, 39 s/l-allele carriers, and 32 l/l-allele carriers. Age, sex, and educational status did not differ between the three groups (all $P > .397$). There was no significant deviation from Hardy–Weinberg Equilibrium ($x^2_{(1)} = 0.16$; $p > 0.05$). In addition, we analyzed the fMRI data using a tri-allelic classification, since several studies used this strategy [Munafò et al., 2008]. Overall, the tri-allelic results only changed slightly (all significant results remain stable; additionally bilateral trends between the s-allele and the l/l-allele were found in the ventral striatum; see Table II). As a supplement, we exploratively investigated group differences between all three genotype groups (e.g. s/s vs. s/l; s/s vs. l/l), adding more findings to the current heterozygosis debate [Uher and McGuffin, 2008].

Conditioning Procedure

A differential conditioning procedure with 42 trials was used (21 per CS). Two colored squares [one blue, one yellow; cf. Phelps et al., 2004] served as CS and were counterbalanced as CS+ and CS− across subjects. The CS+ was followed by a set of 21 erotic pictures (100% reinforcement). Due to the clear association of serotonin to sexual processing and sexual motivation [Abler et al., 2011; Ahrold and Meston, 2009; Hull, 2011; Pfaus, 2009], we used erotic pictures as stimuli [Both et al., 2008a,b; Hoffmann et al., 2004; Klucken et al., 2009b]. All pictures depicted scenes with couples (always one male and one female) practicing vaginal intercourse in different positions. These pictures were successfully rated as highly positive and sexually arousing for males and females in a pilot study ($n = 200$). All pictures were presented in color, and had identical (800 × 600) pixel resolution. The stimuli were projected onto a screen at the end of the scanner (visual field = 18°) using an LCD projector. Pictures were viewed through a mirror mounted on the head coil. The CS stimulus duration was 8 s. The erotic pictures appeared immediately after the CS+ (100% reinforcement) without delay (trace conditioning) for 2.5 s followed by the intertrial-interval (ITI) that ranged from 12 to 14.5 s. For each subject, a pseudo randomized stimulus order was used with the following two restrictions: (1) no more than two presentations of the same CS in a row and (2) equally distributed CS presentations within each half of the acquisition. The first two trials (one CS+, one CS− trial) were excluded from the analyses because learning could not yet have occurred, resulting in a relatively short acquisition phase of 20 trials for each CS [Phelps et al., 2004]. An MRI-compatible video camera was used to control whether subjects watched the stimuli.

Skin Conductance Measuring

SCRs were sampled simultaneously with MR scans using Ag/AgCl electrodes filled with isotonic (0.05 M NaCl) electrolyte medium, placed hypothenar at the nondominant (left) hand. SCRs were defined in three analysis windows: the maximum response within the time window 1–4 s after the CS (CS+ or CS−) onset was counted as the first interval response (FIR), the maximum responses within the time window 4–8 s as the second interval response (SIR)—reflecting the CRs, and the time window within 8.5–12.5 s as the unconditioned responses. Statistical analyses were performed via analysis of variance (ANOVA) in a 2 (stimulus: CS+ vs. CS−) × 2 (group: s-group vs. l/l-group) design followed by post-hoc tests in SPSS 19 (IBM Corporation, Armonk, USA).

Subjective Ratings

Prior to the experiment and immediately after the conditioning procedure, participants rated valence and arousal of the CS+ and the CS− on a nine-point and their UCS expectancy on a ten-point Likert scale. To avoid potential UCS pre-exposure effects that diminish CRs [Bonardi et al., 2010; Franklin and Hall, 2011; Rodriguez and Hall, 2008], erotic pictures were rated after the conditioning procedure only. For the CS ratings, statistical analyses were performed by ANOVA in a 2 (stimulus: CS+ vs. CS−) × 2 (time: pre-acquisition vs. post-acquisition) × 2 (group: s-allele vs. l/l-allele carriers) design followed by post-hoc tests in SPSS 19 for each rating. Appropriate Bonferroni corrected post-hoc *t*-tests were conducted to further analyze significant effects. Regarding the erotic pictures, one sample and two sample *t*-tests were performed to analyze valence and arousal effects and genotype differences.

Magnetic Resonance Imaging

Functional and anatomical images were acquired with a 1.5 Tesla whole-body tomograph (Siemens Symphony with a quantum gradient system) with a standard head coil. Structural image acquisition consisted of 160 T1-weighted sagittal images (MPRage, 1 mm slice thickness). For functional images, a total of 420 images were registered using a T2*-weighted gradient echo-planar imaging (EPI) sequence with 25 slices covering the whole brain (slice thickness = 5 mm; 1 mm gap; descending slice procedure; time of repetition (TR) = 2.5 s; echo time (TE) = 55 ms; flip angle = 90°; field of view = 192 × 192 mm; matrix size = 64 × 64). The first two volumes were discarded due to an incomplete state of magnetization. The orientation of the axial slices was parallel to the OFC tissue–bone transition in order to minimize susceptibility artefacts in prefrontal areas. Data were analyzed using Statistical Parametric Mapping (SPM8, Wellcome Department of Cognitive Neurology, London UK; 2008) implemented in MATLAB 7.5 (Mathworks, Sherbourn, MA). Prior to all analyses, data were preprocessed including realignment and unwarping (b-spline interpolation), slice-time correction, coregistration of functional data to each participant's anatomical image, and normalization to the standard space of the Montreal Neurological Institute (MNI) brain. Spatial smoothing was executed with an isotropic three-dimensional Gaussian filter with a full width at half maximum of 9 mm to allow for corrected statistical inference.

Functional data were analyzed using the mixed effect GLM approach. In the first level, the experimental conditions were CS+, CS−, the erotic pictures, and the control condition (defined as the time window after CS presentation corresponding to the time window of the erotic pictures presentation after the CS+; [e.g. Klucken et al., 2012; Merz et al., in press; Schweckendiek et al., 2011], modelled as events. Regressors were convolved with the hemodynamic response function. The six movement parameters of the rigid body transformation obtained by the realignment procedure were introduced as covariates in the model. The voxel-based time series was filtered with a high pass filter (time constant = 128 s). In the first level of analysis, the following contrasts were analyzed for each subject: CS+ > CS− and erotic pictures > control condition and introduced as dependent variables in the group analyses (second level analyses). We further analyzed absolute values of cosine of angles between CS+ and erotic pictures to account for potential problems of collinearity between the regressors (maximum at 0.19). Estimating models with collinear regressors result in regression coefficients that express the influence of the orthogonal part of a regressor, finally increasing Type II errors but not Type I errors.

In the group level analysis, a full-factorial model was used in order to avoid potentially biased Type I errors in second level analyses due to the use of pooled errors [Barcikowski and Robey, 1984; Boik, 1981]. Contrasts from first level GLM were analyzed by full-factorial ANOVA using partitioned errors [Friston et al., 2007; Penny and Henson, 2007]. The full factorial model included the group factor 5-HTTLPR genotype and was analyzed for main effects of stimulus (e.g., CS+ > CS−). We further conducted a correlation analysis between the contrast CS+ > CS− and differential SCRs (FIR and SIR) as well as between CS+ > CS and differential subjective ratings. The genotype effects on hemodynamic responses of appetitive conditioning were analyzed as follows: First, all s-allele subjects were compared with l/l-allele subjects because all conditioning studies used this classification strategy [Crişan et al., 2009; Garpenstrand et al., 2001; Lonsdorf et al., 2009]; sex and expectancy scores for the erotic pictures were included as covariates in order to account for potential confounding effects on putative influences of the 5-HTTLPR genotype [Cahill, 2006; Merz et al., 2010; Milad et al., 2010]. Second, adding findings to the current heterozygosis debate [Uher and McGuffin, 2008], we exploratively investigated, group differences between all three genotype groups (e.g. s/s vs. s/l; s/s vs. l/l), which are in the Supporting Information. Because the sample sizes differed between the genotype groups, additionally effect sizes (point biserial correlation) for the respective peak voxels were calculated, which are more independent of sample sizes. On the other hand, effect sizes could be overestimated, because they are taken from the peak voxels.

Exploratory whole brain analyses were conducted using an uncorrected $P < 0.0001$ and $k > 10$ voxel; regions of interest (ROI) analyses were performed using the small volume correction in SPM8 (family-wise-error (FWE) corrected $P < 0.05$; $k > 5$ voxel). The amygdala, the ACC, the insula, the OFC, the thalamus, and the ventral striatum were chosen as ROIs based on the following criteria: First, prior studies repeatedly showed that these structures are involved in appetitive conditioning [Cox et al., 2005; Kirsch et al., 2003; Klucken et al., 2009b; for review see: Martin-Soelch et al., 2007]. Second, neuroimaging studies indicate that emotional processing (especially appetitive/sexual) is linked to structural and/or functional alterations within these brain regions [Craig, 2011; Kagerer et al., 2011; Karama et al., 2002; Kühn and Gallinat, 2011; O'Doherty, 2007]. Anatomical masks for ROI analyses of the amygdala, the OFC, the ACC, the insula, and the thalamus were taken from the "Harvard-Oxford cortical and subcortical structural atlases" provided by the Harvard Center for Morphometric Analysis (probability threshold 50%) and from the Human Brain Project Repository database based on the BrainMap database [Fox and Lancaster, 1994; Nielsen and Hansen, 2002]. Since no mask of the ventral striatum exists in the Harvard-Oxford atlases, this mask was designed from predefined anatomical regions using MARINA [Walter et al., 2003]. Due to extensive head motion and technical problems, nine subjects (three s/s-, four s/l, and two l/l-carriers) were excluded from the fMRI analysis, finally resulting in 86 subjects for the fMRI analysis.

TABLE I. Hemodynamic responses for the main effect of stimulus for the contrast CS+ > CS− in the ROI-analyses and results from the correlation analysis between the contrast CS+ > CS− and skin conductance responses of the second interval responses (SIR)

Analysis	Structure	Side	k	x	y	z	z_{max}	P_{corr}
main effect of task CS+> CS−	Amygdala	L	35	−21	−1	−26	2.58	0.075
	Amygdala	R	30	21	2	−20	2.59	0.067
	ACC	R	349	9	14	34	5.13	<0.001
	Insula	L	154	−39	14	−2	4.57	<0.001
	Insula	R	129	36	14	4	5.70	<0.001
	Thalamus	L	84	−3	−25	1	3.42	0.026
	Thalamus	R	171	6	−25	1	3.82	0.007
	OFC	L	548	−24	38	−11	3.73	0.037
	OFC	R	633	33	47	−5	5.03	<0.001
	Ventral Striatum	L	57	−12	5	−2	3.78	0.003
	Ventral Striatum	R	108	18	11	−2	4.07	0.001
correlation CS+ > CS− * SIR	OFC	L	208	−24	50	−5	4.47	0.003
	OFC	R	241	24	53	−8	3.87	0.027

The threshold was $P < 0.05$ (small volume correction according to SPM8; FWE-corrected at the voxel level; additional trends are shown up to a more liberal threshold of $P \leq 0.075$). All coordinates are given in MNI space. L: left hemisphere, R: right hemisphere k: cluster size.

RESULTS

Hemodynamic Responses

Main effect of stimulus (CS+ > CS−)

Whole-brain results showed a significant main effect of stimulus (CS+ > CS−) in the fusiformis gyrus ($x/y/z$= 30/−70/−11, $z_{max} = 6.73$; $P < 0.001$), the posterior cingulate cortex ($x/y/z = -6/-28/28$, $z_{max} = 6.22$; $P < 0.001$), the supplementary motor area ($x/y/z = 6/14/46$, $z_{max} = 6.18$; $P = 0.001$), the middle frontal gyrus ($x/y/z = -30/53/28$, $z_{max} = 4.85$; $P = 0.019$), and the cerebellum ($x/y/z = -39/-55/-50$, $z_{max} = 4.83$; $P < 0.021$). Regarding ROI analyses, we found a significant main effect of stimulus in the contrast CS+ > CS− in the bilateral ventral striatum, the ACC, the OFC, the insula, the thalamus and a trend in the bilateral amygdala (see Table I), thus indicating successfully conditioned hemodynamic responses**.** Further, we observed a positive correlation between differential hemodynamic responses (CS+ > CS−) and differential SIR within the bilateral OFC (see Table I). Main effects of the erotic pictures are presented in the Supporting Information.

Main effect of the 5-HTTLPR genotype

ANOVA revealed main effects of the 5-HTTLPR genotype in the contrast CS+ > CS− in several ROI (see Table II). The s-allele group showed increased hemodynamic responses in the bilateral amygdala, the thalamus, the OFC, and the right insula as compared to the l/l-allele group (see Table II and Fig. 1), whereas the l/l-allele group did not show significantly enhanced activations compared to the s-allele carriers (all $P > 0.05$). In addition, we exploratively compared all three genotype groups (e.g., s/s vs. s/l; s/s vs. l/l). We found strong differences between the l/l-allele and the other two groups in the ventral striatum (see Supporting Information and Fig. 2). Regarding the genotype-dependent group differences for the erotic pictures, main effects of 5-HTTLPR genotype were found in the bilateral amygdala (Table II). In detail, the s-allele group showed increased hemodynamic responses in the bilateral amygdala as compared to the l/l-allele group.

Skin Conductance Responses

ANOVA for the SCRs revealed a significant main effect of stimulus regarding the conditioned FIR ($F_{(1,81)} = 16.89$; $P < 0.001$), the SIR ($F_{(1,81)} = 6.29$; $P < 0.05$), and the unconditioned ($F_{(1,81)} = 22.96$; $P < 0.001$) SCRs, showing greater SCRs to the CS+ as compared to the CS− in the FIR (see Fig. 3), the SIR, and respectively to the erotic pictures. We did not find a main effect of the 5-HTTLPR genotype in the conditioned ($F_{(1,81)} = 0.04$; $P > 0.90$) or unconditioned ($F_{(1,81)} = 0.157$; $P > 0.69$) SCRs as well as no significant genotype × stimulus interaction effect for the conditioned ($F_{(1,81)} = 0.04$; $P > 0.90$) and unconditioned ($F_{(1,81)} = 0.67$; $P > 0.42$) SCRs.

Subjective Ratings

ANOVA revealed strong main effects of stimulus-type for valence ($F_{(1,93)} = 24.69$; $P < 0.001$), arousal ($F_{(1,93)} = 36.58$; $P < 0.001$), and expectancy ratings ($F_{(1,93)} = 223.52$; $P < 0.001$; see Table III). Further, we found main effects of time for arousal ($F_{(1,93)} = 41.69$; $P < 0.001$) and for expectancy ratings ($F_{(1,93)} = 7.40$; $P < 0.01$). Importantly, stimulus × time interaction effects were found for valence ($F_{(1,93)} = 51.36$; $P < 0.001$), arousal ($F_{(1,93)} = 63.58$; $P < 0.001$), and expectancy ratings ($F_{(1,93)} = 276.05$; $P < 0.001$). Follow-up tests showed that the CS+ was rated significantly more positive as compared to the CS− after the experiment, but

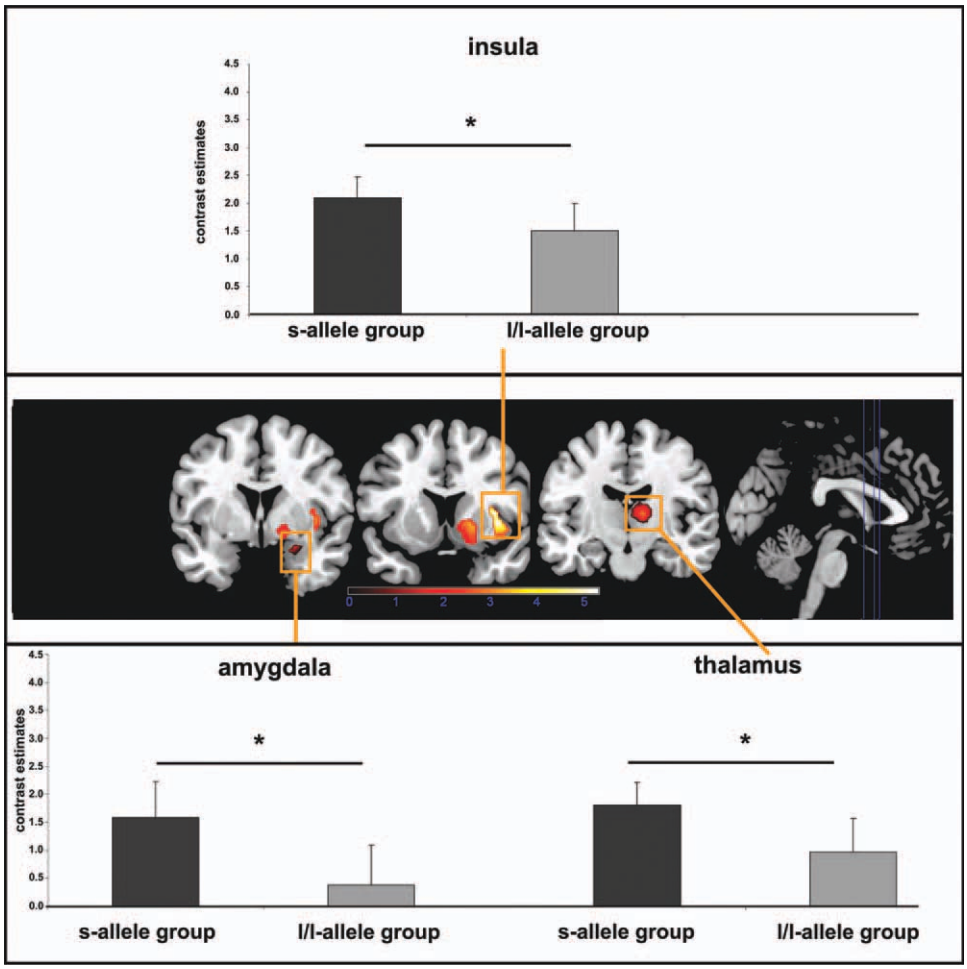

Figure 1.

Hemodynamic responses for the main effect (CS+ > CS−) for the two 5-HTTLPR genotype groups (s-allele vs. l/l-allele). The bar graphs show mean group contrast estimates (and SE of the mean) derived from the peak voxel of the second level analysis (see Table I for exact coordinates). The color bar depicts the *t*-values for this contrast. For illustration reasons, the data were thresholded at $t = 2.00$. *$P < 0.05$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

not before the experiment (all $P < 0.05$). Regarding the erotic pictures, we found strong significant increased valence ($T_{(94)} = 28.14$; $P < 0.001$) and arousal ratings ($T_{(93)} = 19.31$; $P < 0.001$), but no significant genotype differences regarding valence ($T_{(93)} = 0.47$; $P = 0.963$) and arousal ratings ($T_{(93)} = -0.555$; $P = 0.580$, see Table IV).

DISCUSSION

The present study is the first to investigate hemodynamic responses of appetitive conditioning as a function of the 5-HTTLPR genotype. Our results indicate a significant association between the different variants of the 5-HTTLPR polymorphism and blood oxygen level dependent signal change (BOLD)-responses during appetitive conditioning in the amygdala, the thalamus, the insula, and the OFC. As hypothesized, s-allele carriers appeared to be more reactive as compared to the l/l group, supporting a current model [Homberg and Lesch, 2011]. Before focusing on the 5-HTTLPR genotype effects, we briefly discuss some interesting results of the main effect of task in appetitive conditioning (CS+ > CS−).

Main Effect of Stimulus (CS+ > CS−)

The current findings confirm the assumed model of appetitive conditioning [Martin-Soelch et al., 2007] in a large human sample by showing strong subcortical and cortical hemodynamic responses. The most important finding is the significant bilateral striatal hemodynamic responses, indicating that the ventral striatum plays a

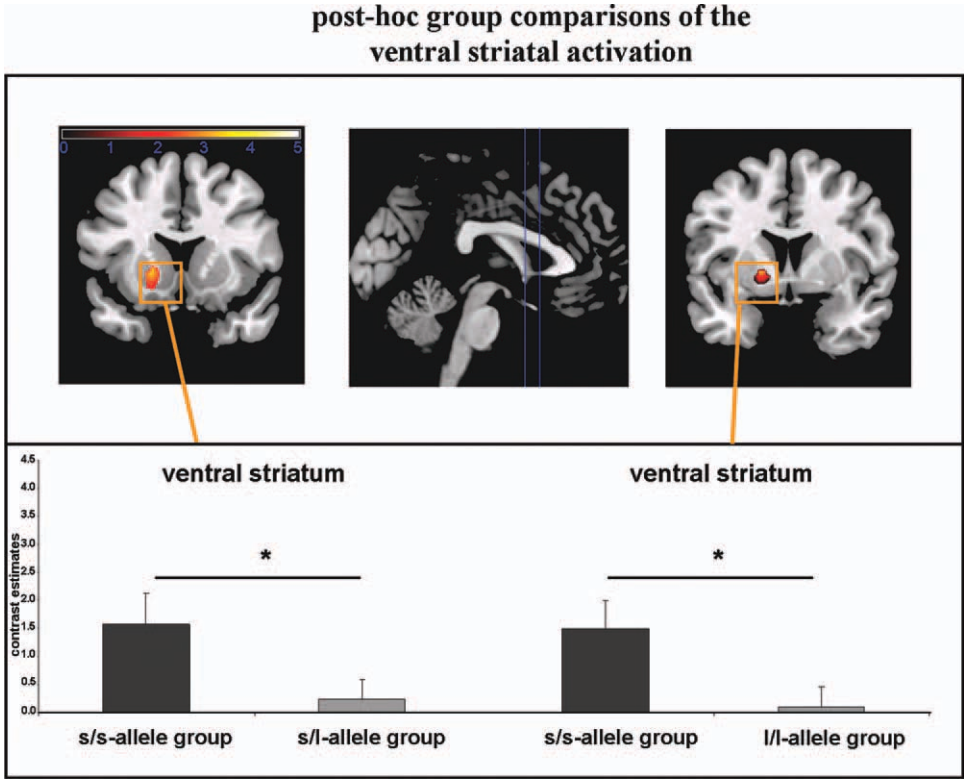

Figure 2.

Hemodynamic responses for the main effect of genotype in the contrast CS+ > CS− in the ventral striatum. The bar graphs show mean group contrast estimates (and SE of the mean) derived from the peak voxel of the second level analysis analyses for each genotype group separately. The color bar depicts the *t*-values for this contrast. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

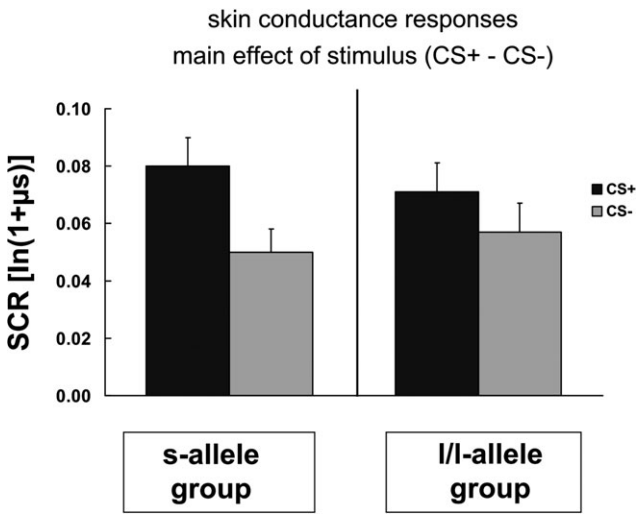

Figure 3.

Main effect of stimulus (CS+ vs. CS−) in SCRs for the s-allele and the l/l allele group. Error bars represent standard errors of the mean.

crucial role in appetitive conditioning. This underlines the role of the ventral striatum in the processing of reward anticipation, prediction, and approaching behavior [Martin-Soelch et al., 2007]. It has been hypothesized that striatal activation plays a key role for addiction-relevant learning processes [Chauvet et al., 2011; Day and Carelli, 2007; Peciña, 2008]. Nevertheless, several current findings have extended the assumed role of the striatum by showing increased activation in aversive conditioning [Klucken et al., 2009a,c; Mechias et al., 2010; Schiller and Delgado, 2010; Schiller et al., 2008]. Therefore, it has been suggested that the striatum is involved in the development and/or modification of CS–UCS contingencies [Klucken et al., 2009a, Klucken et al., 2009b; Schiller and Delgado, 2010; for an alternative view see: Tabbert et al., 2011]. In the present study, subjects had to learn a (simple) stimulus–stimulus association. Therefore, it is assumable that the reported striatal activation could reflect both, approaching and the development of contingency awareness.

In addition, we also observed increased hemodynamic responses in the insula, the ACC, and the OFC. The insula activity could reflect the fact that the former neutral CS+ gained an individual salience for the participants, which

TABLE II. Hemodynamic responses for the main effect of the 5-HTTLPR genotype

Analysis	Contrast	Structure	Side	*k*	*x*	*y*	*z*	z_{max}	Effect size	P_{corr}
s-allele > l/l allele	CS + > CS−	Amygdala	L	57	−27	−7	−14	3.60	0.39	0.005
		Amygdala	R	64	24	−4	−11	3.14	0.34	0.021
		Insula	R	116	39	−4	−8	3.29	0.36	0.039
		Thalamus	L	212	−12	−31	1	3.93	0.42	0.005
		Thalamus	R	190	12	−13	7	3.50	0.38	0.021
		OFC	L	559	−30	56	−2	4.52	0.48	0.002
		OFC	R	461	42	53	−2	4.48	0.47	0.003
		Ventral striatum[a]	L	33	−21	14	−5	2.68	0.29	0.075
		Ventral striatum[a]	R	36	15	−7	1	2.82	0.31	0.054
l/l-allele > s-allele	CS + > CS-	No significant activation								
s-allele > l/l allele	erotic stimuli	Amygdala	L	61	−18	−10	−14	3.81	0.39	0.002
	> non-erotic stimuli	Amygdala	R	66	21	−10	−14	3.51	0.38	0.007
l/l-allele > s-allele	erotic stimuli	No significant activation								
	> non-erotic stimuli									

The threshold was $p < 0.05$ (small volume correction according to SPM8; FWE-corrected at the voxel level; additional trends are shown at a more liberal threshold at $P \leq 0.075$). Effect sizes are given at point biserial correlation of the respectively peak voxel. All coordinates are given in MNI space. L: left hemisphere, R: right hemisphere. k: cluster size.
[a]Additional results using the tri-allellic classification.

provokes many bodily sensations. A recent meta-analysis linked insula activation to such physiological responses and interoceptive processing [Vytal and Hamann, 2010]. For instance, hemodynamic responses within the insula were observed in many studies employing appetitive stimuli and seem to be related more to the processing of arousal-inducing material than to (non-arousing) visual processing per se [Critchley, 2004; Kagerer et al., 2011; Kühn and Gallinat, 2011; Ortigue et al., 2010]. The increased CS+ salience is supported by the observed CS ratings, showing significantly increased arousal ratings of the CS+ as compared to the CS− after conditioning but not before. Regarding the ACC and the OFC, current studies emphasize that these structures are involved in CS–UCS contingency awareness and appraisal processes [Etkin et al., 2011; Kalisch, 2009; Mechias et al., 2010; Milad et al., 2007] and are also required in the expression of conditioned SCRs [Milad et al., 2007]. In addition, differential hemodynamic OFC responses positively correlated with the SIR. The finding that hemodynamic responses correlate with the SIR, but not with the FIR is in accordance with the assumed relationship of the SIR and the OFC. The SIR is generally assumed as a response related to the conscious anticipation of the UCS, whereas the FIR might rather reflect orienting responses [Knight et al., 2009; Pineles et al., 2009; Tabbert et al., 2011]. A similar role has been proposed for the OFC [O'Doherty, 2007], which is

TABLE III. (A) Mean (SE) valence ratings for the CS+ and for the CS− (1: "very unpleasant" to 9: "very pleasant") before (pre-rating) and after the conditioning (post-rating) procedure for both genotype groups separately. (B) Mean (SE) arousal ratings for the CS+ and for the CS− (1: "not arousing at all" to 9: "very arousing") before the conditioning (pre-rating) and after the conditioning (post-rating) procedure for both genotype groups separately. (C) Mean (SE) expectancy ratings for the CS+ and for the CS− (1: "erotic pictures did not appear after the CS" to 9: "erotic pictures appeared very likely after the CS") before the conditioning (pre-rating) and after the conditioning procedure (post-rating) for both genotype groups separately

	Pre-rating		Post-rating	
	CS+	CS−	CS+	CS−
(A) valence				
s-allele	5.30 (0.20)	5.13 (0.19)	6.29 (0.19)	4.25 (0.21)
l/l-allele	5.28 (.029)	5.47 (0.24)	6.47 (0.28)	4.16 (0.24)
(B) arousal				
s-allele	2.92 (0.19)	3.34 (0.18)	5.23 (0.28)	2.90 (0.22)
l/l-allele	3.06 (.031)	2.87 (0.29)	5.40 (0.43)	2.63 (0.34)
(C) expectancy ratings				
s-allele	4.84 (0.29)	5.35 (0.18)	5.35 (0.28)	2.03 (0.25)
l/l-allele	5.59 (.038)	5.38 (0.29)	5.40 (0.38)	1.97 (0.32)

TABLE IV. Mean (SE) valence (1: "very pleasant" to 9: "very unpleasant") and arousal ratings (1: "not arousing at all" to 9: "very arousing") for the erotic pictures after the conditioning procedure (post-rating) for both genotype groups separately

	Post-rating	
Erotic pictures	Arousal	Valence
s-allele	5.22 (0.20)	3.67 (0.18)
l/l-allele	5.19 (0.35)	3.34 (0.31)

associated with conscious stimulus evaluation, but not with orienting responses, further supporting a potential association between SIR and OFC.

Association Between the 5-HTTLPR Genotype and Conditioned Responses

5-HTTLPR-dependent group differences in CRs were observed in a distributed network of brain structures that has previously been implicated in appetitive processing including the amygdala, the insula, the OFC, and the thalamus [Homberg et al., 2008; Kranz et al., 2010; Murrough and Charney, 2011; Pfaus, 2009]. The present findings extend the view of the 5-HTTLPR on five important issues.

First, the presented data fit well with the recently proposed idea of a valence-independent and general hypervigilance to salient stimuli in s-allele carriers, which is not limited to fear processing [Homberg and Lesch, 2011]. Further support for this idea has been provided by recent findings showing serotonergic influences on reward anticipation and reward learning [Boureau and Dayan, 2011; Hayes and Greenshaw, 2011]. Consequently, in addition to the central role of dopamine in reward learning and reward processing, serotonergic influences have to be considered more carefully in the future [Boureau and Dayan, 2011; Hayes and Greenshaw, 2011]. Interestingly, within this context, we found the strongest significant hemodynamic responses in the ventral striatum within the s/s group as compared to the other groups (see Supporting Information). Because the ventral striatum is modulated by dopaminergic transmission, we suggest that these differences are driven by activations in other cortical and subcortical structures. In accordance with this view, detailed anatomical studies in rodents and recent computational neuroscience models provide evidence for close interrelationships of especially the amygdala and the OFC with the ventral striatum [Haber and Knutson, 2010; Martin-Soelch et al., 2007; for a model see Turnock and Becker, 2008].

Second, we found elevated conditioned and unconditioned responses in the subcortical affective learning network in the s-allele as compared to the l/l-allele group, supporting previous studies that indicate facilitated conditioning processes in s-allele carriers [Crişan et al., 2009; Garpenstrand et al., 2001; Lonsdorf et al., 2009]. However, whereas these studies investigated fear conditioning, a potential relationship between the 5-HTTLPR genotype and appetitive conditioning has been neglected so far. Interestingly, Lonsdorf et al. (2009) found group differences between s-allele and l/l-allele carriers in startle responses but not in SCRs, which is supported by our findings: Since conditioned startle is associated with amygdala activation and SCRs with ACC activation [Milad et al., 2007], these findings fit well with our findings of amygdala differences. In addition, since the amygdala is not only essential for the CS–UCS association process, but also for the expression of CR [Delgado et al., 2006], this finding helps to explain previous picture-perception studies reporting an amygdala hyper-reactivity in s-allele carriers in response to salient stimuli [Munafò et al., 2008]. Assuming that emotional responses to secondary reinforcement (e.g., emotional pictures like in picture-perception paradigms) develop through conditioning, it is conceivable that the exaggerated hemodynamic responses within the amygdala may at least partly result from increased learning and sensitization processes in the past.

Third, in addition to the association between the 5-HTTLPR genotype and subcortical activation patterns, s-allele carriers displayed elevated responses in cortical areas. In detail, we observed increased hemodynamic responses in the OFC in s-allele carriers, which is a correlate of conscious evaluation and appraisal processes [Burke et al., 2008; O'Doherty, 2007; Rolls, 2004]. These findings altogether support the idea that the 5-HTTLPR findings may concern bottom–up and top–down processes likewise. Evidence for a top–down approach has been reported by three recently published studies, indicating an association between the 5-HTTLPR genotype and emotion regulation, probably conveyed by altered OFC activation [Gillihan et al., 2010; Lemogne et al., 2011; Schardt et al., 2010]. In sum, the 5-HTTLPR genotype seems to be related to subcortical and cortical activations, which may presumably affect a broad range of cognitive and non-cognitive processes highlighting the importance of the 5-HTTLPR genotype on human behavior.

With respect to clinical implications, it seems conceivable that facilitated appetitive learning processes possibly contribute to the development of psychiatric disorders, e.g. addiction [Day and Carelli, 2007; Martin-Soelch et al., 2007; Peciña, 2008]. Because appetitive conditioning is facilitated in s-allele carriers, this group is likely to exhibit approach behavior more often, which might explain the higher number of addiction-related disorders in this group. Several studies confirmed an association of the 5-HTTLPR [Feinn et al., 2005; Lerman et al., 2000; van der Zwaluw et al., 2010] with psychiatric disorders related to appetitive conditioning processes. Thus, the present data might contribute to a neuronal model integrating risk factors for increased vulnerability to addiction-related disorders. However, it is important to note that this idea remains preliminary, because the present study did not investigate patients exhibiting any of the described psychiatrically relevant behaviors.

Finally, a meta-analysis reported an interaction effect between the 5-HTTLPR and stressful life events on major depression [Uher and McGuffin, 2008]. Notably, no main effect of the 5-HTTLPR was found. The authors assumed that the significant interaction effect and the lacking main effect of genotype could be explained by the assumption that individuals with the s-allele not only suffer more likely from negative life events than individuals with the l-allele, but additionally profit more from positive life events [Uher and McGuffin, 2008]. Our data fit with this conclusion indicating that individuals with the s-allele might expand their set of positive associated stimuli through facilitated learning processes.

Nonetheless, the findings of the present study are restricted by some limitations that have to be taken into account. First, even though we did not find genotype-related group differences in erotic picture ratings after the experiment, potential differences before the experiment might have influenced the appetitive conditioning results. In addition, effect sizes should be interpreted with caution; they are likely to be overestimated, since they are based on the respective peak voxels.

In conclusion, we found an association between the 5-HTTLPR genotype and hemodynamic responses of appetitive conditioning. Our results are in line with the assumption that s-allele carriers exhibit increased reactivity to salient environmental cues in general, rather than specifically to fear. This could at least partially explain the increased vulnerability for various psychiatric disorders like addiction. Nevertheless, one should keep in mind that the 5-HTTLPR genotype is not only associated with emotional processing, but also with many other functions of human life, including decision making or stress reactivity [Caspi et al., 2010; Homberg, 2012; Homberg and Lesch, 2011]. The s-allele should not be considered the "causally bad" allele, but rather an allele that may interact with other genes, neurotransmission, brain functioning, and behavior in a complex manner.

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