Serotonin Transporter (5-HTTLPR) Genotype and Amygdala Activation: A Meta-Analysis

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Background: We evaluated the magnitude of the reported associations between amygdala activation and the serotonin transporter gene linked polymorphic region (5-HTTLPR) and the likely effect size of this relationship.

Methods: We used meta-analytic techniques to combine data from existing published and unpublished studies. We also tested for possible publication bias and explored possible moderating influences on any association, such as sample ancestry.

Results: Our results provide support for the association of the 5-HTTLPR polymorphism and amygdala activation and suggest that this locus may account for up to 10% of phenotypic variance. Although we did not observe evidence for potential publication bias in our main analysis, this was due in part to efforts to obtain unpublished data pertinent to this meta-analysis, and when three unpublished data sets were excluded we did observe evidence of such bias. We also observed evidence that the first published study may provide an overestimate of the true effect size, which is consistent with findings from genetic association studies of other phenotypes.

Conclusions: Although our analysis provides support for the association of the 5-HTTLPR polymorphism and amygdala activation, it also suggests that most studies to date are nevertheless lacking in statistical power. Increasing the sample sizes of future imaging genetics studies will allow a more accurate characterization of any true effect size and afford adequate power to examine the impact of multiple polymorphisms that likely work in concert to affect gene function and, in turn, bias neural processes mediating dispositional traits such as temperament and personality.

Key Words: 5-HTTLPR, amygdala, fMRI, meta-analysis, serotonin transporter gene

Individual differences in trait negative affect are important predictors of vulnerability for a spectrum of health-related disorders including depression, anxiety, and cardiovascular disease (1,2). As such, identifying biological variables contributing to the emergence of such interindividual variability holds great potential for elucidating both the etiology and pathophysiology of these disorders. Moreover, certain biological variables may offer clinical utility by serving as predictive markers of increased disease risk. Converging evidence from rodent and nonhuman primate as well as extensive human research has implicated variability in serotonin (5-HT) neurotransmission as a key predictor of individual differences in multiple, overlapping behavioral constructs related to trait negative affect (3). Research employing pharmacologic challenge of the 5-HT system (via specific receptor agonism/antagonism or general reuptake blockade), for example, has indicated that these manipulations can modulate peripheral stress responses and subjective negative affect (4). These and other findings have subsequently spurred intensive efforts to identify genetic polymorphisms in 5-HT subsystems, which ultimately control the regulation of 5-HT neurotransmission as a function of both homeostatic drive and environmental feedback, that predict trait negative affect, as well as differentiate relative risk for disease.

Of particular importance in these efforts has been the 5-HT transporter (5-HTT), which is responsible for the active clearance of synaptic 5-HT and thus regulation of presynaptic and postsynaptic 5-HT receptor stimulation. In 1996, Lesch and colleagues (5,6) identified a relatively common functional promoter polymorphism in the human 5-HTT gene (*SLC6A4*). The so-called 5-HTT gene linked polymorphic region, or 5-HTTLPR, is typically defined by two variable nucleotide tandem repeat elements, a short (S) allele comprising 14 copies of a 20-23 base pair repeat unit and a long (L) allele comprising 16 copies. Although initial in vitro and in vivo assays revealed relatively diminished 5-HTT density associated with the S allele, recent work has indicated that more complex mechanisms (e.g., regional up- and down-regulation of specific 5-HT receptors) and not altered 5-HTT density may mediate the long-term impact of the 5-HTTLPR on 5-HT neurotransmission (7). Regardless of the underlying mechanisms of action, a modest association has been reported between the 5-HTTLPR S allele and relatively increased trait negative affect (8–11). Moreover, the 5-HTTLPR S allele has been associated with relatively increased risk for depression in the context of environmental adversity (12–15), a relationship that may be mediated by increased neuroticism (16), a psychometrically robust index of trait negative affect.

Although positive associations between the 5-HTTLPR S allele and increased trait negative affect or risk for depression have not been consistently demonstrated across studies (17–19), recent data from the emerging field of imaging genetics (20,21) has provided apparently consistent evidence for a link between the 5-HTTLPR S allele and relatively heightened amygdala activation to emotional stimuli relative to neutral stimuli, a key neural process underlying the generation of behavioral and physiologic arousal to environmental threat. Since the original demonstration of this relationship (22) using functional magnetic resonance imaging (fMRI) in 2002, several studies have reported a link between increased amygdala activation and the 5-HTTLPR S allele using a variety of neuroimaging technologies (i.e., fMRI, positron emission tomography [PET], perfusion MRI), provocative stimuli (e.g., emotional facial expressions, pictures and words) and subject populations (e.g., controls as well as patients with social phobia, panic disorder, and major depression). The

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collective results of these studies suggest that relatively heightened amygdala activation to environmental threat may mediate the association between the 5-HTTLPR S allele and increased trait negative affect, as well as risk for mood and anxiety disorders, especially in response to chronic or severe stress. Recent evidence of traitlike stability for individual differences in amygdala activation measured with fMRI over both short (2- and 8-week) and long (> 1 year) intervals (23,24) further underscores the possible and potentially unique role of the amygdala in mediating effects of the 5-HTTLPR on enduring aspects of temperament and personality.

A perennial difficulty in the field of psychiatric genetics is nonreplication of initially promising findings, in part due to the small magnitude of single gene effects on complex behavioural phenotypes (25). Although endophenotype measures, such as amygdala activation, may offer larger effect sizes, this assumption requires further exploration, and the small sample sizes typical of studies of this kind raise the possibility of Type I error (26). Even in the case of associations that have been replicated with reasonable robustness, there is evidence that the first published study often suggests an effect size that in time proves to be an overestimate of the true effect size (27). Publication bias, in which findings that fail to achieve statistical significance or that are in the opposite direction to that which is predicted are less likely to be published, may also undermine the integrity of published data (28). Meta-analysis is an increasingly common method for addressing these issues, allowing an assessment of the overall strength of evidence for association and formal testing for evidence of publication bias (25), as well as the exploratory investigation of possible sources of between-study heterogeneity such as moderation by measurement instrument (29).

In light of these findings and their potential to inform neurogenetic pathways for disease risk, we were motivated to evaluate formally the reported associations between amygdala activation and the 5-HTTLPR S allele and the likely magnitude of this relationship using meta-analytic techniques. We also tested for possible publication bias, and explored possible moderating influences on any association, such as sample ancestry.

Methods and Materials

Selection of Studies for Inclusion

Genetic association studies of the 5-HTTLPR polymorphism and amygdala activation were included. Studies reporting data on either single-sex or both male and female participants of any ethnic origin drawn from both healthy and psychiatric populations were included. The principal outcome measure was the standardized mean difference in amygdala activation between short (SS and SL, combined) versus long (LL) genotype groups.

Search Strategy

The search was performed on two databases: PubMed and PsycINFO. These databases were searched from the first date available in each database up to 30 April 2007, using the search terms "amygdala," "serotonin transporter," "5-HTT," "5-HTTLPR," and "genet* or genot*." After articles had been collected, bibliographies were then hand-searched for additional references. In addition, researchers active in the field were contacted directly to ascertain whether there were relevant unpublished data that could contribute to the meta-analysis.

The abstracts of studies identified by these search strategies were then examined with reference to the inclusion and exclusion criteria. Duplications were deleted, and the whole text of each reference was then checked to establish further whether the study met the study inclusion criteria. Studies that reported previously published data were excluded.

Data Extraction

For each study, the following data were extracted independently by two authors (M.R.M. and S.M.B.) using standard forms: 1) author(s) and year of publication; 2) methods (country of origin, dominant ancestry of sample, sample size, measure of amygdala activation, Hardy-Weinberg equilibrium); and 3) data (mean and standard deviation of amygdala activity in short and long genotype groups, number of participants, mean age and male/female ratio). Genotype frequencies were used to calculate whether these deviated significantly from Hardy-Weinberg equilibrium in cases in which this was not reported in the original article. Ancestry was coded as European, East Asian, or Other (which included cases in which ancestry was stated as mixed or not stated). Discrepancies were resolved by mutual consent.

Analysis of Data

Data were analyzed using the Comprehensive Meta-Analysis (v.2) statistical software package. A p value of .050 was retained throughout.

Data were initially analyzed within a fixed effects framework, and standardized mean differences (d) pooled using inverse variance methods to generate a summary d and 95% confidence interval (CI). A fixed-effects framework assumes that the effect of genotype is constant across studies, and between-study variation is considered to be due to chance or random variation. The assumption was checked using a chi-square test of goodness of fit for homogeneity. The significance of the pooled d was determined using a Z test.

Where there was evidence of a significant association between 5-HTTLPR genotype and amygdala activation in the presence of significant between-study heterogeneity, a random effects framework was employed, with ds pooled using DerSimonian and Laird methods. A random effects framework assumes that between-study variation is due to both chance or random variation and an individual study effect. Random effects models are more conservative than fixed effects models and generate a wider confidence interval. The significance of the pooled d was determined using a Z test.

Analyses stratified by sample ancestry, measure of amygdala activation, and deviation from Hardy-Weinberg Equilibrium were conducted to assess potential moderating effects of these variables. In addition, the effect size estimate of the first published study was compared with the pooled effect size of the remaining studies using a Z test, and a meta-regression of individual study effect size against year of publication conducted, because there is evidence for a substantially greater estimate of effect size in the first published study (27). Funnel plots were created to assess potential ascertainment bias (as might be caused by publication bias) by plotting individual study effect size against the standard error of the effect size. Ascertainment bias was also assessed using Egger's test (30).

Results

Description of Studies

Fourteen studies published between 2002 and 2007 (22,31–43) and three unpublished data sets (MB Stein, personal communication, August 20, 2006; S. Surguladze, personal communication, April 17, 2007; SE Taylor, personal communication, March 22, 2007) were identified by the search strategy and met the

Table 1. Characteristics of Included Studies

Study	Year	Short			Long			Ancestry	Age	M/F	HWE	Method	Measure
		M	SD	*n*	M	SD	*n*						
Hariri (22)	2002	.28	.08	14	.03	.05	14	European	31.2	29	Y	fMRI	
Furmark (38)	2004	Data Not Available						European	n/a	53	n/a	PET	
Heinz (40)	2004	.05	.08	20	.00	.09	9	European	40.0	100	Y	fMRI	Negative-neutral
Bertolino (31)	2005	.09	.24	18	.04	.29	10	European	33.5	36	Y	fMRI	
Canli (33)	2005	.12	.30	28	−.02	.12	13	European	n/a	46	n/a	fMRI	Negative-neutral
Hariri (39)	2005	.16	.17	65	.03	.30	27	European	30.7	49	Y	fMRI	
Brown (32)	2006	1.38	1.07	34	.74	.86	21	European	45.1	40	Y	fMRI	
Canli (34)	2006	54.10	18.10	13	29.50	14.50	8	Other	30.4	67	n/a	Perfusion MR	
Dannlowski (36)	2006	.19	.29	18	−.15	.55	9	European	36.7	26	Y	fMRI	Negative-neutral
Domschke (37)	2006	.09	.35	13	−.29	.42	7	European	36.8	40	Y	fMRI	
Heinz (41)	2006	Data reported in Heinz 2004						European	40.0	100	Y	fMRI	
Dannlowski (35)	2007	.12	.32	44	−.03	.53	12	European	37.7	41	Y	fMRI	Negative-neutral
Smolka (42)	2007	Data partially reported in Heinz 2004						European	41.2	100	Y	fMRI	
Rao (43)	2007	58.90	8.30	13	51.50	8.30	13	European	20.3	50	N	Perfusion MR	
Stein (unpub.)	Unpub.	.19	.21	15	−.06	.19	3	European	18.1	n/a	n/a	fMRI	Fear-shape
Surguladze (unpub.)	Unpub.	.10	.03	22	.09	.03	7	European	37.1	59	Y	fMRI	Fear
Taylor (unpub.)	Unpub.	.31	1.07	22	2.39	1.22	7	Other	20.9	n/a	Y	fMRI	

F, female; fMRI, functional magnetic resonance imaging; M, male; N, no; PET, positron emission tomography; unpub, unpublished; Y, yes.

Amygdala activation by 5-HTTLPR genotype (short: SS or SL; long: LL) is presented, with sample ancestry, mean age, male:female ratio (expressed as a percentage of males in the sample), whether the genotype frequencies reported were in approximate Hardy-Weinberg Equilibrium (HWE), and imaging method employed. In cases in which more than one behavioral contrast was used in a single study, the measure used in the meta-analysis is reported.

inclusion criteria. The characteristics of these studies are described in Table 1.

Fifteen studies reported data on samples of predominantly European ancestry, and two reported data on a sample of Other ancestry (34; SE Taylor, personal communication, March 22, 2007). One study reported 5-HTTLPR genotype frequencies that deviated significantly from Hardy-Weinberg equilibrium (43). Thirteen studies used fMRI methods to assess amygdala activation, whereas one used PET (38) and two used perfusion MR (43) methods (grouped as Other). One study (38) did not report data in a format that enabled inclusion in the meta-analysis, and two studies reported data that were reported either partially or in their entirety elsewhere (41,42), so that a total of 14 studies contributed to the final analysis. The majority of studies reported data on right hemisphere amygdala activation only, and four provided a figure for both hemispheres without summary statistics (33,35,36,42), of which two reported summary statistics for global amygdala activation only (36,42). Only one study (40) provided summary statistics for left and right hemisphere amygdala activation separately.

Meta-Analysis

When all studies ($k = 14$) were included, there was evidence of a significant association between 5-HTTLPR genotype and amygdala activation ($Z = 6.09$, $p < .001$, $d = .63$, 95% CI .42 – .83). There was evidence of significant between-study heterogeneity (χ^2 [13] = 57.04, $p < .001$), but when the analysis was rerun within a random-effects framework, the evidence for association remained statistically significant ($Z = 3.15$, $p = .002$, $d = .71$, 95% CI .27 – 1.15). These results are presented graphically in Figure 1.

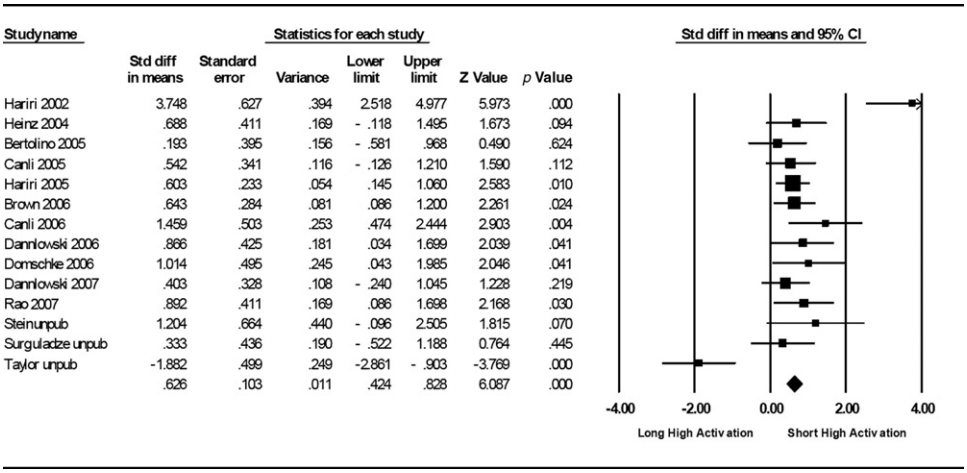

Figure 1. Meta-analysis of association studies of 5-HTTLPR genotype and amygdala activation. Meta-analysis indicates significant association between 5-HTTLPR genotype and amygdala activation ($p < .001$). Bars represent individual study 95% confidence intervals, with a central block proportional to study size. The summary diamond bar represents the pooled effect size estimate and 95% confidence interval (CI).

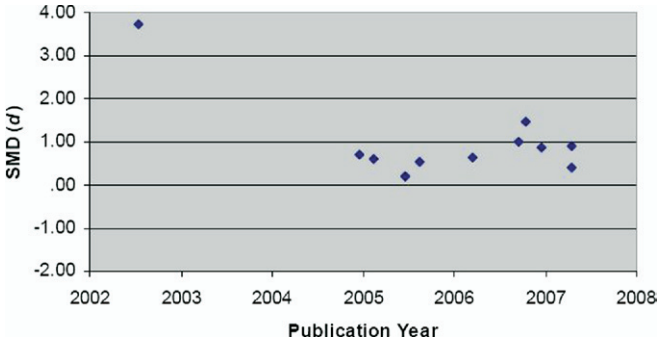

Figure 2. Association between year of publication and effect size estimate. Year of publication is negatively associated with individual study effect size ($p = .028$), with this trend reflecting a decrease in individual study effect size over time. Removal of the first published study results in a significant reduction in pooled effect size estimate ($p < .001$), although the effect of 5-HTTLPR genotype remains significant.

Sensitivity Analyses

When the first published study (22) was removed from the analysis ($k = 13$), there was evidence of a significant association between 5-HTTLPR genotype and amygdala activation ($Z = 5.18$, $p < .001$, $d = .54$, 95% CI .33–.74). There was evidence of significant between-study heterogeneity (χ^2 [12] = 31.61, $p = .002$), but when the analysis was rerun within a random-effects framework, the evidence for association remained statistically significant ($Z = 3.00$, $p = .003$, $d = .53$, 95% CI .18–.87).

The comparison of the effect size indicated by the first published study ($d = 3.75$) with the pooled effect size for subsequent studies ($d = .54$) indicated a significant difference within both a fixed-effects ($p < .001$) and a random-effects framework ($p < .001$). Meta-regression, excluding unpublished data sets, indicated a significant negative association between year of publication and individual study effect size ($Z = -2.20$, $p = .028$), with this trend reflecting a decrease in individual study effect size over time. These data are presented graphically in Figure 2.

The comparison of the pooled effect size for studies that used fMRI methods to assess amygdala activation ($d = .57$), compared with other methods ($d = 1.12$), did not indicate a significant difference ($p = .10$).

The removal of one study that reported 5-HTTLPR genotype frequencies that deviated significantly from Hardy-Weinberg equilibrium (43) did not alter these results substantially. Similarly, when two studies that recruited participants of multiple ancestries (34; SE Taylor, personal communication, March 22, 2007) were removed from the analysis, these results were not altered substantially.

Two studies (22; SE Taylor, personal communication, March 22, 2007) were clear outliers (see Figure 1), and when these studies were removed from the analysis ($k = 12$), there was evidence of a significant association between 5-HTTLPR genotype and amygdala activation ($Z = 6.10$, $p < .001$, $d = .65$, 95% CI .44–.86), with no evidence of significant between-study heterogeneity (χ^2 [11] = 7.01, $p = .80$).

Publication Bias

A visual inspection of a funnel plot of $1/SE$ against effect size estimate did not suggest evidence of ascertainment bias. Egger's test [$t(12) = .86$, $p = .41$) also did not indicate the presence of such bias. Notably, when the three unpublished data sets were excluded from this analysis, there was evidence of publication bias ($p = .030$). This suggests potential bias in the currently available, published literature.

Laterality

Although only one study (40) reported summary statistics separately for left and right hemisphere amygdala activation, these data were available for an additional two studies (39,43). When these studies ($k = 3$) were included, there was evidence of a significant association between 5-HTTLPR genotype and amygdala activation in the left ($Z = 1.98$, $p = .047$, $d = .36$, 95% CI .00–.71) and right ($Z = 3.71$, $p < .001$, $d = .68$, 95% CI .32–1.03) hemispheres. Although the effect size estimate for left hemisphere amygdala activation was considerably smaller, this did not differ significantly from the estimate for right hemisphere amygdala activation ($p = .21$).

Discussion

Our results provide support for the association of the 5-HTTLPR polymorphism and amygdala activation and suggest that this locus may account for up to 10% of phenotypic variance. As such, they underscore the biological relevance of genetically driven variability in 5-HT function and its resultant modulation of neural circuitries involved in the generation of complex emotional behaviors associated with risk for mood and anxiety disorders. Specifically, our results indicate that alterations in 5-HT signaling associated with the 5-HTTLPR appear to contribute significantly to variability of amygdala activation in response to a broad range of salient environmental stimuli. However, they also indicate that the direction of this association may be influenced by genetic background (i.e., ancestry) as the only study reporting greater amygdala activation in L allele homozygotes in comparison with S allele carriers was comprised largely of individuals of East Asian ancestry (SE Taylor, personal communication, March 22, 2007), although it should be noted that removal of studies of non-European ancestry did not alter our findings substantially. Interestingly, the opposite direction of this association from that observed in samples of European ancestry has been mirrored in some pharmacogenetic studies in which greater efficacy of selective serotonin reuptake inhibitors has been linked with the S allele in Asians (44,45), although the evidence for this is mixed (46). This highlights the potential importance of considering genetic background in association studies of single genetic polymorphisms.

More generally, our results bolster the potential importance of in vivo neuroimaging assays of brain function in determining the relevance of specific genetic polymorphisms in the emergence of interindividual variability in circumscribed biobehavioral pathways. Notably, the method used to assess amygdala activation did not appear to modify this association. We also did not observe formal evidence for laterality effects on the association between 5-HTTLPR genotype and amygdala activation, although the pooled effect size was qualitatively greater for the right hemisphere than for the left. Unfortunately, this analysis was restricted to only three studies, of which only one explicitly reported results for left and right hemisphere amygdalae separately, so that this analysis lacked statistical power. Future studies should report data for both hemispheres to allow for laterality effects to be fully explored. Although such effects, if they exist, may be an artifact of the stimuli employed (e.g., visual), task processing demands (e.g., perceptual matching), or both, which requires further systematic investigation.

Although we did not observe evidence for potential publication bias in our main analysis, this was due in large part to efforts

to obtain unpublished data pertinent to this meta-analysis. When three unpublished data sets were excluded from our analysis of potential publication bias, we did observe evidence of such bias. Egger's test is a formal test of the association between individual study effect sizes and their accuracy (i.e., size). The three unpublished studies were of small size and reported either no association or an association in an opposite direction to that originally reported. It is exactly studies such as these that are likely to be more difficult to publish, resulting in publication bias and possible distortion of the corpus of available data. Given that formal tests of publication bias lack statistical power and the relatively small number of data sets that contributed to our analysis, this emphasizes the importance of the publication of nonsignificant findings to avoid distorting the corpus of publicly available evidence.

We also observed evidence that the first published study may provide an overestimate of the true effect size, which is consistent with findings from genetic association studies of other phenotypes (27). As well as observing a reduced pooled effect size estimate when the first published study was removed, we observed a negative association between year of publication and individual study effect size. The latter observation suggests that our estimate of the true effect size may need to be revised further as more data become available. This reinforces the potential importance of meta-analysis as a means by which the true effect size of a genetic association with a given phenotype may be estimated with greater accuracy. On the basis of the pooled effect size estimate from our main analysis, any given study would require a sample of more than 70 participants (assuming equal numbers of short and long genotypes) to achieve 80% power to detect association with amygdala activation at an alpha level of .05. It is notable that only one of the studies (39) included in our analysis included a sample as large as this, suggesting that even in the context of genetic associations with neural phenotypes of relatively large effect sizes the majority of studies to date are underpowered. This suggests a need for investment in larger sample sizes to achieve more accurate estimates of the magnitude of the true effect.

The magnitude of any association may be further affected by additional occult polymorphisms within *SLC6A4* (e.g., rs25531, VNTR-2), which have the potential to moderate the effects of the 5-HTTLPR on gene expression (47,48) and subsequent biological processes (including amygdala activation) potentially sensitive to alterations in serotonergic neurotransmission. Indeed, the effect of such polymorphisms on 5-HT availability has recently been confirmed using PET techniques with [^{11}C]-3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile to measure serotonin transporter binding potential (49). Increasing the sample sizes of future imaging genetics studies, as well as allowing a more accurate estimation of the true effect size, will afford adequate power to examine the impact of multiple polymorphisms that likely work in concert to affect gene function and, in turn, bias neural processes mediating dispositional traits such as temperament and personality.

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