Manuscript Information

Journal name: Psychophysiology
NIHMSID: NIHMS254477
Manuscript Title: Serotonin Transporter Promoter Region (5-HTTLPR) Polymorphism Predicts Resting Respiratory Sinus Arrhythmia
Principal Investigator:
Submitter: John Wiley And Sons Publishing (CDT-help@wiley.com)

Grant/Project/Contract/Support Information

Name	Support ID#	Title

Manuscript Files

Type	Fig/Table #	Filename	Size	Uploaded
manuscript		PsyP-2010-0007R3_Ellis.doc	56320	2010-11-24 07:57:23
table	Table 01	Ellis_Table 1.doc	28160	2010-11-24 07:57:23

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RUNNING HEAD: 5-HTTLPR and Respiratory Sinus Arrhythmia

Serotonin Transporter Promoter Region (5-HTTLPR) Polymorphism Predicts Resting Respiratory Sinus Arrhythmia

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Abstract

Respiratory sinus arrhythmia (RSA) is often conceptualized as an index of physiological flexibility that has been related to emotion regulatory capacity. Although behavioral genetics research indicates that RSA is partly heritable, relatively few molecular genetics studies have been conducted. We examined whether the serotonin transporter promoter region (5-HTTLPR) polymorphism was associated with resting RSA among healthy young adults ($N = 71$). Short 5-HTTLPR allele carriers had significantly lower resting RSA than long 5-HTTLPR homozygotes. Genotype explained 5% of the variance in resting RSA. Although firm conclusions depend on further study, the short allele of the 5-HTTLPR polymorphism may contribute to individual differences in RSA and its behavioral correlates.

Keywords: 5-HTTLPR; serotonin; respiratory sinus arrhythmia; emotion regulation

Serotonin Transporter Promoter Region (5-HTTLPR) Polymorphism Predicts Resting Respiratory Sinus Arrhythmia

Respiratory sinus arrhythmia (RSA) has been associated with an individual's ability to physiologically regulate emotion (e.g., Beauchaine, 2001). RSA is variation in respiration-linked beat-to-beat intervals in heart rate. These between beat variations are the result of fluctuations in vagal efference to the heart. If vagus function is disrupted or reduced, sympathetic activity remains unopposed by the inhibitory effect of the parasympathetic nervous system and capacity for emotion regulation decreases (Porges, 1995).

Behavioral genetics studies of individual differences in resting RSA suggest that it has a partly heritable origin. Twin and family studies report 25 - 65% of the variance in resting RSA among Caucasians is due to heritable influences (Snieder, et al., 1997). Further, similar levels of heritability have been observed for resting RSA among European and African American twins (Wang et al., 2009). Despite this, however, the specific genetic influences of RSA remain unclear.

A central autonomic network is purported to support goal-directed behavior and adaptability via parasympathetic efference to the heart (Thayer & Lane, 2000). This network is comprised of the anterior cingulate and ventromedial prefrontal cortices, insular cortex, and central nucleus of the amygdala (Thayer & Lane, 2000). The amygdala communicates bidirectionally with prefrontal, cingulate, and insular cortices. Inhibitory control of the amygdala occurs via prefrontal vagal pathways (Thayer & Lane, 2009). When this inhibitory control is reduced, sympathetic and subsequent

parasympathetic responses remain unopposed (Thayer & Lane, 2009). Genetic influences on structures within the autonomic network may therefore affect RSA regulation.

Prefrontal coretx and amygdala function have previously been associated with the serotonin transporter (5-HTTLPR) polymorphism (Pezawas, et al., 2005). This is a promising candidate for influencing resting RSA since reduced serotonergic activity in the central nervous system has been posited to influence physiological activity through decreased parasympathetic nervous system responsivity (Williams, 1994). A common insertion/deletion polymorphism in the promoter region of this gene is associated with altered transport/reuptake of serotonin. More specifically, the short (s) allele variant has been linked with reduced transcriptional efficiency and decreased serotonin reuptake compared to the long (l) allele variant (Lesch et al., 1996).

5-HTTLPR short allele status (i.e., ss, sl) has been associated with smaller amygdala and perigenual anterior cingulate (pACC) volumes and reduced functional connectivity between these regions compared to l allele homozygotes. The pACC is believed to inhibit amygdala reactivity (e.g., Pezawas et al., 2005). Further, s 5-HTTLPR allele carriers have less white matter connectivity between medial/orbital prefrontal cortex and the amygdala than l homozygotes (Pacheco et al., 2009). Altered function and connectivity within these circuits may contribute to lowered RSA among s 5-HTTLPR allele carriers.

In the only previous study of resting RSA and 5-HTTLPR, no differences between genotype groups were reported for baseline RSA, despite genotype differences in RSA change following a 35% carbon dioxide challenge (Schmidt et al., 2000). Although resting RSA and RSA reactivity tend to be correlated and influenced by the

same genes (Wang, et al., 2009; De Geus et al., 2007), the paucity of work examining links between the 5-HTTLPR polymorphism and resting RSA suggest further investigation is warranted.

Thus, we examined whether the 5-HTTLPR polymorphism is associated with resting RSA in a sample of healthy, unmedicated, young adults. Such a sample is well suited for identifying genetic associations, as disease state can influence physiology and complicate studying associations between 5-HTTLPR and RSA (cf. Way & Taylor, 2009). Based on theoretical work linking serotonin function with physiological functioning (Williams, 1994), a plausible biological pathway that could connect the 5-HTTLPR polymorphism to lowered RSA (i.e., altered amygdala – PFC function and connectivity; Thayer & Lane, 2009), we expected s allele carriers (with putatively less efficient 5-HT transport) to have lower resting RSA than l homozygotes.

Method

Participants

Seventy-one introductory psychology students at a large, southwestern university participated to partially fulfill course requirements. Average age was 18.95 (SD = 1.12) years. The ll participants were 62% female with 62% Caucasian (Asian: 4.8%; African American 4.8%; Hispanic: 19%). The ss and sl participants were 54% female with 60.3% Caucasian (Asian: 15.9%; African American 1.6%; Hispanic: 17.5%). 5-HTTLPR allele groups did not significantly differ on age ($F(1, 70) = 2.17$, $p = 0.15$), race ($\chi^2(4, N=68) = 2.90$, $p = 0.57$) or sex ($\chi^2(1, N=71) = 0.48$, $p = 0.58$). When sex was included as a covariate in analyses, results remained unchanged.

Physiological Assessments

Data were obtained using a Biopac MP 150 system and processed with Acqknowledge v3.9 software (Biopac Systems Inc., Santa Barbara, CA).

RSA. Following established guidelines (cf., Task force proceedings, 1996), electrocardiographic activity (ECG) was recorded with a Biopac ECG100C Electrodcardiogram amplifier. Ag-AgCl electrodes on the right wrist and left ankle provided ECG activity that was sampled at 1000 Hz while participants were seated upright and still across a single 5- min epoch.

ECG data were band pass filtered between 0.5 and 35Hz. A QRS detector using a modified Pan and Tompkins algorithm (Pan & Tompkins, 1985) generated a tachogram which was visually inspected for artifacts (i.e., presence of an unusual R-R interval). Artifacts identified were corrected by adjusting peak values to within threshold or eliminating them completely. Missed beats were corrected by one of two methods: 1) splitting erroneously long beats into separate RR intervals or 2) interpolating the missing R-waves from the surrounding beats (Berntson et al., 1997). After detrending, power spectral analysis in the frequency spectrum 0.04 to 0.5 Hz was computed using fast Fourier transformation. Total power in the high frequency range (0.15-0.4 Hz) was computed. A log transformation normalized these data. A Shapiro-Wilk test indicated that the log-transformed RSA distribution did not significantly differ from a normal distribution ($W = 0.97$, $p = 0.13$).

Genotyping

Serotonin transporter promoter region polymorphism (5-HTTLPR). The SLC6A4 gene contains an insertion/deletion polymorphism in the 5' regulatory region of the gene (Heils *et al.*, 1996). This polymorphism is associated with differential transcriptional

activity: the long (l) variant has approximately three times the basal activity of the shorter (s) variant (Lesch et al., 1996). We have previously reported the details of the collection method and genotyping assay (Pacheco et al., 2009). Genotype frequencies were SS = 19 (28%), SL = 34 (50%), LL = 15 (22%) and did not differ from Hardy Weinberg Equilibrium (X^2 = .0008, p = 0.98).

For analyses, two groups were formed based on the presence (ss, sl) or absence (ll) of the s 5-HTTLPR variant. A dichotomous grouping was used because serotonin uptake in human lymphoblastoid cells is similar for cells carrying either one or two copies of the 5-HTTLPR short variant (Lesch et al, 1996). Cells homozygous for the 5-HTTLPR long variant have approximately 2-fold higher uptake (Lesch et al., 1996). Nevertheless, we examined whether ss and sl groups differed in RSA since research has documented an allele dose-response relationship for some outcomes (e.g., depression vulnerability) (Caspi, et al., 2003).

Procedure

Upon arrival, participants were oriented to the laboratory and consent was obtained. Participants were seated, fitted with electrodes and instructed to find a comfortable position in a sound attenuated room. They completed self-report measures for 15 min, providing time for the electrode gel to adhere to skin and facilitate conductivity. This also provided a rest period prior to physiological data collection. Participants were then re-evaluated for comfort and remained still in a seated position for 5 min during which heart rate was obtained. The experimenter initiated data collection from an adjacent control room. Following this, participants provided a DNA sample. The Internal Review Board at the University of Texas approved all procedures.

Results

Univariate analyses of variance examined whether genotype groups differed on physiological measures. For RSA, the main effect for 5-HTTLPR group was significant, $F(1, 70) = 3.85$, $p = 0.05$, partial $\eta^2 = 0.05$; s 5-HTTLPR allele carriers had lower resting RSA than long homozygotes. Further, comparisons of the ss and sl 5-HTTLPR groups revealed no significant group differences on RSA, $F(1, 54) = 0.04$, $p = 0.87$, partial $\eta^2 = 0.00$ (ss: mean = -0.18, SD = 0.15; sl: mean = -0. 17, SD = 0.21). These results indicate that s allele carriers have lower RSA than l homozygotes (see Table 1). In contrast, the genotype effect was not significant for heart rate, $F(1, 70) = 2.64$, $p = 0.11$, partial $\eta^2 = 0.04$.[1]

Discussion

The current study examined relations between the 5-HTTLPR polymorphism and resting physiological functioning. Short 5-HTTLPR allele carriers had significantly lower resting RSA than long homozygotes. Consistent with support that RSA has a genetic component (e.g., Wang et al., 2009), this is the first evidence of an association between the 5-HTTLPR and resting RSA in healthy young adults. Although the effect size was relatively modest (genotype explained approximately 5% of the variance in resting RSA), small effects of individual polymorphisms on phenotypes are common and expected (Cardon & Bell, 2001). In fact, most genetic association studies account for only about 1-5% of the variance in a phenotype (Maher, 2008), highlighting the relative importance of our results.

The 5-HTTLPR polymorphism may influence RSA via its impact on PFC function, an important structure in the central autonomic network. Studies document that

PFC function, particularly in the medial region, influences hypothalamic-pituitary-adrenal axis and autonomic nervous system activity (reviewed in Thayer, Hansen, Saus-Rose, & Johnsen, 2009). Interestingly, s 5-HTTLPR allele carriers appear to have increased cortisol response and delayed recovery following stress (Gotlib et al., 2008) and higher waking cortisol (Chen et al., 2009). Resting RSA is inversely associated with cortisol response to stress (Thayer, Hall, Sollers, & Fischer, 2006). Thus, the 5-HTTLPR polymorphism may influence multiple physiological and neural systems critical for regulating responsivity to environmental challenge. Future research might simultaneously measure genetics, physiology, neural function, and stress reactivity so that complex associations among these variables can be directly tested (see Thayer et al., 2009).

The current study has several limitations. First, participants consisted of a relatively small, convenient sample of undergraduates, which may limit generalizability. Further, we only examined resting RSA. Future research should examine the 5-HTTLPR polymorphism and RSA reactivity during an environmental challenge (e.g., Schmidt et al., 2000). Moreover, our assessment procedure may have been improved by using a more rigorous analysis of change in RSA throughout the 5-min baseline (e.g., growth curve modeling of change in resting RSA over time).

Another limitation is that we only examined a single polymorphism. Given our limited sample size we did not genotype individuals for the single nucleotide polymorphism that occurs (A to G) in the l 5-HTTLPR allele (Wendland, Martin, Kruse, Lesch, & Murphy, 2006). Future work with larger sample sizes should consider examining the 5-HTTLPR and other genetic variants so that additive and interactive effects can be explored. Other promising candidates include the choline transporter gene

(Neumann et al., 2005) and the angiotensin converting enzyme gene insertion/deletion polymorphism (Thayer et al., 2003). Finally there is the potential risk of unmeasured third variables driving the associations. Potential third variables include population stratification and the possibility of linkage disequilibrium of the 5-HTTLPR and another functional polymorphism. Future studies might use genomic control or stratified association methods to address the former risk and haplotype mapping to address the latter.

Despite these limitations, findings from this study suggest a modest effect of the 5-HTTLPR polymorphism on resting RSA. Future research should examine whether 5-HTTLPR also predicts RSA reactivity to a variety of environmental contexts, as previous associations have yielded inconsistencies (Schmidt et al., 2000; Murakami et al., 2009). Intriguingly, the 5-HTTLPR may function more like a plasticity gene (rather than a vulnerability gene), such that s 5-HTTLPR allele carriers are both more responsive to negative effects under adverse conditions and positive effects under supportive conditions (Boyce & Ellis, 2005). Thus, the 5-HTTLPR may be associated with greater changes in RSA in negative and positive environmental contexts. Finally, research is needed across levels of analysis (e.g., genetic, physiological, neural, environmental) so a more comprehensive etiological model of adaptive and maladaptive physiological regulation can be achieved.

References

Beauchaine, T. (2001). Vagal tone, development, and Gray's motivational theory: Toward an integrated model of autonomic nervous system functioning in psychopathology. *Development and Psychopathology, (13),* 183-214.

Berntson G. G., Bigger T. J., Eckberg D. L., Grossman P., Kaufmann P. G., Malik M., et al., (1997). Heart rate variability: Origins, methods, and interpretive caveats. *Psychophysiology, 34*, 623–648.

Boyce, W. T., & Ellis, B. J., (2005). Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. *Development and Psychopathology, 17* (2), 271-301.

Cardon, L.R., & Bell, J.I. (2001). Association study designs for complex diseases. *Nature Reviews Genetics, 2,* 91-99.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., McClay, J, Mill, J., Martin, J., Braithwaite, A., Poulton, R. (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science.* 301, 386–389.

Chen, M.C., Joormann, J., Hallmayer, J., Gotlib, I.H., (2009). Serotonin transporter polymorphism predicts waking cortisol in young girls. *Psychoneuroendocrinology.*

De Geus, E. J., Kupper, N., Boomsma, D. I., & Snieder, H. (2007). Bivariate genetic modeling of cardiovascular stress reactivity: Does stress uncover genetic variance? *Psychosomatic Medicine*, 69, 356–364.

Gotlib, I.H., Joormann, J., Minor, K.L., Hallmayer, J., 2008. HPA axis reactivity: A mechanism underlying the associations among 5-HTTLPR, stress, and depression. *Biological Psychiatry. 63***,** 847-851.

Heils, A., Teufel, A., Petri, S., Stober, G., Riederer, P., Bengel, D., Lesch, K.P., 1996. Allelic variation of the human serotonin transporter gene expression. *Journal of Neurochemistry. 66***,** 2621-2624.

Lesch, K.P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., Benjamin, J., Muller, C.R., Hamer, D.H., Murphy, D.L., 1996. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science. 274***,** 1527-31.

Maher, B. (2008). The case of the missing heritability. *Nature, 456* (6),18-21.

Murakami, H., Matsunaga. M., Ohira, H. (2009). Association of serotonin transporter gene polymorphism and emotion regulation. *Neuroreport, 20* (4), 414-418.

Neumann, S.A., Lawrence, E.C., Jennings, J.R., Ferrell, R.E., Manuck, S.B., 2005. Heart rate variability is associated with polymorphic variation in the choline transporter gene. *Psychosomatic Medicine 67,* 168–171.

Pan, J., & Tompkins, W. J. (1985). A Real-Time QRS Detection Algorithm. *Biomedical Engineering*, IEEE Transactions on, 32 (3) 230–236.

Pacheco, J., Beevers, C.G., Benavides, C., McGeary, J., Stice, E., Schnyer, D.M., 2009. Frontal-limbic white matter pathway associations with the serotonin transporter gene promoter region (5-HTTLPR) polymorphism. *Journal of Neuroscience. 29***,** 6229-33.

Pezawas, L., Meyer-Lindenberg, A., Drabant, E.M., Verchinski, B.A., Munoz, K.E., Kolachana, B.S., Egan, M.F., Mattay, V.S., Hariri, A.R., Weinberger, D.R., 2005. 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression. *Nature Neuroscience. 8*, 828-834.

Porges, S.W., 1995. Orienting in a defensive world: mammalian modification of our evolutionary heritage. A polyvagal theory. *Psychophysiology 32*, 301_318.

Schmidt, N. B., Storey, J., Greenberg, B. D., Santiago, H. T., Li, Ql, Murphy, D. L. (2000). Evaluating gene x psychological risk factor effects in the pathogenesis of anxiety: a new model approach. *Journal of Abnormal Psychology, 109* (2), 308-320.

Snieder, H., Boomsma, D. L., Van Doornen, L. J. P., de Geus, E. J. C., (1997). Heritability of respiratory sinus arrhythmia: Dependency on task and respiration rate. *Psychophysiology, 34,* 317-328.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electophysiology. (1996). Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. *European Heart Journal, 17*, 354-381.

Thayer, J. F., Hall, M., Sollers, J. J., Fischer, J. E. (2006). Alcohol use, urinary cortisol, and heart rate variability in apparently healthy men: Evidence for impaired inhibitory control of the HPA axis in heavy drinkers. *International Journal of Psychophysiology, 59* (3), 244-250.

Thayer, J. F., Hansen, A. L., Saus-Rose, E., Johnsen, B. H. (2009). Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. *Ann Behav Med, 37*(2), 141-153.

Thayer, J.F., Lane, R.D., (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *Journal of Affective Disorders. 61***,** 201-216.

Thayer, J. F., & Lane, R. D., (2009). Claude Bernard and the heart-brain connection: Further elaboration of a model of neurovisceral integration. *Neuroscience and Biobehavioral Reviews, 33* 81-88.

Thayer, J.F., Merritt, M.M., Sollers 3rd, J.J., Zonderman, A.B., Evans, M.K., Yie, S., Abernethy, D.R., 2003. Effect of angiotensin-converting enzyme insertion/deletion polymorphism DD genotype on high-frequency heart rate variability in African Americans. *American Journal of Cardiology 92*, 1487–1490.

Wang, X., Ding, X., Su, S., Li, Z., Riese, H., Thayer, J.F., Treiber, F., Snieder, H., (2009). Genetic influences on heart rate variability at rest and during stress. *Psychophysiology. 46***,** 458-465.

Way, B. M., & Taylor, S. E. (2009). The serotonin transporter promoter polymorphism is associated with cortisol response to psychosocial stress. *Biological Psychiatry, PMID: 20006325.*

Wendland, J. R., Martin, B. J., Kruse, M. R., Lesch, K. P., Murphy, D. L. (2006). Simultaneous genotyping of four functional loci of human SCL6A4, with a reappraisal of 5-HTTLPR and rs25531. *Molecular Psychiatry, 11* (3), 224-226.

Williams, R. B. (1994). Neurobiology, cellular and molecular biology, and psychosomatic medicine. *Psychosom Med*, *56*, 308–15.Authors' Note

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Footnote

[1] 5-HTTLPR group effects were not significant for respiration rate, $F(1, 70) = 0.00$, $p = 0.98$, partial $\eta^2 = 0.00$.

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Table1. Means (standard deviations) of resting physiological assessments for 5-HTTLPR allele groups.

	LL	SL	SS	F-Statistic LL vs. □L+SS	F-Statistic SL vs. SS
RSA	-0.07 (0.18)	-0.16 (0.21)	-0.18 (0.15)	F (1, 71) = 3.85*	F (1, 55) = 0.10
Heart Rate (b/min)	68.99 (8.65)	72.39 (9.46)	74.07 (9.59)	F (1, 71) = 2.63	F (1, 55) = 0.03

* indicates $p < .05$