

Ethnicity, gender, genotype, and anger as related to nocturnal dipping

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Abstract

Bishop, Pek, and Ngau (2005) found a significant interaction in Singapore between anger and nocturnal dipping among Indians but not Chinese and Malays. The current study examines the role of 5-HTTLPR genotype in this relationship. Two hundred thirty-one undergraduates participated in up to 4 days of 24-h ambulatory monitoring, completed the State-Trait Anger Expression Inventory, and provided blood samples for genotyping of 5-HTTLPR. Results indicated individuals with two copies of the short allele (SS) showed reduced dipping when they were high in Outward Anger (OA) but increased dipping when they were low in OA. Further, for Indian men only, dipping was reduced for individuals having the SS genotype when they were low on Anger In and increased when they were high on Anger In. These data provide further evidence for the role of 5-HTTLPR in cardiovascular risk as well as ethnic differences in the 5-HTTLPR–phenotype relationship. They also provide further evidence for 5-HTTLPR as a “plasticity gene.”

Descriptors: Nocturnal dipping, 5-HTTLPR, Ethnicity, Anger, Singapore, Plasticity

Nocturnal blood pressure (BP) dipping is a well-established phenomenon related to the diurnal cycle of blood pressure and related functions (O'Brien, Sheridan, & O'Malley, 1988). Research evidence points to marked individual differences in the extent of BP dipping (Staessen et al., 1997), and there is accumulating evidence that these individual differences are predictive of cardiovascular risk. Specifically, individuals showing less than a 10% reduction of systolic blood pressure (SBP) from daytime to nighttime have been found to be at elevated risk for hypertension (Timio et al., 1995), left ventricular hypertrophy (Verdecchia et al., 1995), myocardial infarction, heart failure, stroke, and sudden death (Staessen et al., 1999) as well as overall cardiovascular mortality (Ohkubo et al., 1997; Palatini et al., 1992; Staessen et al., 1999).

There is also evidence for racial differences in nocturnal dipping that parallel differences in cardiovascular risk. Several studies have found that African Americans show a reduction in nocturnal dipping that parallels their higher rates of hypertension (Fumo et al., 1992; Herbert et al., 1996; Ituarte, Kamarck, Thompson, & Bacanu, 1999). Further, there is evidence that psychosocial variables such as anger and hostility play a role in reduced dipping (Thomas, Nelesen, & Dimsdale, 2004) and that

the relationship between anger and dipping may vary by race. Bishop, Pek, and Ngau (2005) found that higher levels of trait anger were associated with reduced blood pressure dipping for Indians in Singapore whereas this was not true for Chinese and Malays. This reduced dipping appeared to be the result of continued vasoconstriction among nondippers during the night. The reduced blood pressure dipping among Indians high in trait anger is particularly interesting in light of the fact that Indians are at significantly higher risk of cardiovascular disease (CVD) as compared with Chinese and Malays in Singapore (Hughes, Lun, & Yeo, 1990). Indeed, high rates of CVD have been found for South Asians relative to other groups in the United Kingdom (Marmot, Adelstein, & Bulusu, 1984), South Africa (Walker, 1980), Trinidad (Miller, Beckles, Alexis, Byam, & Price, 1982), and Canada (Anand et al., 2000).

The present study was undertaken to further examine the interaction of race with dispositional anger and to examine the potential role of the serotonin transporter gene (5-HTTLPR) in this relationship. As such, the first purpose of the current study was to replicate the findings of Bishop et al. (2005) showing reduced SPB dipping for Indians with high trait anger using a larger sample that underwent multiple 24-h periods of BP monitoring. In line with this, we hypothesized that Indians would show reduced BP dipping when they were high in trait anger.

The second purpose of this study was to explore the role of 5-HTTLPR in the relationship of BP dipping with race and trait anger. Although there is now evidence that blood pressure dipping is a heritable trait (Fava et al., 2005), there has been little research on the specific genetic markers involved with what little research that has been done focusing on the angiotensin

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converting enzyme (ACE) gene insertion/deletion polymorphism (Czupryniak et al., 2008; Spiering, Zwaan, Kroon, & de Leeuw, 2005). The findings reported here are from a larger study of the role of serotonergic genes in emotion and cardiovascular reactivity. The serotonin system plays a key role in emotions as well as in emotion-related disorders (Murphy et al., 2008), and there is growing evidence for the role of 5-HTTLPR in cardiovascular responses to stress (Williams et al., 2003, 2008). Further, there is emerging evidence that 5-HTTLPR is associated with sleep quality (Brummett et al., 2007), with racial differences in sleep quality shown to accompany racial differences in nocturnal dipping (Hughes, Kobayashi, & Deichert, 2007). On this basis, there appeared to be reason to investigate the role that 5-HTTLPR might play in nocturnal dipping.

5-HTTLPR is a 44-base pair insertion/deletion polymorphism in the promoter region of the serotonin transporter gene. Two variants of this gene have been identified, the "short" (S) and "long" (L) alleles, with the L allele associated with increased transcriptional efficiency and lower neuroticism in Caucasians (Lesch et al., 1996). With each person having two copies of the gene, this results in three genotype groups (SS, LS, and LL). In this study, we examined differences in nocturnal dipping as a function of genotype group.

Methods

Participants

The total sample consisted of 328 undergraduates (93 Indians, 154 Chinese, 81 Malays; 49.7% female) at the National University of Singapore. Of these, 74 did not have genotyping data and 5 lacked valid State-Trait Anger Expression Inventory (STAXI) scores. To ensure high quality data, to be included in the analyses for any 24-h period, participants were required to have gone to bed between 10 p.m. and 6 a.m., slept at least 4 hours, and have at least six valid daytime blood pressures and three valid night time readings. Also because daytime posture was used as a covariate, participants were required to have completed at least six diaries during waking hours. An additional 18 participants who did not meet these criteria for at least one monitoring period were eliminated from the analyses, resulting in a final sample size of 231 (62 Indians, 116 Chinese, and 53 Malays; 51.9% female). Age ranged from 18 to 27 years ($M = 21.1$).

Genotyping

Genotyping was done from blood samples using procedures described by Lesch et al. (1996). Following DNA extraction, the 5-HTT-linked polymorphism was amplified from genomic DNA as a 484/528bp polymerase chain reaction (PCR) product. PCR amplification was carried out with primers 5'-GGCGTTG CCGCTCTGAATTGC-3' (forward) and 5'-GAGGGACTGA GCTGGACAACCCAC-3' (reverse).

In the total sample, 36 (13.9%) participants were LL, 115 (44.4%) were LS, and 108 (41.7%) were SS. For the final sample, the distribution was 31 (13.6%) LL, 102 (44.2%) LS, and 98 (42.4%) SS. Analysis of these distributions showed that the allele frequencies did not significantly depart from Hardy-Weinberg equilibrium and were equivalent between ethnic groups. Given the rarity of the LL genotype and that fact that preliminary analyses suggested no readily discernable allele dominance pattern, individuals with the LL genotype were not included in analyses.

Measurement of Anger

The STAXI (Spielberger, 1988; Spielberger et al., 1985) was used to measure dispositional anger. The STAXI is a 44-item inventory with five subscales: State Anger, Trait Anger (TA), Anger In (AI), Anger Out (AO), and Anger Control (AC). As we were interested in dispositional anger, we used only the TA, AI, AO, and AC subscales. An initial principal components analysis of these scales indicated two components accounting for 79.5% of the variance. Varimax rotation of these components showed high loadings on the first component for TA (.78), AO (.84), and AC (-.72). These three subscales were combined into an index labeled Outward Anger (OA) by first taking the z scores for each component and then averaging them after reversing the z scores for AC. The second component consisted of only AI (.96) and was analyzed separately.

Sleep Questionnaire

To obtain information on sleeping times and quality of sleep upon returning to the laboratory after each 24-h monitoring period, participants were asked to complete a questionnaire that asked what time they went to bed the night before, the time they got up that morning, whether they had trouble falling asleep, if they indicated trouble falling asleep how long it took them to fall asleep, how many times they woke up in the night, when they woke up did they get out of bed, when they woke up during the night did they have difficulty going back to sleep, and whether they felt rested on waking in the morning. The question on difficulty falling asleep was combined with the time taken to get to sleep and coded as 0 = *No or < 15 min to get to sleep*, 1 = *Yes, 15–30 mins*, 3 = *Yes, 31–60 min*, 4 = *Yes, > 60 min*. Waking up in the night was coded as 0 = *No*, 1 = *1–2 times*, 2 = *3–4 times*, and 3 = *> 4 times*. Getting out of bed was coded as 0 = *Did not wake up during the night*, 1 = *Woke up but did not get out of bed*, 2 = *Got out of bed 1–2 times*, 3 = *Got out of bed > 2 times*. Difficulty getting back to sleep was coded as 0 = *Did not wake up*, 1 = *Woke up but no difficulty going back to sleep*, 2 = *Difficulty getting back to sleep*. Finally the question on feeling rested on awaking was coded as 0 = *No*, 1 = *Yes*.

Blood Pressure Measurement

Participants underwent 24-h BP monitoring for up to 4 days using Spacelabs 90217 ambulatory blood pressure monitors (Spacelabs Medical, Redmond, WA). The Spacelabs 90217 is a lightweight ambulatory blood pressure monitor using the oscillometric method for BP determination. SBP and DBP were obtained every 30 min. When the monitor was unable to obtain a valid reading, a second attempt was made 2 min later. Participants were told to avoid movement if possible during the time the blood pressure cuff was inflated so as to reduce movement artifact. Participants also wore an AIM-8 ambulatory impedance monitor (Bio-Impedance Technology, Chapel Hill, NC) in this study. However, because of technical problems with the AIM-8 and data produced by it, data from the AIM-8 are not reported here.

Procedures

Study procedures were approved by the National University of Singapore Institutional Review Board. Participants reported to a psychophysiology laboratory, where they were briefed on the procedures for the ambulatory monitoring. After the procedures of the study were explained, participants signed an informed consent form. Participants were then instrumented with the am-

bulatory monitors and given instruction on the use of the Palm Zire palmtop computer for filling out a computerized diary that they were to complete after each waking BP measurement.

As part of their orientation to the ambulatory monitoring, participants were instructed that when the blood pressure cuff began to inflate they should try to move as little as possible until the blood pressure cuff was completely deflated. They were also instructed to begin filling out the questionnaire on the palmtop computer only once the blood pressure cuff had completely deflated. This was done to reduce arm movements that might interfere with the blood pressure readings. Participants were instructed not to get the equipment wet and were provided with written instructions to remind them of various aspects of the use of the palmtop computer along with the researcher's contact number should there be any problems. Finally, the participant was given an appointment for returning approximately 24 h later.

To encourage filling out the diaries, participants were paid on a graduated scale according to their cooperation in completing the diaries. For wearing the monitors and completing up to 50% of the diaries they were paid S\$10 (US\$6.06) for each day of monitoring. They were then paid S\$2.00 (US\$1.21) for each percentage above 50% of diaries completed across all days up to a maximum payment of S\$140 (US\$84.80). In order to be counted, each diary was required to be started within 5 min of the time recorded for the BP reading. In cases where the first BP reading was inconclusive the 5 min was counted from the time of the second reading, which was automatically initiated 2 min following an inconclusive reading.

Data Screening and Reduction

The Spacelabs 90217 automatically checks readings for possible artifacts and eliminates those determined to be erroneous. To further test for possible artifacts, the criteria proposed by Marler, Jacob, Lehoczy, and Shapiro (1988) were used to eliminate likely artifactual blood pressure readings. Both SBP and DBP values were excluded from analyses if SBP > 250 mmHg or < 70 mmHg, DBP > 150 mmHg or < 45 mmHg, or SBP/DBP > 3 or < [1.065 + (.00125 × DBP)].

Waking and sleeping hours were determined by asking each participant upon returning the monitor what time she or he had gone to bed the night before and gotten up that morning. To verify sleep times, all diary entries were checked to be sure that they did not occur during a time the participant claimed to be sleeping. In cases where diaries were completed after the participant indicated she or he had gone to sleep or before the time given for rising, sleeping times were adjusted to exclude those times.

Altogether, attempts were made to obtain blood pressure measurements for a total of 37,383 time periods, with valid blood pressure readings obtained for 32,965 periods (88.2%). The number of valid blood pressure readings during the day for each participant ranged from 0 to 49, with an average of 30.4. For nighttime readings, the range was 0 to 23, with an average of 9.4. Based on the criteria stated above for amount of sleep, sleep times, and number of valid BP measurements, usable data were obtained for 549 monitoring days from 231 participants for an average of 2.4 monitoring days per participant.

Daytime averages for each day for each participant were then obtained by taking the average of readings from the beginning of the monitoring period until the time the participant retired for the night and then those taken from the time the participant got up the next morning until the end of the monitoring period. Sleeping

averages for each monitoring day were obtained by taking the average of all readings taken from the time the participant went to bed until getting up the next morning. Percentage of dipping was then computed by subtracting the sleeping average from the daytime average, dividing by the daytime average, and then multiplying by 100. For each participant, the mean of the averages for all days was then obtained. Participants who showed a drop in SBP of 10% or more were defined as dippers, whereas those exhibiting a drop of less than 10% were categorized as nondippers. By this definition, 153 (66.2%) participants were classified as dippers and 78 (33.8%) were classified as nondippers. This criterion is the one most commonly used in the research literature on nocturnal dipping, as individuals showing less than 10% reduction in nocturnal blood pressure have been shown to be at increased cardiovascular risk (O'Brien et al., 1988; Rääkkönen et al., 2004; Verdecchia et al., 1995).

Statistical Analysis

Comparison of dippers and nondippers was accomplished through the use of χ^2 analysis for categorical dependent variables and *t* tests for continuous dependent variables with the exception of cardiovascular parameters. For daytime cardiovascular parameters, an analysis of covariance (ANCOVA) was performed using body mass index (BMI) and percentage of time lying down as covariates. For nighttime cardiovascular parameters, an ANCOVA was performed using BMI and daytime values as covariates.

The main analyses reported below used percentage of day to night systolic and diastolic dipping (as computed above) as the dependent variables. These data were analyzed using a 3 (Ethnicity) × 2 (Gender) × 2 (Genotype) × Anger factorial ANCOVA model with AI and OA entered as continuous variables in separate analyses. To ensure that results obtained were not biased by differences in demographics, anthropometric variables, daytime activities, or stress, each of these variables was individually tested for their relationship to percentage of systolic dipping in preliminary analyses. Only BMI and percentage of time lying down showed significant relationships with percentage of dipping and were thus used as covariates in the main analyses. In addition, analyses of dipping and nighttime values included daytime values as a covariate, as daytime values showed significant relationships with both dipping and nighttime values. Significant interactions were followed up using simple effects analysis.

Results

Comparison of Dippers and Nondippers

Table 1 shows comparisons between dippers and nondippers. As can be seen from this table, nondippers were more likely to be female (64.1%) than was the case for dippers (45.8%), $\chi^2(1, N = 231) = 6.97, p = .008$. Also, dippers spent less time lying down ($M = 9.91\%$ vs. 12.66%), $t(229) = -2.07, p = .039, \eta_p^2 = .018$, and had higher scores on AC (23.3) than did nondippers (22.0), $t(229) = -2.06, p = .034, \eta_p^2 = .018$. As expected, dippers and nondippers differed significantly on cardiovascular parameters. Waking values for dippers were significantly higher than those for nondippers for heart rate (78.52 vs. 76.03), $F(1,227) = 4.13, p = .043, \eta_p^2 = .018$, SBP (115.62 vs. 112.50), $F(1,227) = 7.60, p = .006, \eta_p^2 = .032$, DBP (72.01 vs. 70.15), $F(1,227) = 7.62, p = .006, \eta_p^2 = .032$, and mean arterial pressure (MAP; 86.54 vs. 84.27), $F(1,227) = 8.98, p = .003, \eta_p^2 = .038$. By

Table 1. Comparison of Dippers and Nondippers

Variable	Dippers (<i>n</i> = 153) (≥ 10% drop in SBP)	Nondippers (<i>n</i> = 78) (<10% drop in SBP)	<i>p</i>
Demographics			
% Indian	30.1	20.5	n.s.
% Chinese	47.7	55.1	
% Malay	22.2	24.4	
% Female	45.8	64.1	.008
Age (years)	21.2 ± 0.15	21.0 ± 0.19	n.s.
% with family history of CHD or hypertension	48.7%	51.6%	n.s.
Genotype			
% LL	14.4	11.5	n.s.
% LS	43.1	46.2	
% SS	42.5	42.3	
Anthropometric			
Height (m)	1.68 ± 0.01	1.66 ± 0.01	.065
Weight (kg)	59.76 ± 0.91	57.82 ± 1.28	n.s.
Body mass index	21.09 ± 0.27	20.87 ± 0.37	n.s.
Ambulatory cardiovascular values			
Awake heart rate (bpm) ^a	78.52 ± 0.71	76.03 ± 1.00	.043
Awake systolic blood pressure (mmHg) ^a	115.62 ± 0.65	112.50 ± 0.92	.006
Awake diastolic blood pressure (mmHg) ^a	72.01 ± 0.39	70.15 ± 0.55	.006
Awake mean arterial pressure (mmHg) ^a	86.54 ± 0.44	84.27 ± 0.62	.003
Sleep heart rate (bpm) ^b	58.91 ± 0.36	59.74 ± 0.51	n.s.
Sleep systolic blood pressure (mmHg) ^b	98.50 ± 0.27	106.52 ± 0.37	<.001
Sleep diastolic blood pressure (mmHg) ^b	57.68 ± 0.25	62.14 ± 0.35	<.001
Sleep mean arterial pressure (mmHg) ^b	71.31 ± 0.22	76.90 ± 0.31	<.001
Waking posture			
Standing (%)	22.98 ± 0.83	20.99 ± 1.14	n.s.
Sitting (%)	67.11 ± 0.92	66.36 ± 1.45	n.s.
Lying down (%)	9.91 ± 0.70	12.66 ± 1.25	.058
Average daytime activity (1–4)	1.56 ± 0.02	1.49 ± 0.03	.058
Time slept (min)	364.22 ± 4.89	358.28 ± 6.87	n.s.
Self-rated stress (1–4)	1.73 ± 0.05	1.67 ± 0.07	n.s.
STAXI			
Trait Anger	19.96 ± 0.36	20.68 ± 0.56	n.s.
Anger In	18.07 ± 0.33	18.20 ± 0.43	n.s.
Anger Out	15.24 ± 0.27	15.21 ± 0.42	n.s.
Anger Control	23.40 ± 0.38	21.99 ± 0.54	.034
Outward Anger	−0.07 ± 0.07	0.07 ± 0.09	n.s.

Note: Numbers in table are means and standard errors for all variables except ethnicity and sex, which are percentages.

^aValues adjusted for body mass index and percentage of time lying down.

^bValues adjust for body mass index and daytime values.

contrast, sleep values for dippers were lower than those for nondippers for SBP (98.50 vs. 106.52), $F(1,227) = 301.56$, $p < .001$, $\eta_p^2 = .571$, DBP (57.68 vs. 61.14), $F(1,227) = 108.65$, $p < .001$, $\eta_p^2 = .324$, and MAP (71.31 vs. 76.90), $F(1,227) = 214.44$, $p < .001$, $\eta_p^2 = .486$.

Main Analyses

On the basis of findings by Bishop et al. (2005), we hypothesized a significant interaction between Ethnicity and OA. However, this hypothesized interaction was not obtained for either systolic dipping, $F(2,173) = 0.33$, $p = .7184$, or diastolic dipping, $F(2,173) = 1.65$, $p = .1946$.

As expected, 5-HTTLPR showed a significant relationship to nocturnal dipping. However, this relationship differed by Ethnicity, Gender, and Anger. Significant Ethnicity × Gender × Genotype × AI interactions were obtained for both systolic, $F(2,173) = 3.13$, $p = .0460$, $\eta_p^2 = .035$, and diastolic, $F(2,173) = 4.78$, $p = .0096$, $\eta_p^2 = .052$, dipping. For systolic dipping, simple interaction effects analysis showed that the only Ethnicity × Gender group that showed a significant Genotype × AI interaction was Indian men, $F(1,21) = 23.39$, $p < .0001$, $\eta_p^2 = 0.527$, with all other groups showing nonsignificant inter-

actions, all $ps > .15$. The pattern of this interaction, shown in Figure 1, indicates that AI shows a positive relationship with systolic dipping for those with the SS genotype, $\beta = 0.91$, $F(1,9) = 44.59$, $p < .0001$, $\eta_p^2 = 0.832$, but has nonsignificant negative relationship for those with the LS genotype, $\beta = -0.38$, $F(1,9) = 2.54$, $p = .1454$, $\eta_p^2 = 0.220$. Examination of daytime and nighttime SBP indicated a significant four-way interaction for nighttime SBP, $F(2,174) = 3.40$, $p = .0357$, $\eta_p^2 = 0.038$. Simple effects analysis showed that the reduced dipping by SS Indian men who are low on AI was due to higher nighttime SBP for these individuals, $\beta = -0.57$, $F(1,10) = 17.53$, $p = .0019$, $\eta_p^2 = 0.637$ (see Figure 2). The parallel four-way interaction for daytime SBP was not statistically significant, $F(2,174) = 0.59$, $p = .5545$.

The pattern for diastolic dipping was slightly different (see Figure 3). As with systolic dipping, the interaction of AI and genotype was significant only for Indian men, $F(1,21) = 8.87$, $p = .0072$, $\eta_p^2 = .297$. For other groups, this relationship was nonsignificant, all $ps > .31$. Further, as with systolic dipping, the relationship between AI and dipping was positive for Indian men with the SS genotype, $\beta = 0.49$, $F(1,9) = 7.45$, $p = .0232$, $\eta_p^2 = .453$. However, different from the results for systolic dip-

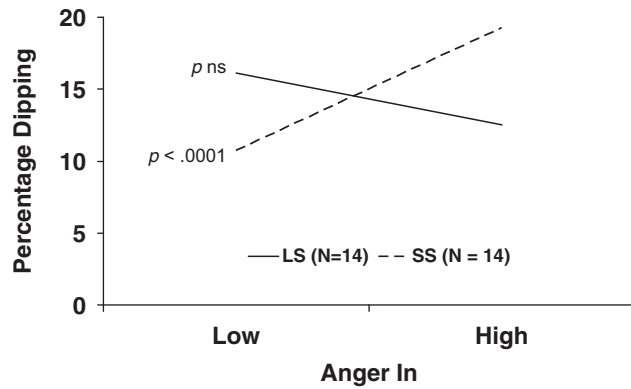


Figure 1. Genotype \times Anger In interaction for systolic dipping, Indian men only. Percentage dipping refers to the percentage drop in blood pressure from day to night.

ping, the relationship between AI and dipping was significantly negative for those with the LS genotype, $\beta = -0.54$, $F(1,9) = 6.53$, $p = .0310$, $\eta_p^2 = .420$. Examination of daytime and nighttime DBP levels indicated that, as with systolic dipping, the differences in dipping were because of higher nighttime BP on the part of those showing reduced dipping.

In addition to these findings for AI, there is evidence that the relationship of OA with systolic dipping is moderated by genotype, as seen in the significant Genotype \times OA interaction, $F(1,173) = 5.72$, $p = .0179$, $\eta_p^2 = 0.032$. The pattern of this interaction is shown in Figure 4. Simple effects analysis indicated that whereas the relationship between OA and SPB dipping was significantly negative for those with the SS genotype, $\beta = -0.23$, $F(1,83) = 6.79$, $p = .0109$, $\eta_p^2 = 0.076$, it was not significant for those with the LS genotype, $\beta = 0.10$, $F(1,87) = 0.90$, $p = .3466$, $\eta_p^2 = 0.010$. Parallel to the Ethnicity \times Gender \times Genotype \times AI interaction, this was because of a positive relationship between OA and nighttime SBP for those with the SS genotype, $\beta = 0.25$, $F(1,83) = 6.60$, $p = .0120$, $\eta_p^2 = 0.074$.

To assess the possibility that the differences in dipping patterns, as well as the related differences for nighttime BP, might be related to participants' quality of sleep, we analyzed responses to the questions on the sleep questionnaires completed by participants when they returned the monitoring equipment. Specifically, we examined participants' responses to the questions of

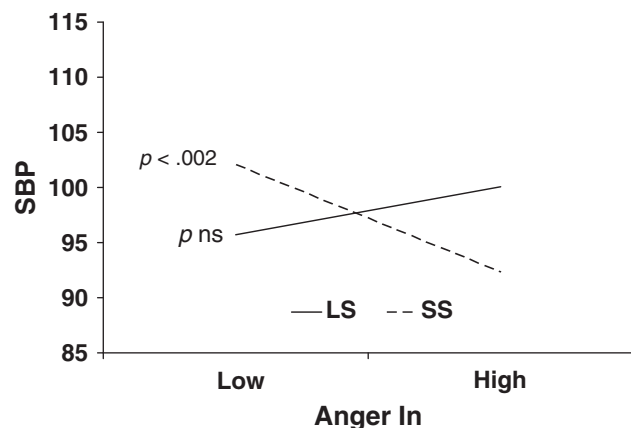


Figure 2. Genotype \times Anger In interaction for nighttime SBP, Indian men only.

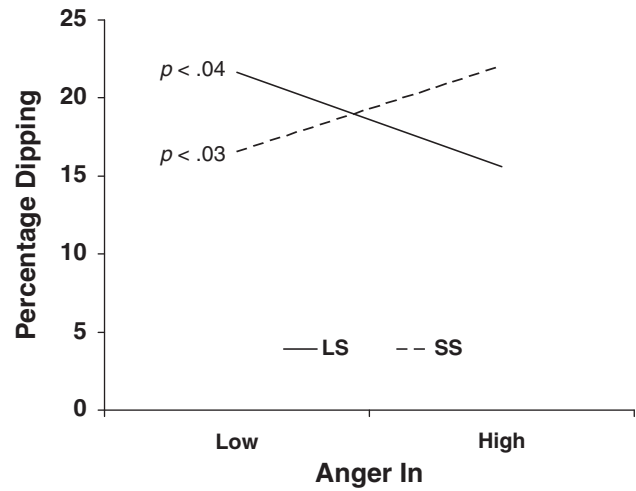


Figure 3. Genotype \times Anger In interaction for diastolic dipping, Indian men only. Percentage dipping refers to the percentage drop in blood pressure from day to night.

whether they had difficulty falling asleep, whether they woke up during the night, and, if they woke up, did they get out of bed, whether they had difficulty going back to sleep, and whether they awoke feeling rested. We also looked at their reported sleeping time adjusted for the presence of diaries filled out during the period they indicated they were sleeping. In only a few cases did the effects found to be significant for nocturnal dipping come close to being significant for these sleep variables, and in no case did the shape of the interaction obtained match that found for dipping. As such, there is no evidence that the effects for dipping and nighttime BP in this study were the result of differences in sleep quality.

Discussion

To summarize, the key findings of this study indicated significant interactions between Ethnicity, Genotype, Gender, and AI for both systolic and diastolic dipping, in which the significant effects were found only among Indian men. Specifically, Ethnicity \times

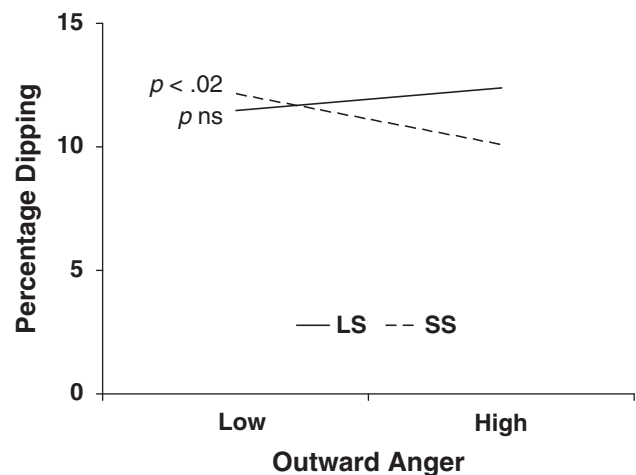


Figure 4. Genotype \times Outward Anger interaction for systolic dipping. Percentage dipping refers to the percentage drop in blood pressure from day to night.

Genotype \times Gender \times AI interactions were obtained in which Indian men with the SS genotype showed reduced dipping when they were low in AI but greater dipping when they were high in AI, an effect that was not found in any other group. In addition an interaction was obtained between Genotype and OA for systolic dipping, in which individuals with the SS genotype showed reduced dipping when they were high on OA. In all cases, these effects appear to be the result of BP remaining high at night for the groups with reduced dipping as compared to those showing more dipping. Analyses of quality of sleep found no evidence that the reduced dipping or related higher nighttime BP was a function of poorer sleep quality. Finally, there was no evidence that reduced dipping was related to daytime BP.

The results of this study do not replicate the findings of Bishop et al. (2005) showing that Indians high in trait anger (TA) showed reduced SBP nocturnal dipping. It is not clear why the results of Bishop et al. were not replicated. This is something that needs to be examined in future research. One possibility is that the differences between the results of Bishop et al. and the findings here come from differences in the methodology in that in Bishop et al. dipping was measured for only one 24-h period whereas in the present study participants were monitored for up to 4 days. The greater stability obtained from multiday monitoring would argue that the results from Bishop et al. may have been a chance finding and hence can be discounted.

Although the Bishop et al. (2005) finding was not replicated, a conceptually related finding was obtained. Specifically, a different pattern of dipping was obtained for Indians than for Chinese and Malays, one that fits with the higher CHD rates found among Indians. Specifically, the Ethnic \times Gender \times Genotype \times AI interactions showed that the relationship between anger and dipping depended on ethnicity, gender, and genotype, with the relationship between AI and dipping found only for Indians men with the SS genotype. This finding needs to be replicated, but it provides the first evidence we know of linking 5-HTTLPR to nocturnal dipping and to ethnic differences in CVD risk.

At this point, it is not known whether Indian men with CHD are more likely than other groups to have low Anger In combined with the SS genotype. Follow-up analyses of the participants in the present study, however, are consistent with this possibility in showing that Indian men with the SS genotype did tend to have low AI scores ($M = 16.1$) relative to the mean across all participants (18.12). This is consistent with higher CHD risk for this group, because the lowest dipping and hence higher CHD risk was found with low AI scores. Future research should examine whether this pattern is found in relationship to diagnosed CHD.

Another question that needs to be addressed in future research is why higher levels of AI are protective for Indian men with the SS genotype. One possibility is that, because AI can be interpreted as representing a suppression of anger, individuals with low AI may have more of a tendency to express angry feelings, with this expression of angry feelings then being associated with greater CHD risk (cf. Siegman, 1994). This interpretation suggests that Indian men with the SS genotype would tend to be more expressive of their anger, which would then lead to greater CHD risk.

In addition, a Genotype \times OA interaction was obtained that showed that systolic dipping was reduced for individuals high in OA but only when they had the SS genotype. As this effect was not qualified by any higher order interaction, this is an effect that applies equally to the three ethnic groups and also

equally to men and women. Because OA reflects the extent to which a person experiences and expresses anger outwardly, this is a further suggestion that it is the tendency to express angry feelings that increases CHD risk.

When interpreting these interactions between genotype and anger, it is important to note that, for both the Genotype \times AI interaction for Indian men and the Genotype \times OA interaction, it is principally individuals with the SS genotype who show a relationship between anger and dipping. The exception to this is that for diastolic dipping Indian men with the LS genotype showed a negative relationship between AI and dipping. For all results involving systolic dipping, however, no relationship was obtained between anger and dipping for those with the LS genotype. In addition, individuals with the SS genotype show extremes of dipping in that Indian men who are high on AI show the greatest percentage of dipping in that ethnic-gender group whereas those low on AI show the least dipping. This fits a pattern recently identified by Belsky et al. (2009) as representing “for-better-or-for-worse” or plasticity. In other words, individuals with the SS genotype do not just show greater CVD risk (in this case reduced nocturnal dipping) when they are high in OA or, if Indian and male, low in AI, but also show reduced risk when they are at the other end of the anger scale. This suggests that for individuals with the SS genotype intervention for helping them deal better with anger may go beyond simply reducing risk to conferring greater resilience than would be the case for individuals with the LS genotype.

The fact that relationships between anger and dipping were only found for individuals with the SS genotype fits with other evidence related to 5-HTTLPR. Characteristically, it is individuals with the S allele that show enhanced vulnerability or, in many cases, plasticity in their responses to the environment (Belsky et al., 2009). This has been found to be the case for depression (Brummett et al., 2008; Caspi et al., 2003), anxiety (Gunthert et al., 2007), and ADHD (Retz et al., 2008). The results of the present study add additional evidence for considering the low activity allele of 5-HTTLPR to be a plasticity gene, that is, a gene that renders the person more responsive to environmental influences whether for better or for worse.

At this point in time, the pathway connecting the SS genotype to variations in nocturnal dipping is not clear. One possibility is that the differences in nocturnal dipping may be because of differences occurring during sleep. Differences in dipping were not associated with daytime SBP values but appeared to be a direct reflection of nighttime SBP, with individuals low in dipping showing sustained nighttime SBP. Although we found no evidence that differences in sleep quality accounted for the higher levels of nighttime SBP for those with reduced dipping, it is quite possible that the ad hoc sleep questionnaire used was not sensitive enough to detect relevant differences. It is instructive to note that Brummett et al. (2007) found sleep quality to reflect an interaction between 5-HTTLPR genotype and stress, with individuals homozygous for the S allele showing reduced sleep quality when experiencing the stress of being a caregiver for a spouse or parent with dementia as compared to controls or individuals with at least one L allele. The differences observed by Brummett et al. (2007) could easily be reflected in higher SBP at night. This is a possibility that needs to be explored in future research.

The findings of this study need to be interpreted in light of its limitations. First the participants in this study were all young adults within a limited age range. As such, it is important to replicate these results in an older sample with a broader age

range. Second, all of the participants were Asian and living in Singapore. It is thus possible that these findings apply only to Asians. Future studies should replicate these findings in other ethnic groups so as to test for their generalizability. Third, the small number of individuals with the LL genotype and the dissimilarity of this group with the LS group during preliminary analyses made it impossible to include them in the reported analyses, as this would have implied an L-dominant model for which there is no evidence. As such, these analyses are of necessity incomplete, and there is no evidence for the LL genotype concerning the relationship of anger to dipping. Given the relative rarity of the L allele in Asian populations, future research with Asians will require substantially larger samples in order to

include individuals with the LL genotype in analyses. Finally, the use of an ad hoc sleep questionnaire reduces our confidence in the null findings obtained from this instrument and argues for the use of a more sensitive and preferably well-validated instrument for measuring sleep variables in future research.

In conclusion, this study obtained evidence for interactions between anger and 5-HTTLPR genotype such that, across ethnicity and gender, individuals with the SS genotype who were high in OA showed reduced systolic dipping. Further Indian men with the SS genotype showed reduced dipping when they were low in AI but greater dipping when they were high in AI. These findings fit the interpretation of the low activity allele of the serotonin transporter gene as being a plasticity gene.

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