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► **To cite this version:**

C. Rob Markus, Rudi de Raedt. Differential effects of 5-HTTLPR genotypes on inhibition of negative emotional information following acute stress exposure and tryptophan challenge.. *Neuropsychopharmacology*, 2010, 10.1038/npp.2010.221 . hal-00598943

HAL Id: hal-00598943

<https://hal.science/hal-00598943>

Submitted on 8 Jun 2011

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Differential effects of 5-HTTLPR genotypes on inhibition of negative emotional information following acute stress exposure and tryptophan challenge.

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Abstract: 208 words

Main text: 3000 words

Figures: 4

Tables: 0

Supplementary material: zero

KEY WORDS: serotonin; emotion processing, 5-HTTLPR; stress; tryptophan

Running Title: 5-HTTLPR, stress and attention bias

ABSTRACT

Previous data suggest that a polymorphism at the serotonin (5-HT) transporter gene (5-HTTLPR) may influence stress resilience and stress-related depression symptoms due to interactions between brain 5-HT dysfunction and stress exposure. Although attentional bias for emotional information has been reliably observed in depression, the interaction between 5-HTTLPR, brain 5-HT vulnerability and acute stress on affective information processing has not yet been investigated. This study examines the effects of tryptophan augmentation (indicating 5-HT manipulation) on inhibition of negative emotional information under stress in mainly female S'/S' versus L'/L' allele carriers.

Fifteen female homozygotic short-allele 5-HTTLPR (S'/S'= S/L_G, L_G/L_G) and 13 female homozygotic long-allele 5-HTTLPR (L'/L'= L_A/L_A) subjects were tested for mood and inhibition of emotional information in a double-blind placebo-controlled design before and after stress exposure following tryptophan manipulation.

Stress exposure significantly impaired inhibition of negative affective information only in S'/S' carriers, whereas L'/L' carriers even showed increased inhibition of negative information.

The S'/S' allele 5-HTTLPR genotype increases cognitive-attentional bias for negative emotional information under acute stress. Since this bias is an important component of depression, this may be a mediating mechanism making S'/S'-allele carriers more vulnerability for stress-induced depression symptoms. Moreover, current data suggest that L'/L'-allele genotypes are more resilient, even increasing cognitive emotional (inhibitory) control after stress.

INTRODUCTION

The identification of biological vulnerability factors for depression is an important challenge facing biological psychiatry today. Among the various biochemical processes involved, brain serotonin (5-HT) dysfunction appears to be a meaningful risk factor (Maes and Meltzer, 1995; Van Praag, 2004). 5-HT dysfunction manifests in depressed patients as lowered brain tryptophan concentrations (e.g. Agren and Reibring, 1994; Maes *et al*, 1990); impaired 5-HT synthesis, release, reuptake, or metabolism (e.g. Maes and Meltzer, 1995; Malison *et al*, 1998; van Praag *et al*, 1970); or 5-HT receptor disturbances (e.g. Cowen *et al*, 1994; Sargent *et al*, 2000), whereas antidepressant drugs generally act by improving brain 5-HT function (Delgado *et al*, 1990; Delgado *et al*, 1993; Maes and Meltzer, 1995).

5-HT dysfunction in depression is promoted by a genetic vulnerability involving a polymorphism in the 5-HT transporter-linked promoter region (5-HTTLPR). This region encodes the 5-HT transporter protein (5-HTT) that controls 5-HT reuptake and function and which is the main target mechanism for antidepressant drugs (Serretti *et al*, 2007). The short-allele (S) variant of 5-HTTLPR is associated with fewer 5-HTT binding sites compared to the long-allele (L) variant (Heils *et al*, 1996; Mann *et al*, 2000) and is involved in 5-HT dysfunction during depression (Jans *et al*, 2007). Recently, the presence of an A>G single nucleotide polymorphism has been identified in the L allele, rendering an Lg variant (as opposed to La) that is functionally equivalent to the S-allele (Hu *et al*, 2005; Wendland *et al*, 2006). However, 5-HT dysfunction in depression is also promoted by stress due to interactions between 5-HT and the hypothalamus-pituitary-adrenal (HPA) system (Firk and Markus, 2007; Van Praag, 2004). Hence, depression is often preceded by stress (Van Praag, 2004), and there is a positive association between the number of copies of the S-allele 5-HTTLPR and self-reports of past depressive responses to stressful events

(Caspi et al, 2003; Jacobs et al, 2006; Wilhelm *et al*, 2006). However, attempts to replicate these findings have yielded mixed results (Risch *et al*, 2009).

Despite the suggestion from previous studies that stressful events might promote depression symptoms mostly in homozygous S-allele compared to homozygous L-allele 5-HTTLPR carriers, actual interactions between genetic 5-HT vulnerability, stress exposure and depression have barely been investigated. A recent experimental study reveals evidence for enhanced HPA stress-responses exclusively in homozygous S-allele 5-HTTLPR carriers (Gotlib *et al*, 2008), but this experiment did not include the measurement of depression-specific cognitive-affective changes or of brain 5-HT vulnerability.

Depression specific changes can be adequately measured by negative affective priming (NAP) tasks that measure attentional bias for emotional information (Goeleven *et al*, 2006; Joormann, 2004). Hence, attentional bias for negative information is a hallmark feature of MDD (see (Scher C, 2005) which relates to impaired cognitive inhibition towards negative emotional material (Goeleven *et al*, 2006); (Gotlib, 2005); (De Raedt, 2010). In support, 5-HT is implicated in decreased attentional control over negative stimuli by modulating prefrontal-amygdala connectivity (see (Cools, 2007), and S/S show enhanced emotional amygdala responses {Hariri, 2006} and impaired disengagement to emotional information (Beavers, 2009).

Brain 5-HT vulnerability relates to an enhanced sensitivity to alterations in the 5-HT system (Jans *et al*, 2007). This can be measured by acute tryptophan depletion (Biggio *et al*, 1974; Carpenter *et al*, 1998; Nishizawa *et al*, 1997; Williams *et al*, 1999) that has been found to increase depression symptoms in individuals at risk for depression (Ruhe *et al*, 2007) including homozygous S-allele 5-HTTLPR carriers (Neumeister *et al*, 2002); (Neumeister *et al*, 2006). However, because S/S-allele

carriers may exhibit altered 5-HT sensitization to compensate lower 5-HTT expression (David *et al*, 2005); (Moore P, 2000), tryptophan challenge may be an alternative method to reveal 5-HT vulnerability associated with 5-HTTLPR. In support, tryptophan administration improved mood in S/S not L/L-allele carriers (Markus, 2009).

The current study is the first to investigate the interaction between acute stress, 5-HTTLPR and 5-HT vulnerability on the inhibition of negative emotional information. Based on previous assumptions, it is hypothesized that stress reduces the inhibition of negative information in S/S not L/L-allele carriers and that TRP administration reduces this effect only in S/S-allele carriers.

METHODS

Participants

Students from Maastricht University completed a questionnaire package concerning general information (health, personal or family history of medical or psychiatric complaints, smoking and drinking habits, caffeine consumption, weight and height, use of psychoactive drugs) and concerning relevant symptoms and psychopathology (Beck Depression Inventory [BDI], Inventory of College Students' recent life experiences [ICSRLE], Inadequacy/neuroticism Scale of the Dutch Personality Inventory [IN] and the Social Complaints List [SCL]). Exclusion criteria were health or medical complaints; personal or family history of psychiatric illness; history of medical illness; medication use; metabolic-, hormonal- or intestinal diseases; irregular diet; deviant eating habits or excessive alcohol or drug use. Following this first selection, 90 participants attended a buccal sample extraction session to genotype for 5-HTTLPR (resulting in 26% S/S, 46% S/L and 28% L/L). Since brain 5-HT vulnerabilities for stress are mainly found in homozygotes S compared to homozygotes L allele carriers

(e.g. (Neumeister *et al*, 2002); (Caspi *et al*, 2003); (Wilhelm *et al*, 2006); (Zalsman *et al*, 2006), only homozygous S-allele (S/S, S/L_G, L_G/L_G; classified as S'/S') and homozygous L-allele (L_A/L_A; classified as L'/L') were then invited for a psychiatric structured interview (MINI: (Sheehan *et al*, 1994) to double-check exclusion compliance and to receive information about the experiment. After invitation, before the psychiatric interview, a number of 8 S'/S' and 12 L'/L' subjects refrained from further participation due to shortage of time or other non-specified reasons.

Fifteen S'/S' and 13 L'/L' female participants completed the experiment. Both groups included Caucasian participants with European (mainly Dutch) background. They all revealed normal body-mass indexes (BMI in kg/m² between 20-25; mean 22±2), were non-smokers and were requested not to use alcohol or any kind of drugs 24 hours prior to and during the study. Both groups did not differ in age, BMI and additional relevant symptoms. The study was approved by the Medical Ethics Committee of the Academic Hospital Maastricht (CTCM azM; Maastricht; The Netherlands) and the procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983. All subjects gave their written informed consent and were paid for participation.

Design and procedure

A placebo-controlled, double-blind, crossover design was used. During two sessions, subjects were monitored for negative affective priming (NAP) before and after stress exposure following intake of tryptophan (TRP) or placebo (PLC). The order of presentation of the treatment conditions was counterbalanced within groups; both sessions were separated by one week. All subjects were tested in the mid-late follicular phase of their menstrual cycle or when actually taking oral contraceptives. An illustration of the design is given in Figure 1.

----- FIGURE 1 -----

All participants fasted overnight; except for water or tea without sugar. On each experimental day, three participants arrived at 08:00 a.m., 09:00 a.m. and 10:00 a.m., respectively. Capsules were consumed 1 hour before arrival. After arrival and a subsequent 15min rest period, mood and NAP was assessed. Then, participants were exposed to a stress-inducing procedure followed by a second mood and NAP measure.

Stress exposure

Subjects were placed in front of a camera for 20min while completing 2-min serial subtraction sessions and cold-pressor exposures. At unpredictable occasions, they were signaled to start at a certain number and count backwards by seven as quickly as possible. If they made a mistake, they were interrupted by the experimenter and had to start over. In addition, at unpredictable occasions while continuing counting, they had to place their non-preferred hand on a 1.5 °C cold plate for as long as possible (with a maximum of 2 min). For cold-pressor exposure, a cooler device was used composed of a copperplate surface (150x230mm) that was thermoelectrically cooled at a constant level by way of Peltier (TEC) devices. Backward counting was initiated at four different unpredictable 180±30sec time intervals using different numbers (400, 425, 530 or 840) that were counterbalanced within-subjects. Cold-pressor signals appeared at six unpredictable 120±60sec time intervals either during (85%) or between (15%) counting sessions.

Tryptophan challenge

To increase plasma tryptophan, 0.8g tryptophan (TRP) as compared to 0.8g placebo (Cellulose) capsules were used. Comparable intake of tryptophan has been shown to increase plasma TRP availability (TRP/LNAA ratio) with 190% 60-90min after intake (Markus *et al*, 2008). Capsules were identical and subjects were instructed to swallow them whole with water.

Positive and Negative Affect Scale (PANAS).

The PANAS (Watson *et al*, 1988) consists of two 10 items mood scales, a positive mood scale and a negative mood scale. These items are randomly presented and participants have to respond on a scale ranging from 1 (totally disagree) to 5 (totally agree).

Negative Affective Priming (NAP)

The NAP task included (5x5.5cm) colored pictures of positive (n=33), negative (n=33) or neutral (n=22) faces that were selected from the Karolinska Emotional Directed Faces database (Lundqvist, 1998) and based on prior validation (Goeleven *et al*, 2006). Pictures were divided into eight lists (based on stimulus type) of 11 randomly chosen pictures sharing the same valence: one negative and one positive prime target list, one negative and one positive prime distracter list, one negative and one positive probe target list and two neutral probe distracter lists. Pictures were indicated as target or distracter by a black or grey frame. Participants were instructed to evaluate the valence of the target picture –indicated by the colour of the frame– as accurately as possible by a response key while ignoring the distracter picture. Each trial started with a fixation cross (for 1000 ms) after which two emotional faces appeared in the upper and the lower half of the screen (one surrounded by a grey frame and one by a black frame) until responding (see Figure 2).

----- FIGURE 2 -----

After training (including 32 trials), a sequence of 256 test trials (separated by a 1000 ms blank screen) followed that were divided into eight blocks of 16 prime and probe trials. The response cue (grey or black frame) and key assignment was counterbalanced between subjects and the spatial position of the target and the distracter –as well as sequence of congruent and incongruent prime-probe trials- were randomly assigned from trial to trial. Reaction time (RT) to the probe trials for both the positive and negative trials were reordered (RT below 300ms and above 2000ms were excluded, see (Goeleven *et al*, 2006) and NAP scores were calculated by subtracting RT to control trials from RT to experimental trials.

Buccal cells for tri-allelic 5-HTTLPR polymorphism

Buccal cell samples for 5-HTTLPR genotype determination were obtained using sterile swabs (Omni Swabs, Whatman, 's Hertogenbosch, The Netherlands). Genomic DNA was isolated by QIamp DNA Mini Kits from Qiagen (Westburg, Leusden, The Netherlands) and 5-HTTLPR genotyping was performed using the polymerase chain reaction (PCR) protocol according to Glatz *et al*. (2003). In compliance with previous work (e.g. Neumeister *et al*, 2006; Walderhaug *et al*, 2007; Zalsman *et al*, 2006), tri-allelic variants were reclassified into a bi-allelic model as follows: S/S, S/L_G and L_G/L_G were classified as S'/S' and L_A/L_A as L'/L'.

Statistical analysis

Data were first examined for accuracy of data-entry, missing values and normal distributions. Hardy-Weinberg equilibrium was determined on the original 5-HTTLPR

data-base (N=90) using X^2 tests, revealing that the genotype frequencies of S'/S' (N=24), S/L (N=40) and L'/L' (N=26) did not depart from Hardy-Weinberg equilibrium ($X^2_{(1)}=1.45$; $p=0.23$). Data was analyzed by means of repeated measures anova (GLM: SPSS 12.0 for Windows) with one between-subjects factor *Genotype* (S'/S' vs L'/L') and two within-subjects factors *Treatment* (TRP vs PLC) and *Stress* (pre-stress vs. post-stress) on mood (PANAS) and NAP. Regarding NAP, repeated measures were conducted on difference reaction time (RT) scores between control and experimental probe trials; separately for the positive and negative trials. Although we counterbalanced for the order of treatment, *Order of Treatment* was preliminary taken as a between-subjects factor. Because *Order of Treatment* did not contribute to any of the scores, final analyses were performed with only *Genotype* as between-subjects factor. All statistics are evaluated at a two-tailed significance level of 5%. Data are reported as means \pm SD.

RESULTS

PANAS

Repeated measure analyses were conducted on the PANAS scores for positive and negative mood with *Genotype* (S'/S' vs L'/L') as between-subjects factor and *Treatment* (TRP vs PLC) and *Stress* (pre-stress vs. post-stress) as within-subjects factors. Analysis revealed an effect of *Stress* on positive feelings [$F(1,26)=13.49$, $p=0.001$; Power=.95] and negative feelings [$F(1,26)=15.52$, $p=0.001$; Power=.94]; indicating a reduction in positive feelings post-stress (2 ± 0.7) compared to pre-stress (2.6 ± 0.7) and an increase in negative feelings post-stress (1.3 ± 0.4) compared to pre-stress (1.0 ± 0.2). Regarding negative mood, analysis revealed an interaction of *Treatment* x *Genotype* [$F(1,26)=6.01$, $p=0.02$; Power=.70], *Treatment* x *Stress* [$F(1,26)=4.58$, $p=0.042$; Power=.60] and a marginally three-way interaction of

Treatment x Stress x Genotype [$F(1,26)=4.2$, $p= 0.041$; Power=.50]. As shown in Fig 3, S'/S' reported a modest increase in negative feelings post-stress (1.6 ± 0.9) compared to pre-stress (1.0 ± 0.12) after PLC that was absent after TRP (1.1 ± 0.3 post-stress compared to 1.1 ± 0.2 pre-stress).

----- FIG 3 -----

NAP: Negative Affective Priming

Repeated measure analyses were conducted on the NAP scores for positive and negative trials with *Genotype* (S'/S' vs L'/L') as between-subjects factor and *Treatment* (TRP vs PLC) and *Stress* (pre-stress vs. post-stress) as within-subjects factors. Analysis revealed an interaction of *Stress x Genotype* [$F(1,26)=16.84$, $p < 0.001$; Power= .95] for the negative trials; indicating an effect of stress exposure on inhibition of negative emotional information depending on 5-HTTLPR. As illustrated in Figure 4, stress exposure significantly impaired inhibition of negative emotional information in S'/S' ($P < 0.0001$), whereas in L'/L' inhibition of negative information improve (higher NAP) by stress exposure ($P=0.027$).

----- FIG 4 -----

DISCUSSION

The aim of the present study was to explore whether the S'/S' compared to the L'/L'-allele variant of the 5-HTTLPR increases the negative effects of stress exposure on the inhibition of affective information. Assuming 5-HTergic vulnerability in carriers of the low expressing 5-HTTLPR, S'/S' allele carriers were also expected to be less affected by stress following TRP compared to PLC. Results revealed that stress exposure significantly reduced mood and that only in S'/S'-allele carriers did stress impair the successful inhibition of negative affective information.

Stress and NAP

To induce acute stress, subjects completed serial subtraction sessions in combination with frequent cold-pressor exposures. Both the cold-pressor exposures (Isowa *et al*, 2005) and counting tasks (Dickerson and Kemeny, 2004) have been shown to be sufficient to induce stress; repeated exposures given in a variable and unpredictable manner have induced even more severe stress responses (Markus, 2007). In addition, the stress procedure in the current study was sufficient to induce negative mood responses.

In line with our expectation, exposure to the stress procedure increased the facilitation of negative information processing only in S'/S'-allele carriers. Hence, exclusively in S'/S', acute stress speeded up recognition for negative facial expressions during experimental NAP conditions (responding to negative probe targets that are preceded by prime distracters with the same negative valence) compared to control conditions (responding to negative probe targets that are preceded by prime distracters with an opposite valence). Because this reduced NAP effect represents an impaired inhibitory function towards negative affective information (Joormann, 2004); (Goeleven *et al*,

2006), the current findings suggest that S'/S'-allele carriers are more susceptible to the deteriorating effects of stress exposure on the successful inhibition of negative information. In contrast, L'/L'-allele carriers not only did not show these vulnerabilities, they were more successful at inhibiting negative information under stress (indicating that this variant could be a marker of resilience against the detrimental effects of stress).

The current 'boosting' effect of acute stress on the facilitation of negative information processing in S'/S'-allele carriers complies with recent findings that homozygous S'-allele are most susceptible to the negative affective (Firk , 2009) and hormonal (Gotlib *et al*, 2008) effects of stress exposure and to the depressogenic effects of life events (Caspi *et al*, 2003); (Uher R, 2008). In addition, the current study elaborates on previous findings by showing that the enhanced vulnerability in S'/S'-allele carriers for stress-related depression may be mediated by a stress-induced impairment in the ability to inhibit negative information. This also complies with findings from imaging studies showing that S-allele carriers exhibit enhanced amygdala responses to negative facial expressions (Pezawas *et al*, 2005); (Dannlowski *et al*, 2008). This result has been attributed to a reduced amygdala-to-prefrontal-cortex connectivity (a reduced inhibition of the amygdala) that is also observed in depression (Siegle *et al*, 2007).

The current stress-induced increase in negative information processing in S'/S'-allele carriers can be explained by the mood-congruent hypothesis that negative affective interruption facilitates continued processing of negative information. In addition, the previously demonstrated depressogenic effects of life events on S/S-allele carriers (Caspi *et al*, 2003); (Uher R, 2008) may be moderated by a stress-induced increase in attentional bias allowing for maintained attention on negative material. Thus, in S'/S'-allele carriers, stress may reduce the successful inhibition of distracting negative

stimuli. In fact, this stress-induced disturbed inhibitory functioning is thought to be a core factor that is related to the previously observed difficulties in depressed patients to disengage attention away from negative information (Koster *et al*, 2005). In addition and comparable both with our current findings in healthy S'/S'-allele carriers and with the assumption that S'/S'-allele carriers are more prone to develop depression, depressed subjects have also been shown to exhibit a reduced ability to inhibit negative information during a NAP task (Goeleven *et al*, 2006); (Leyman, in press).

To reveal whether S'/S' are more susceptible to the effects of stress due to brain 5-HT vulnerability, a TRP challenge was used in the current study. Based on previous studies reporting a 5-HT vulnerability to the mood-lowering effects of ATD in S'/S'-allele carriers (Neumeister *et al*, 2006; Neumeister *et al*, 2002; Roiser *et al*, 2006), it was hypothesized that TRP may reduce the negative stress effects. The current findings however did not reveal significant TRP effects on NAP and only marginally (reducing) effects on negative mood in S'/S' carriers.

Limitations

One point that deserves special attention is the relatively small sample size of the S'/S' and L/L groups. Even though the current findings reveal powerful genotype x stress interaction effects on NAP, the sample sizes might have been too small to also detect TRP x stress interactions on NAP. Another point of attention is that the current findings only included female subjects. It therefore remains necessary to conduct a comparable experiment in a comparable sample of male participants.

Conclusion

The current findings reveal that stress exposure in female S'/S' not L'/L'-allele carriers reduces inhibitory functioning for negative information. Because S'/S' carriers are found to be vulnerable to the negative depressogenic effects of stress, the current data is in line with the assumption that a stress-by-depression interaction in S'/S'-allele carriers may be promoted by a stress-induced facilitation of negative emotional information.

DISCLOSURE/CONFLICTS OF INTEREST

All authors ensure the integrity of the work and none of them has any direct or indirect financial or personal interests, or conflicts of interest, to the subject matter of the manuscript

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FIGURE LEGENDS

Figure 1. Schematic overview of the study design.

Figure 2. Negative Affective Priming Design

Figure 3. S'/S' reported an increase in negative feelings post-stress compared to pre-stress (1.0 ± 0.13) after PLC that was prevented after TRP (1.2 ± 0.3 post-stress compared to 1.1 ± 0.2 pre-stress).

Figure 4 Acute stress exposure significantly impaired inhibition of negative emotional information (reduced NAP) only in S'/S' genotypes ($P < 0.0001$), whereas in L'/L' genotypes inhibition of negative information improved (higher NAP) by stress exposure ($P = 0.025$).

FIGURE 1

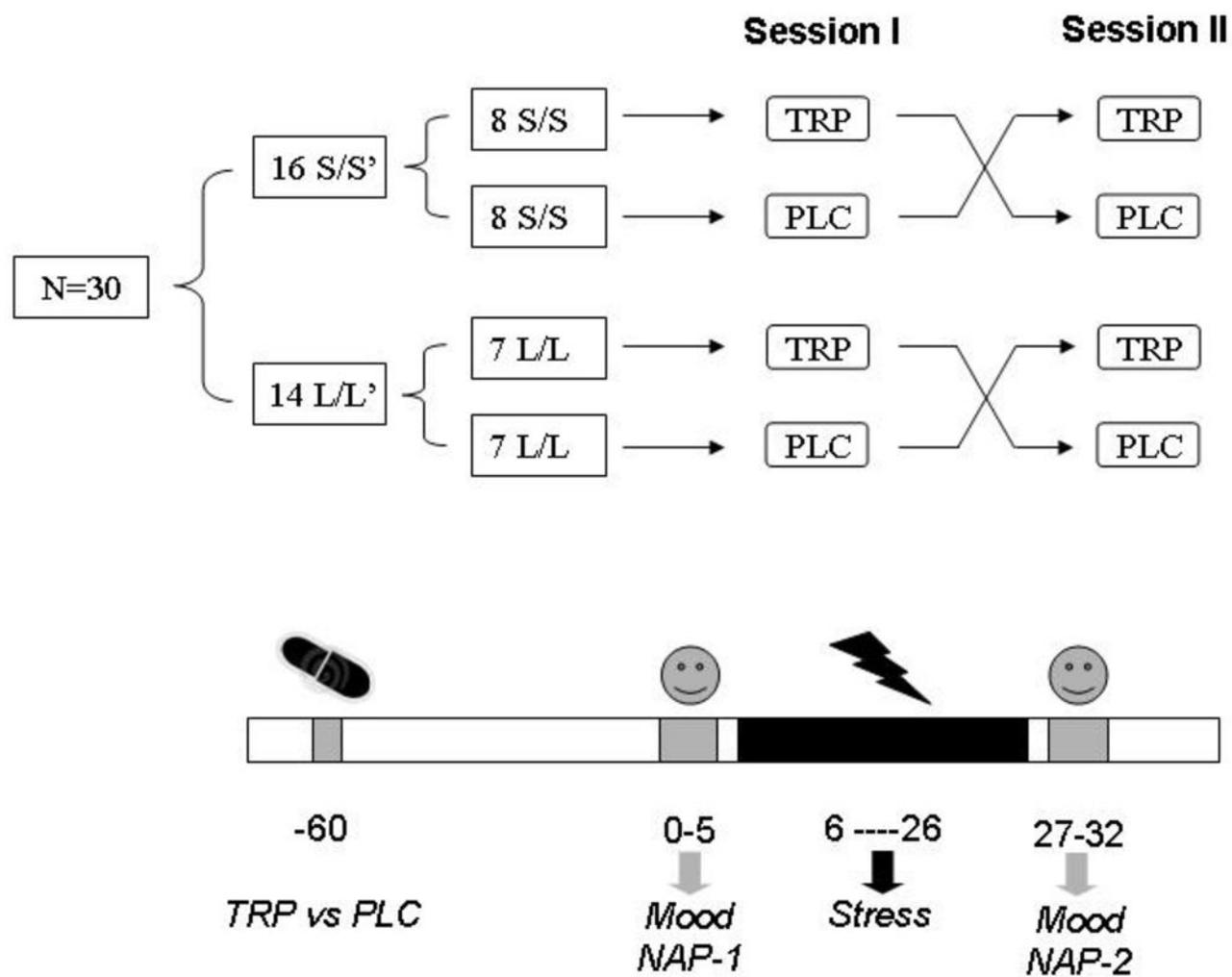


FIGURE 2

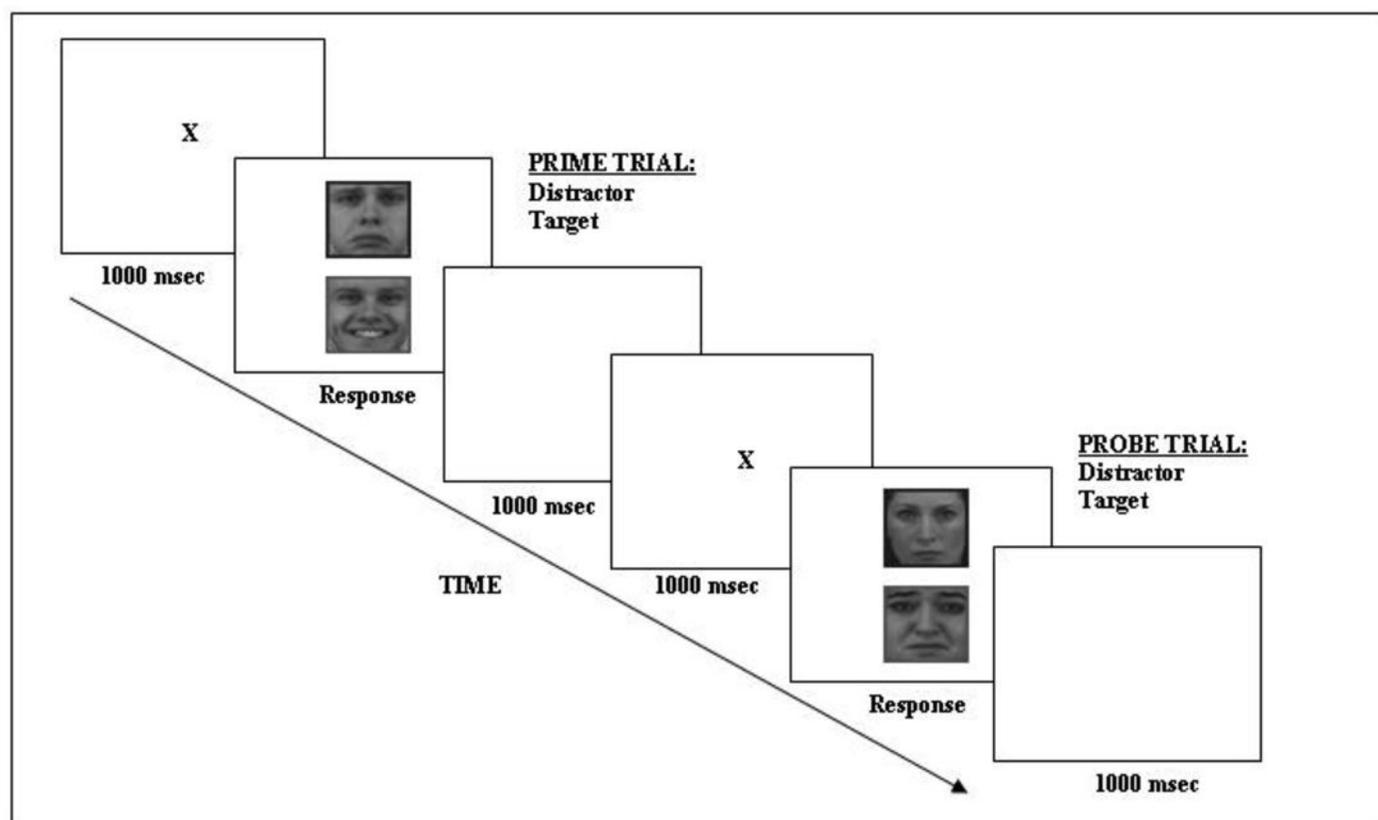


FIGURE 3

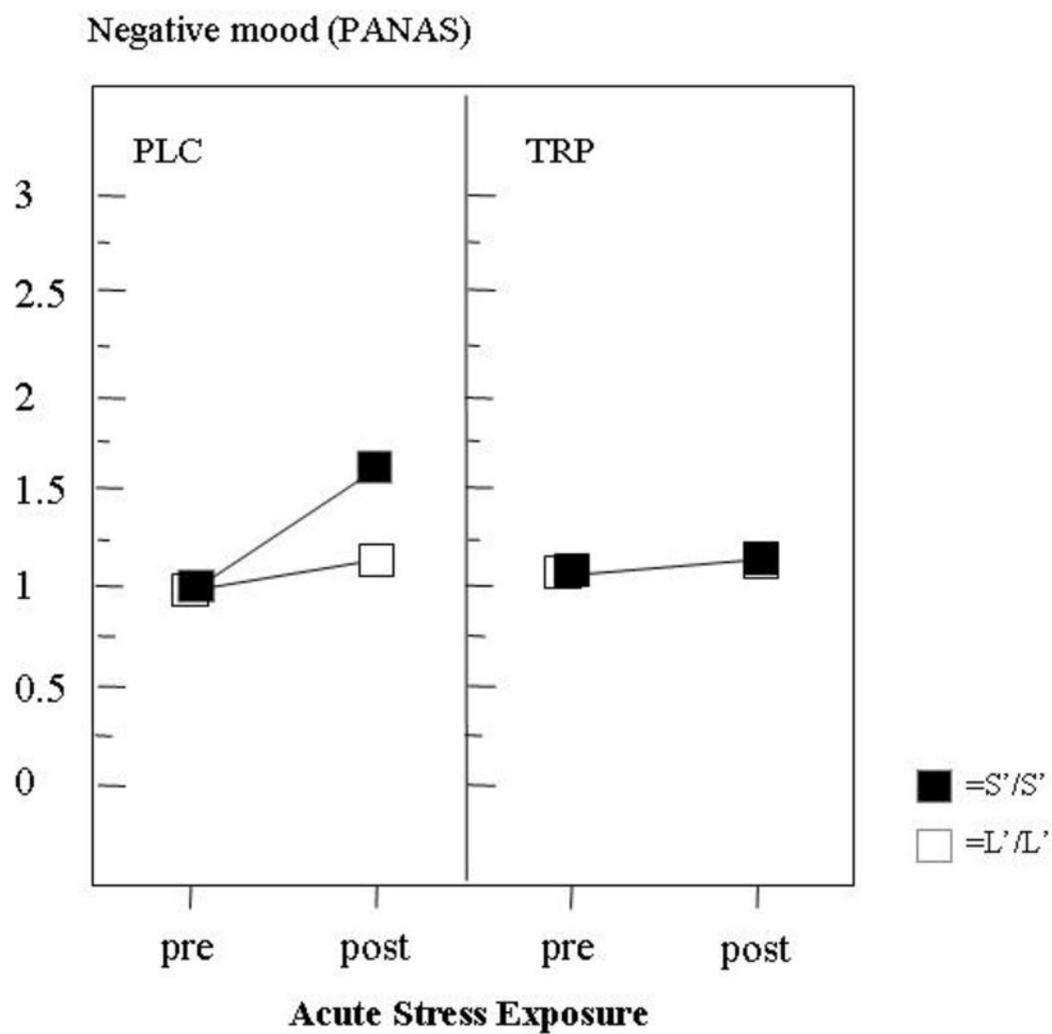


FIGURE 4

