Serotonin transporter gene (5-HTTLPR) polymorphisms are associated with emotional modulation of pain but not emotional modulation of spinal nociception

Shreela Palit a, Robert J. Sheaff b, Christopher R. France c, Sarah T. McGlone c, William T. Potter b, Allan R. Harkness a, John L. McNulty a, Emily J. Bartley a, Rachel Hoffmann b, Julie K. Monda b, Jamie L. Rhudy a,*

a Department of Psychology, The University of Tulsa, 800 South Tucker Drive, Tulsa, OK 74104, United States
b Department of Biochemistry, The University of Tulsa, 800 South Tucker Drive, Tulsa, OK 74104, United States
c Department of Psychology, Ohio University, 245 Porter Hall, Athens, OH 45701, United States

Abstract

The short allele of the serotonin transporter gene (5-HTTLPR) is associated with greater negative emotionality. Given that emotion modulates pain, short allele carriers (s-carriers) may also demonstrate altered pain modulation. The present study used a well-validated emotional picture-viewing paradigm to modulate pain and the nociceptive flexion reflex (NFR, a measure of spinal nociception) in 144 healthy genotyped participants. As expected, pain/NFR responses were largest during unpleasant pictures and smallest during pleasant pictures. However, relative to l/l-carriers, s-carriers demonstrated greater pain inhibition during pleasant pictures and greater pain facilitation during unpleasant pictures. Neither emotional modulation of NFR nor NFR threshold was associated with 5-HTTLPR polymorphisms. Results also indicated that men who were s-carriers had a higher pain threshold and tolerance than other participants. Taken together, our results indicate 5-HTTLPR polymorphisms may influence pain modulation at the supraspinal (not spinal) level; however, the influence on pain sensitivity may be sex-specific.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Pain perception is determined, in part, by ascending nociceptive signals, but also by inhibitory and facilitatory processes that continuously modulate nociceptive inputs. Thus, characterizing pain modulatory processes can contribute greatly to our understanding of pain, because a disruption of pain modulation could promote chronic pain. Consistent with this, exaggerated pain facilitation has been noted in several pain disorders (e.g., Fusco et al., 1997; Raphael et al., 2009; Sarlani and Greenspan, 2005; Staud et al., 2001).

1.1. Emotion as a tool to study pain modulation

Emotional processes are known to modulate pain (e.g., Keefe et al., 2001; Klossika et al., 2006; Rhudy and Meagher, 2001; Villemure and Bushnell, 2002). For example, several laboratories have demonstrated that viewing pleasant pictures (e.g., erotica) elicits positive emotions and pain inhibition, whereas viewing unpleasant pictures (e.g., attack scenes) elicits negative emotions and pain enhancement (de Wied and Verhagen, 2001; Kenntner-Mahiaia and Pauli, 2005; Meagher et al., 2001; Mini et al., 1995; Rhudy et al., 2006a,b, 2005; Wunsch et al., 2003). Our laboratory has also shown that viewing emotional pictures modulates a physiological correlate of spinal nociception, the nociceptive flexion reflex (NFR), in the same direction as pain (Rhudy et al., 2005): unpleasant pictures enhance NFR, whereas pleasant pictures inhibit NFR. Because the NFR is a physiologically measured, spinally mediated, withdrawal reflex elicited following A-delta nociceptor (pain fiber) activation (Sandrini et al., 2005), any change in NFR exhibited during emotional picture-viewing is evidence of descending brain-to-spinal cord modulation of spinal nociceptive processes (Fig. 1). Willer et al. (1981) were the first to demonstrate that the NFR could be down-regulated following intense, emotional stress. Our laboratory extended this work and was the first to show that NFR and pain can be down-regulated or up-regulated in parallel by sub-tle emotional pictures (Rhudy et al., 2005). Since then, we have replicated our findings numerous times (Rhudy and Bartley, 2010; Rhudy et al., 2006b, 2008). For brevity, we refer to the modulation of pain and NFR by emotional pictures as the Emotional Controls of Nociception (ECON) paradigm (Rhudy et al., 2008).

Recently, an independent laboratory found that several supraspinal structures were activated during ECON modulation...
(e.g., prefrontal cortex, insula, amygdala, thalamus, brainstem), but that unique neural circuits were responsible for pain modulation (orbitofrontal cortex, subgenual cingulate cortex, cuneus, and insula) vs. NFR modulation (dorsolateral prefrontal cortex, parahippocampal gyrus, brainstem nuclei) (Roy et al., 2009). Thus, when taken together, ECON procedures appear to be an ideal tool for studying individual differences in supraspinal modulation of pain and brain-to-spinal cord modulation of NFR. Interestingly, a recent ECON study suggested that altered supraspinal modulation of pain may be more associated with chronic pain than altered brain-to-spinal cord modulation of NFR, because fibromyalgia patients failed to emotionally modulate pain, but had intact emotional modulation of NFR (DelVentura et al., 2010).

1.2. 5-HTTLPR polymorphisms and emotional modulation of pain

Identifying genetic determinants of individual differences in pain modulation has clear importance because of the potential insights into the etiology and maintenance of chronic pain (e.g., Edwards, 2005). However, to our knowledge, there have been no genetic or heritability studies of emotional modulation of pain. Given the role of serotonin (5-HT) in the regulation of emotion and pain (Fields et al., 2006; Murphy et al., 2008; Sommer, 2004; Suzuki et al., 2004), genetic modulators of serotonergic activity might influence emotional modulation of pain. Indeed, serotonergic neurons ascend from the dorsal raphe to influence multiple brain regions involved with mood and emotion (Kandel, 2000), and selective serotonin reuptake inhibitors (SSRIs) are prescribed to treat affective disorders (Nelson, 1999; Nemeroff, 2007; Whittington et al., 2004). Likewise, there are serotonergic neurons that descend from the brain stem to the dorsal horn which can inhibit and facilitate spinal nociceptive transmission (Fields et al., 2006; Suzuki et al., 2004). And, serotonin and norepinephrine reuptake inhibitors, and to a lesser extent SSRIs, are prescribed to treat some chronic pain (Arnold et al., 2002; Goldenberg et al., 1996; Sindrup et al., 1992).

The serotonin transporter (5-HTT) is an important determinant of individual differences in 5-HT signaling because it is involved with clearance of 5-HT from the synapse. In the human 5-HTT gene (i.e., SLC6A4) an insertion/deletion in the 5-HTT linked promoter region (5-HTTLPR) leads to two common alleles, one short (s) and one long (l), that influence the availability of 5-HT (Heils et al., 1996; Murphy et al., 2008). Relative to the l-allele, the s-allele reduces transcriptional efficiency of the 5-HTT gene promoter which leads to decreased 5-HTT expression and availability (Lesch et al., 1996). As a result, s-carriers are at increased risk for affective disturbances, such as anxiety and depression (Heils et al., 1996; Lesch et al., 1996; Lotrich and Pollock, 2004; Schinka et al., 2004; Sen et al., 2004), and also appear to have higher risk for pain disorders, such as tension-type headache (Park et al., 2004), fibromyalgia (Buskila and Neumann, 2005), and irritable bowel syndrome [IBS] (Yeo et al., 2004) – although the association with IBS is not always found (Van Kerkhoven et al., 2007).

Even healthy s-carriers not affected by depression, anxiety, or chronic pain show exaggerated amygdala activation in response to unpleasant stimuli (for a review, Munafo et al., 2008). Though less robust than responses to unpleasant stimuli, some studies even indicate s-carriers have an exaggerated amygdala response to pleasant stimuli (Canli et al., 2008), suggesting an enhanced response to all emotional stimuli. One compelling hypothesis for amygdala hyperreactivity in s-carriers is that there are changes in top-down control from prefrontal regions (Heinz et al., 2005; Pezawas et al., 2005). For example, anatomical and functional changes in the circuit comprising the anterior cingulate cortex (ACC) and amygdala have been noted in s-carriers, specifically an uncoupling of the correlation in activity between the ACC and amygdala during emotional processing (Pezawas et al., 2005). Given the involvement of the amygdala and ACC in emotion and pain modulation (Apkarian et al., 2005; Fields et al., 2006; LeDoux, 2000; Pezawas et al., 2005; Roy et al., 2009; Zald, 2003), polymorphisms of the 5-HTTLPR gene could influence this circuit and thus influence emotional modulation of pain. Consistent with this, a recent study found that, relative to l-carriers, s/s-carriers demonstrated enhanced activity in prefrontal regions, including the ACC, during experimentally induced visceral pain (i.e., colorectal distention) (Fukudo et al., 2009), suggesting a disruption of pain modulation in s-carriers.

Taken together, it is possible that healthy s-carriers might show greater pain facilitation while viewing unpleasant pictures relative to individuals who are l/l-carriers. However, because some reports have also noted greater amygdala activity to pleasant stimuli in s-carriers (Canli et al., 2008), it is also possible that they might show relatively greater pain inhibition during pleasant pictures. If true, this “enhanced” emotional modulation of pain by s-carriers might serve as a heritable diathesis that interacts with stress to promote the development of chronic pain. Specifically, s-carriers might show a greater disposition for both pain facilitation and greater pain inhibition than l/l-carriers; but, following exposure to psychological or physical stressors, the balance becomes biased toward negative affectivity (e.g., depression, anxiety) and pain facilitation. There are several lines of evidence that support this idea. First, s-carriers who have been exposed to stressful events are more likely to develop depression than l/l-carriers or s-carriers not exposed to stress (e.g., Caspi et al., 2003; Karg et al., 2011). Second, chronic pain is often comorbid with affective disorders such as depression (Henningsen et al., 2003), and depression appears to increase risk

---

1 It should be noted that there are alternative explanations for the difference in amygdala activity between emotional vs. neutral stimuli. Specifically, Canli and Lesch (2007) have hypothesized that s-carriers actually show tonically exaggerated amygdala activity and that amygdala activity actually decreases during neutral stimuli, giving the appearance that amygdala activity increases during positive and negative stimuli. While this alternative explanation may be correct, it does not significantly affect our hypothesis that s-carriers may show activation in brain regions responsible for emotional modulation of pain that is different than l/l-carriers.
for chronic pain (e.g., Currie and Wang, 2005). Third, early life stress and trauma are associated with hyperalgesia and chronic pain (Al-Chaer and Weaver, 2009). And fourth, a relationship between the s-allele and chronic pain has been noted (Buskila and Neumann, 2005; Park et al., 2004).

1.3. The present study

The present study tested whether healthy s-carriers display enhanced emotional modulation of pain and NFR. To do so, NFR threshold, electrocutaneous pain threshold, and electrocutaneous pain tolerance were assessed and then ECON procedures were carried out in 144 genotyped men and women. We predicted that, relative to l/l-carriers, s-carriers would show: (1) enhanced facilitation of pain and NFR during unpleasant pictures, (2) enhanced inhibition of pain and NFR during pleasant pictures, and (3) greater pain sensitivity (e.g., lower electrocutaneous pain threshold and tolerance). However, given the relationship between the s-allele and depression (Caspì et al., 2003; Heils et al., 1996; Karg et al., 2011; Lotrich and Pollock, 2004), and other evidence indicating depression is associated with higher electrocutaneous pain threshold and tolerance (hyposensitivity) (Bar et al., 2005a), it is possible that s-carriers may show hypoalgesia on measures of electrocutaneous pain sensitivity.

2. Methods

To maximize our sample size, data were combined from two ECON studies that used slightly different methodologies. The Ohio ECON study was interested in the influence of family history of hypertension on emotional modulation of pain/NFR (McGlone et al., 2009), whereas the Oklahoma ECON study was interested in the influence of participant sex (sex differences in emotional modulation) and a wide array of picture contents on emotional modulation of pain/NFR (Rhudy et al., 2010b). In the parent studies, emotional modulation was noted in both samples, but neither sex nor family history of hypertension was found to influence emotional modulation of pain and NFR, so the data were combined for the present study.

2.1. Participants

All participants provided written and verbal informed consent after the study procedures were fully described to them. Data collection was conducted at The University of Tulsa and Ohio University. Participants at The University of Tulsa were recruited from the community by newspaper advertisements, email distribution, and fliers, while participants at Ohio University were recruited from the Ohio University Psychology Department online participant pool. Participants were excluded if they were <18 yrs old (Ohio University also excluded participants who were >25 yrs old due to other study aims at that site); self-reported a history of neurological disease, or given research credit for psychology courses (Ohio). If they were <18 yrs old due to other study aims at that site); self-reported a history of neurological disease, or given research credit for psychology courses (Ohio).

2.2. Apparatus, electrode application, and signal acquisition

Experiments monitored physiological signals, experimental timing, and participant behavior from an adjacent room. All data acquisition, as well as stimuli and questionnaire presentation, were controlled by a PC equipped with dual monitors and A/D board. One video output from the computer was used to present pictures to the participant. A second video output was displayed to a monitor so that an experimenter could monitor physiological signals and experimental timing.

Noxious electrocutaneous stimuli were delivered via a bipolar stimulating electrode (Nicolet, 019-401400, Madison, WI) to the left ankle over the retromalleolar pathway of the sural nerve. At the Tulsa site, stimulations were delivered by a Grass Technologies stimulator (Model S88 or Model S48, West Warwick, RI), stimulus

Note: Group comparisons on continuous variables were made with independent samples t-tests, whereas comparisons on categorical variables were made with chi-square tests.

2 Conclusions were the same if analyses were restricted to Caucasians.
after picture onset and 11–21 s after inter-picture interval onset, also to reduce predictability. The 3–5 s post-picture-onset interval was chosen based on prior research that it produces the largest emotional modulation effects on the acoustic startle reflex (Bradley et al., 1993; Coida et al., 2001). Following every picture, the Self-Assessment Manikin was administered to assess subjective emotional responses to the pictures (Bradley et al., 2001a). The numerical rating scale was administered following pictures and inter-picture intervals in which a suprathreshold stimulus was delivered. At study completion, participants were thanked and provided their compensation.

2.7. Data analysis

Participant sex was included as a predictor in all analyses given prior observations that, relative to men, women have greater pain sensitivity (Ellermeier and Weber, 1995; France and Suchowicki, 1999; Paulson et al., 1998; Riley et al., 1998) and react with greater displeasure to threatening pictures and less pleasure to erotic pictures (Bradley et al., 2001b). Removing this sex-related variance from the error term of the ANOVA models should improve statistical power; however, we did not have specific hypotheses about the interaction of sex and genotype. Analyses of NFR threshold, pain threshold, and pain tolerance were conducted using 2 (Genotype: s-carriers vs. l/l-carriers) × 2 (Participant Sex) ANOVAs. Consistent with previous studies (Rhudy et al., 2005, 2006b, 2008) and studies of emotional modulation of startle (Bradley et al., 2001a), pain ratings and NFR manipulations evoked during pictures were converted to within-subject changes in spinal nociceptive processes during picture-viewing (Rhudy et al., 2006b).

3. Results

3.1. Basal pain and nociceptive sensitivity

3.1.1. NFR threshold

There were no significant effects of sex, genotype, or Sex × Genotype observed for NFR threshold (all ps > .35, n²s < .007; Fig. 2).

3.1.2. Electrocutaneous pain threshold

As can be seen in Fig. 2, examination of electrocutaneous pain thresholds revealed a significant main effect of sex, F(1, 137) = 5.17, p = .03, n² = .04, such that males had a higher pain threshold than females (M = 14.80, SEM = .94 vs. M = 11.85 mA, SEM = .90). Although the main effect of genotype was non-significant (F = .48, n² = .004), a significant Sex × Genotype interaction was found, F(1, 137) = 5.69, p = .018, n² = .04. Decomposition of this interaction found the simple effect of genotype was significant for men (p = .03) but not women (p = .23). Men who were s-carriers had a higher pain threshold than men who were l/l-carriers. Also, men who were s-carriers had a higher pain threshold than women who were s-carriers (p < .001), but men and women who were l/l-carriers did not differ (p = .94). In sum, pain threshold was influenced by genotype, but only in men.

3.1.3. Electrocutaneous pain tolerance

Examination of electrocutaneous pain tolerance levels revealed no main effects of sex or genotype (ps > .08, n²s < .023). However, there was a significant Sex × Genotype interaction, F(1, 136) = 4.14, p = .04, n² = .03. The pattern of means was the same as pain thresh-
interaction indicated that females rated the attack pictures as more unpleasant than males, and males rated the erotic pictures as more pleasant than females ($F(2, 139) = 13.00, p < .001, \eta^2 = .16$; Bonferroni follow-up $p < .001$). Males and females did not differ in their ratings of neutral pictures ($p = .31$). There was also a significant Content $\times$ Sex $\times$ Genotype interaction ($F(2, 139) = 3.23, p = .04, \eta^2 = .04$); however, there were no significant simple effects of genotype when this interaction was decomposed with Bonferroni mean comparisons ($p > .07$), indicating that genotype did not moderate the Content $\times$ Sex interaction noted above. The main effect of genotype, the Content $\times$ Genotype interaction, and the Genotype $\times$ Sex interaction were all non-significant ($p > .49, \eta^2_s < .011$). In sum, pictures influenced emotional valence as expected; pleasant pictures increased valence and unpleasant pictures decreased valence. However, men had higher valence ratings to erotica and women had lower valence ratings of attack.

### 3.2. Emotional Controls of Nociception (ECON)

#### 3.2.1. Valence ratings of pictures

As can be seen in Table 2, analysis of valence ratings revealed a significant main effect of content, $F(2, 139) = 193.40, p < .001, \eta^2 = .74$ (Table 2). Erotic and attack pictures were more arousing than neutral pictures (all $p < .001$), but attack was not significantly different from erotica ($p = .12$). However, a Content $\times$ Sex interaction qualified the main effect, $F(2, 139) = 12.64, p < .001, \eta^2 = .15$. Males rated the erotic pictures as more arousing than females ($p = .02$), but there were no sex differences in ratings of attack or neutral ($p > .06$). The main effect of genotype, the main effect of sex, and the interactions of Content $\times$ Genotype, Content $\times$ Genotype $\times$ Sex, and Genotype $\times$ Sex were all non-significant ($p > .14, \eta^2_s < .028$). In sum, pictures influenced emotional arousal as expected; attack and erotic pictures led to higher arousal than neutral pictures. However, men had higher arousal to erotica than women.

#### 3.2.2. Arousal ratings of pictures

As illustrated in Fig. 3, analysis of arousal ratings revealed a significant main effect of content, $F(2, 138) = 31.24, p < .001, \eta^2 = .31$. Pain ratings were lower during erotic pictures than neutral and attack pictures, and pain ratings were higher during attack pictures than neutral and erotic pictures (all $p < .001$). This main effect was qualified by an interaction of Content $\times$ Genotype, $F(2, 138) = 3.29, p = .04, \eta^2 = .05$. Compared to participants who were I/l-carriers, s-carriers experienced a greater increase in pain during attack pictures and a greater decrease in pain during erotic pictures ($p < .041$). The main effect of genotype, the main effect of sex, and the interactions of Content $\times$ Sex, Genotype $\times$ Sex, and Content $\times$ Genotype $\times$ Sex were all non-significant ($p > .21, \eta^2_s < .013$). In sum, pain was modulated by emotional pictures as expected; pain was facilitated by attack pictures and inhibited by erotic pictures. However, this effect was moderated by genotype; s-carriers showed greater facilitation and inhibition. For comparison, unstandardized pain ratings are reported in Table 3.

#### 3.2.3. Pain ratings

Analysis of NFR magnitudes revealed only a significant main effect of content, $F(2, 138) = 7.68, p = .001, \eta^2 = .10$ (Fig. 3). NFRs were smaller during erotic pictures compared to neutral and attack pictures ($p < .005$), but NFRs were similar during neutral and attack ($p = .59$). The main effect of genotype, the main effect of sex, and the interactions of Content $\times$ Genotype, Content $\times$ Genotype $\times$ Sex, and Genotype $\times$ Sex were all non-significant ($p > .21, \eta^2_s < .016$). The Content $\times$ Sex interaction just eluded significance ($p = .061, \eta^2_s = .04$). In sum, NFR was modulated by emotional pictures; NFR was inhibited by erotic pictures compared to neutral and attack pictures. Genotype did not influence emotional modulation of NFR.

**Fig. 2.** NFR threshold, electrocutaneous pain threshold, and electrocutaneous pain tolerance grouped by sex and 5-HTTLPR genotype (s-carrier vs. I/l-carriers). *Genotype differences, $p < .05$.

3.2.4. Nociceptive flexion reflex (NFR) magnitudes

Analysis of NFR magnitudes revealed only a significant main effect of content, $F(2, 138) = 48.09, p < .001, \eta^2 = .50$. Attack and neutral ($p = .86$) were rated as more unpleasant than women who were s-carriers than men who were l/l-carriers ($p = .44$). Also, men who were s-carriers had a higher pain tolerance than women who were s-carriers ($p = .002$), but men and women who were I/l-carriers did not differ ($p = .86$). In sum, pain tolerance was influenced by genotype, but only in men.
### Table 2
Means and standard deviations for valence and arousal ratings by genotype, picture content, and participant sex.

|                     | Attack | | Neutral | | Erotic | |
|---------------------|--------|--------|---------|--------|---------|
|                     |        |        |         |        |         |
|                     | M      | SD     | M       | SD     | M       | SD     |
| Valence ratings     |        |        |         |        |         |
| l/l-Carriers        |        |        |         |        |         |
| Men                 | 3.10   | 1.16   | 5.14    | 0.58   | 7.04    | 1.14   |
| Women               | 2.64   | 1.10   | 4.94    | 0.86   | 5.53    | 1.71   |
| Total               | 2.86   | 1.14   | 5.04    | 0.73   | 6.27    | 1.64   |
| s-Carriers          |        |        |         |        |         |
| Men                 | 3.68   | 1.16   | 5.06    | 0.83   | 6.39    | 1.10   |
| Women               | 2.38   | 1.02   | 4.99    | 0.73   | 5.70    | 1.50   |
| Total               | 2.94   | 1.26   | 5.02    | 0.77   | 6.00    | 1.38   |
| Total Men           | 3.47   | 1.18   | 5.09    | 0.74   | 6.63    | 1.15   |
| Total Women         | 2.46   | 1.05   | 4.97    | 0.77   | 5.64    | 1.56   |
| Total               | 2.92   | 1.22   | 5.03    | 0.76   | 6.09    | 1.47   |
| Arousal ratings     |        |        |         |        |         |
| l/l-Carriers        |        |        |         |        |         |
| Men                 | 5.83   | 1.93   | 2.38    | 1.08   | 6.20    | 2.14   |
| Women               | 5.70   | 2.08   | 2.90    | 1.20   | 4.91    | 1.72   |
| Total               | 5.76   | 1.99   | 2.64    | 1.16   | 5.54    | 2.02   |
| s-Carriers          |        |        |         |        |         |
| Men                 | 5.27   | 2.02   | 2.43    | 1.45   | 5.48    | 1.94   |
| Women               | 6.06   | 1.72   | 2.79    | 1.37   | 5.27    | 1.72   |
| Total               | 5.72   | 1.89   | 2.63    | 1.41   | 5.36    | 1.81   |
| Total Men           | 5.47   | 1.99   | 2.41    | 1.32   | 5.75    | 2.03   |
| Total Women         | 5.95   | 1.83   | 2.82    | 1.31   | 5.15    | 1.72   |
| Total               | 5.73   | 1.92   | 2.64    | 1.33   | 5.42    | 1.88   |

### Table 3
Means and standard deviations for unstandardized pain and NFR during ECON testing.

|                     | Attack | | Neutral | | Erotic | |
|---------------------|--------|--------|---------|--------|---------|
|                     |        |        |         |        |         |
|                     | M      | SD     | M       | SD     | M       | SD     |
| Pain ratings (0–100) |        |        |         |        |         |
| l/l-Carriers        | 43.93  | 23.20  | 41.19   | 21.65  | 40.17   | 21.76  |
| s-Carriers          | 40.33  | 26.05  | 36.40   | 24.15  | 33.76   | 23.50  |
| Total               | 41.54  | 25.10  | 38.01   | 23.37  | 35.91   | 23.06  |
| NFR magnitude (ΔµV) |        |        |         |        |         |
| l/l-Carriers        | 6.67   | 8.05   | 6.28    | 6.41   | 6.18    | 7.61   |
| s-Carriers          | 5.22   | 4.57   | 5.24    | 4.32   | 4.52    | 4.57   |
| Total               | 5.71   | 5.98   | 5.59    | 5.12   | 5.08    | 5.80   |

Note: These unstandardized values are presented for comparison only. Within-subject standardizing has the advantage of increasing statistical power by removing between-subject variance that is not associated with emotional modulation. It is worth noting that none of this between-subject variability was significantly related to 5-HTTLPR polymorphisms.

For comparison, unstandardized NFR magnitudes are reported in Table 3.

4. Discussion

This study was conducted to examine the relationships between 5-HTTLPR polymorphisms and emotional modulation of pain and NFR. To do so, measures of pain and nociceptive sensitivity were first assessed (NFR threshold, pain threshold, pain tolerance) and then noxious electric stimuli set at 120% NFR threshold were delivered during emotionally charged pictures (attack, neutral, erotica).

4.1. 5-HTTLPR polymorphisms and basal pain/nociceptive sensitivity

We found that 5-HTTLPR polymorphisms were associated with pain threshold and tolerance, an effect that was moderated by sex. But contrary to our hypothesis, men who were s-carriers had higher electrocutaneous pain thresholds and pain tolerances than men who were l/l-carriers and women from either genotype group. This implies electrocutaneous pain sensitivity is dampened (hypoalgesia) in s-carriers, but only in men. Because the s-allele is a risk factor for major depression (Caspi et al., 2003; Heils et al., 1996; Karg et al., 2011; Lotrich and Pollock, 2004), this finding is consistent with...

---

**Fig. 3.** 5-HTTLPR polymorphisms and emotional modulation of pain ratings and nociceptive flexion reflexes (NFR) by attack, neutral, and erotic pictures. *Genotype differences, p < .05."
research suggesting persons with major depression or adjustment disorder (a risk for major depression) have increased cold, heat, and electrocutaneous pain thresholds and tolerances (Bar et al., 2006, 2005b; Schwier et al., 2010). However, at this time it is unclear why we observed hypoalgesia only in male s-carriers. While speculative, this could stem from the fact that males at risk for affective disturbance (i.e., males who are s-carriers) have altered pain circuitry, relative to females. Specifically, reduced ACC volumes have been noted in males, but not females, at risk for depression (Boes et al., 2008; Hastings et al., 2004). Given the importance of the ACC in pain processing (Apkarian et al., 2005; Rainville et al., 1997), alterations of the ACC could contribute to the hypoalgesia found only in male s-carriers. However, future research is needed to specifically address this hypothesis. Interestingly, hypoalgesia could also be a risk factor for chronic pain development. For example, chronic activation of opioid systems (which leads to hypoalgesia) may ultimately promote chronic pain by exhausting an individual’s capacity to engage pain inhibitory mechanisms, perhaps through a down-regulation of opioid receptors or a depletion of opioid stores (e.g., Bruehl et al., 2007).

Unlike subjective measures of pain sensitivity, there were no genetic associations with the physiologically determined NFR threshold. The NFR is a spinally mediated, protective reflex elicited by A-delta fiber (pain fibers) activation (Sandrini et al., 2005). Although the NFR can be modulated by supraspinal regions, its elicitation does not require supraspinal input. Indeed, the NFR can be evoked in spinal humans; but, the threshold for elicitation in spinal humans is lower, indicating the NFR is under tonic descending inhibition from supraspinal centers (Sandrini et al., 2005). In light of our findings, it appears that 5-HTTLPR polymorphisms do not exert a direct effect on the reflex arc of the NFR nor do they exert an indirect effect on the NFR via tonic descending inhibition from supraspinal centers (Sandrini et al., 2005). Importantly, because NFR threshold did not differ across genotypes, this means that the stimulus intensity used during ECON (which was set at 120% NFR threshold) did not differ by genotype. Thus, any associations between 5-HTTLPR polymorphisms and emotional modulation of pain cannot be attributed to differences in stimulus intensity used during ECON procedures.

4.2. 5-HTTLPR polymorphisms and emotional modulation of pain and spinal nociception

Consistent with prior research (Rhudy et al., 2010a, 2005, 2006b, 2008), both pain and NFR were modulated by emotional pictures. Pain was less and NFR was smaller during erotic pictures as compared to attack pictures. Interestingly, emotional modulation of pain, but not NFR, was moderated by 5-HTTLPR polymorphisms. As predicted, s-carriers showed enhanced emotional modulation relative to l/l-carriers, such that pain was facilitated to a greater degree by attack pictures and inhibited to a greater degree by erotic pictures. By contrast, there were no differences between s-carriers and l/l-carriers in emotional modulation of NFR. Thus, 5-HTTLPR polymorphisms appear to be associated with the supraspinal mechanisms that modulate pain, but not the brain-to-spinal cord mechanisms that modulate NFR.

Indeed, two separate modulatory mechanisms (supraspinal vs. brain-to-spinal cord) appear to be engaged by ECON picture-viewing. This notion initially stemmed from a study that manipulated whether electric stimulations delivered during emotional pictures were unpredictable or predictable (Rhudy et al., 2006b). When unpredictable electrical stimulations were used to evoke pain and NFR, emotional pictures modulated both pain and NFR (Rhudy et al., 2005, 2006b). However, when electric stimulations were preceded by a cue that signaled their delivery (predictable electric stimulations); modulation of pain and NFR diverged (Rhudy et al., 2006b). Specifically, emotional pictures modulated pain as expected, but NFR magnitude did not vary by picture content. This suggests at least two mechanisms are involved in emotional modulation: (1) brain-to-spinal cord (descending modulation) mechanisms that modulate spinal nociception (NFR) and (2) supraspinal (brain-only) mechanisms that modulate pain perception (Fig. 1).

This notion was supported by Roy et al. (2009) using fMRI and connectivity analysis to study ECON modulation. They noted that descending brain-to-spinal cord modulation of spinal nociception involves the dorsolateral prefrontal cortex, parahippocampal gyrus, and brainstem nuclei. By contrast, modulation of pain experience was associated with activity in the orbitofrontal cortex, subgenual cingulate cortex, cuneus, and insula. Although they found that the amygdala was activated during ECON modulation, it was not clear what role it played because connectivity analysis only included brain regions that were differentially activated by pleasant and unpleasant pictures. Given that both pleasant and unpleasant picture contents can evoke amygdala activation (Zald, 2003), a pleasant vs. unpleasant comparison would not be able to identify amygdala activation involved with emotional modulation of pain and NFR. Nonetheless, the Roy et al. data support our hypothesis that different central mechanisms are involved with supraspinal modulation of pain and brain-to-spinal cord modulation of NFR. Given these separate mechanisms for pain modulation vs. NFR modulation, 5-HT efficiency appears to influence supraspinal pain modulation mechanisms without significantly influencing the brain-to-spinal cord mechanisms that modulate spinal nociception. Put another way, our results indicate the incoming nociceptive signal (as indexed by NFR) is modulated at spinal levels via brain-to-spinal cord circuits, regardless of 5-HTTLPR genotype. However, once the signal is relayed to the brain, additional supraspinal modulation takes place, and this modulation is augmented in s-carriers (Fig. 1).

Based on the prior studies noting an association between 5-HTTLPR polymorphisms and amygdala activation (e.g., Canli et al., 2008; Munafo et al., 2008), we hypothesized that amygdala might be the primary supraspinal region contributing to the association between 5-HTTLPR polymorphisms and emotional modulation of pain. However, other circuits may be involved. For example, Fukudo et al. (2009) found that, compared to l-carriers, s/s-carriers had greater activation in the perigenual ACC and parahippocampal gyrus during moderate visceral pain (colorectal distention at 40 mm Hg) and greater activation in the orbitofrontal cortex (OFC) during mild visceral pain (colorectal distention at 20 mm Hg). Therefore, our findings of differential association between 5-HTTLPR polymorphisms and emotional modulation of pain, but not NFR, may stem from the involvement of the ACC, OFC, and/or hippocampus given that (1) Roy et al. (2009) found activation in the orbitofrontal cortex and the subgenual ACC was associated with emotional modulation of pain but not NFR, and (2) the ACC, OFC, and hippocampus are innervated by serotonergic neurons originating in the dorsal raphe nucleus (Kandel, 2000). Whatever the circuitry, the present study lends further credence to the notion that different mechanisms mediate emotional modulation of pain vs. NFR, and provide new evidence that 5-HTTLPR polymorphisms differentially influence the two mechanisms.

It is worth noting that results from pain threshold, pain tolerance, and NFR threshold are consistent with the idea that 5-HTTLPR polymorphisms influence supraspinal, but not spinal, processing of nociceptive information. 5-HTTLPR polymorphisms were not associated with NFR threshold (spinal processing), but were associated with measures of pain threshold and tolerance (supraspinal processing). At this time, however, it is unclear why there was a sex-specific influence of genotype on pain sensitivity, but not emotional modulation of pain. It does suggest the mechanisms responsible for...
individual differences in pain sensitivity are different than those responsible for individual differences in pain modulation, given that sex influences one but not the other. Supporting this, measures of pain sensitivity correlate poorly with measures of pain modulation (Bhalang et al., 2005), despite the fact that individual differences in both appear to be associated with chronic pain risk (Edwards, 2005; Nielsen et al., 2009).

The genetic differences in emotional modulation of pain that we observed cannot be completely explained by differences in subjective reactions to pictures, because valence and arousal ratings did not differ by genotype. Relative to neutral pictures, attack pictures evoked greater unpleasantness and arousal, and erotic pictures evoked greater pleasure and arousal. Although a significant Content $\times$ Sex $\times$ Genotype interaction was noted for valence ratings, no significant genotype effects were found when the interaction was decomposed using Bonferroni adjusted mean comparisons. This lack of association between 5-HTTLPR polymorphisms and subjective reactions to emotional pictures is consistent with prior research (Brocke et al., 2006) and may reflect the fact that 5-HTTLPR polymorphisms are associated with amygdala function, a supraspinal region whose activity is uncorrelated with subjective reactions to pictures (Zald, 2003). Rather, subjective reactions to pictures appear to correlate more so with activity in the hippocampus and orbitofrontal cortex (Garrett and Maddock, 2006).

4.3. Implications

While speculative at this time, the enhanced emotional modulation of pain in s-carriers could reflect a diathesis for the development of chronic pain. Following a stressful event (e.g., psychological or physical trauma), s-carriers have been shown to be at increased risk for developing affective disorders (e.g., depression) which increases negative affect and decreases positive affect (e.g., Caspi et al., 2003; Karg et al., 2011). Doing so could tip the pain modulatory balance toward relatively greater pain facilitation and less pain inhibition. Thus, enhanced emotional modulation of pain may serve as the diathesis in a stress–diathesis model for chronic pain risk. Future longitudinal studies that take into account both genetic and environmental factors are needed to address this hypothesis.

The lack of an association between 5-HTTLPR polymorphisms and either NFR threshold or emotional modulation of NFR suggests that the descending brain-to-spinal cord mechanisms examined in the present study are not influenced by 5-HT function. Therefore, it appears that the capacity to protect the lower limbs from an acute noxious event (e.g., stepping on a piece of glass) is not affected by 5-HTTLPR polymorphisms, whereas the complex processes involved in the painful perception of such an event may be affected.

4.4. Study limitations

This study had a number of strengths including well-validated methods to study emotional modulation of pain/NFR, subjective and physiological measures of noiceptive processing, and a measure of spinal nociception that allowed us to examine brain-to-spinal cord mechanisms. Nonetheless, a few limitations should be noted. First, the procedures and equipment used were slightly different between testing sites. For example, the Oklahoma site presented more pictures and additional picture contents; thus, the testing session was longer. However, we have shown that a longer testing session does not affect the magnitude of emotional modulation (Rhudy et al., 2010a). Moreover, preliminary analysis indicated that including testing site as a factor in the analyses did not change any of the study conclusions.

Second, we used a single ascending staircase of electric stimulations to assess electrocutaneous pain threshold and tolerance. While these outcomes were not the main focus of the study, these results should be interpreted with caution until a more sophisticated design can be used to improve the assessment (e.g., multiple random staircase procedure) (Gracely et al., 1988). Third, although we did exclude for use of antidepressant and anti-anxiety medications, we did not systematically exclude for psychiatric disorders in our sample.

Fourth, as is often the case in genetic studies of pain (e.g., Diatchenko et al., 2005), the effect sizes in the present study were relatively small. This likely reflects the multitude of gene, environment, and gene $\times$ environment interactions that influence pain processing. For budgetary reasons we focused on only one single nucleotide polymorphism (SNP). Not only is it possible that other genes might influence emotional modulation of pain (e.g., COMT, OPRM1), but it is also possible that other SLC6A4 polymorphisms could contribute. The 5′ promoter repeat difference assessed in this study (5-HTTLPR), indicated as l and s, represents the major variation, but is not the only variant of the SLC6A4 gene. Another common variant is the LA and LC polymorphism (rs25531) (Hu et al., 2006). Interestingly, a recent study by Kosek et al. (2009) found that combinations of the 5-HTTLPR and rs25531 polymorphisms that demonstrate low 5-HT transcriptional efficacy (i.e., LA/Ss and SA/Ss) have greater analgesic response to an exogenous opioid compared to other polymorphism combinations. This suggests an alteration of pain modulatory circuitry which could have implications for emotional modulation. Thus, it is important to recognize that a complete understanding of the SLC6A4 associations with pain modulation will have to include multiple SNPs as well as other influences such as copy number variants (CNV), epigenetic methylations, miRNA-based control parameters, and a thorough consideration of environmental factors. Future research is needed to address these other potential influences. Nonetheless, it is noteworthy that we were able to detect genetic differences in emotional modulation of pain from a single SNP.

5. Conclusions

Despite these limitations, to our knowledge this is the first study to examine genetic factors that influence emotional modulation of pain and spinal nociception. Our results are consistent with prior research noting that s-carriers have altered supraspinal processing in response to emotional stimuli (Canli et al., 2008; Munafò et al., 2008). We found that, compared to l/l-carriers, s-carriers demonstrated greater pain facilitation during unpleasant pictures and greater pain inhibition during pleasant pictures. Further, male s-carriers demonstrated hypoalgesia on supraspinal measures of pain (electrocutaneous pain threshold and tolerance), but there were no 5-HTTLPR polymorphism associations with NFR threshold (spinal nociceptive processing) or emotional modulation of NFR. These alterations in supraspinal pain processing might put healthy s-carriers at risk for developing chronic pain; however, future research is needed to evaluate this hypothesis.

Acknowledgements

This work was partially funded by a Health Research Award (HR06-177) from the Oklahoma Center for the Advancement of Science and Technology awarded to Jamie L. Rhudy, Ph.D. The authors would like to thank Carl Lattimore, Jennifer DelVentura, Ellen Terry, Kara Kerr, Mary Chandler, Ashley Vincent, Emily Main, and Fred Kurzban for their assistance in data collection for this study.

References

Pezawas, L., Meyer-Lindenberg, A., Drabant, E.M., Verchinski, B.A., Munoz, K.E., Kolachana, B.S., et al., 2005. 5-HTTLPR polymorphism impacts human circumlate-
Sarlani, E., Greenspan, J.D., 2005. Why look in the brain for answers to temporo-
Roy, M., Piche, M., Chen, J.-I., Peretz, I., Rainville, P., 2009. Cerebral and 
Rhudy, J.L., Williams, A.E., McCabe, K., Nguyen, M.A., Rambo, P., 2005. Affective mod-
Rhudy, J.L., France, C.R., 2007. Defining the nociceptive flexion reflex (NFR) thresh-