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Emotional modulation of muscle pain is associated with polymorphisms in the serotonin transporter gene

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ABSTRACT

The perception of pain is determined by a combination of genetic, neurobiological, cultural, and emotional factors. Recent studies have demonstrated an association between specific genotypes and pain perception. Particular focus has been given to the triallelic polymorphism in the promoter region of the serotonin transporter gene in relation to pain perception. The aim of this study was to investigate whether the modulatory effect of emotions mediated by visual stimuli on muscular pain perception is genotype dependent. A total of 150 healthy subjects were selected on the basis of their polymorphism in the serotonin transporter gene. First, visual conditioning was performed with positive, negative, and neutral pictures from the International Affective Picture System, and the unpleasantness/pleasantness of the pictures was rated. Second, visual conditioning stimuli were presented while experimental jaw muscle pain was evoked by injection of hypertonic saline into the masseter muscle, and participants continuously rated pain intensity on an electronic visual analogue scale. The pictures induced similar changes in emotions across the 3 genotype groups, and hypertonic saline evoked moderate pain levels in all participants. However, in participants with a high expression of the serotonin transporter protein, conditioning with negative pictures increased pain intensity and positive pictures decreased pain intensity when compared with neutral pictures. In contrast, there were no significant effects of the pictures on pain perception in participants with either intermediate or low expression of the protein. These results suggest that polymorphisms in the serotonin transporter gene play an important role in emotions modulation of muscle pain.

1. Introduction

Sensitivity and expression of pain vary widely between individuals [7]. Studies have reported associations between specific genes and pain perception (eg [22,28–30,44,54]) and pain pathologies [6,23,39,49]. Serotonin (5-HT) is known to modulate nociception through peripheral and central mechanisms [36,37], but it is also known to be highly involved in mood regulation [32,46] and may therefore both directly and indirectly modulate pain.

A key player in 5-HT signaling is the serotonin transporter (5-HTT), which regulates the uptake of serotonin into the presynaptic neuron for recycling or degradation after serotonin has been released, thus playing a critical role in determining the duration and intensity of serotonin communication (for review see [15]). The serotonin transporter is coded by a single gene (*SLC6A4*) located on the long arm of chromosome 17 [25]. A well-described polymorphism in the promoter region of the gene (5-HTTLPR) appears to influence the efficiency with which the 5-HTT returns serotonin into the presynaptic neuron [47] by affecting the expression of *SLC6A4*. This polymorphism consists of a variation of the GC-rich repetitive sequence. A deletion/insertion in the 5-HTTLPR creates a short (S) allele and a long (L) allele. The s-allele is coupled to a reduced gene expression, leading to lower densities of 5-HTT receptors [17,18,26].

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The single-nucleotide polymorphism (SNP) rs25531, which consists of an A/G substitution, is also located in the promoter region of the *SLC6A4*. The G-allele is mostly linked to the L-allele of the 5-HTTLPR and has been shown to reduce its transcriptional efficacy to the level of the s-allele [20,58]. The 5-HTTLPR together with rs25531 are referred as to the "triallelic" 5-HTTLPR and permit a functional division of individuals into having a high, intermediate, or low expression of the 5-HTT [58]. Persons with a low expression of 5-HTT have been associated with higher scores of neuroticism and anxiety traits [14,26,34]. It has been suggested that individuals with a low expression of the protein have a higher predisposition for depression [21], eating disorders [5] and attention deficit disorder [11], among others. It is possible then that the variation in 5-HTT expression can affect pain perception by generating an overall negative emotional state.

In a previous study we reported that observing negative pictures generates an overall negative emotion and an increase in pain perception in healthy subjects; however, this behavior was not observed in all participants [19].

In a recent study, Lindstedt and colleagues reported that healthy subjects with a low expression of 5-HTT exhibited a lower degree of conditioned pain modulation-mediated inhibition of pressure and heat pain sensitivity [27], suggesting that individuals with a low expression of the protein modulate the perception of pain differently from those with a high expression.

The aim of this study was to analyze whether the emotional modulation of muscle pain perception observed in prior studies differs between groups of individuals with different triallelic polymorphism.

2. Methods

2.1. Participants

A total of 150 healthy volunteers were selected from a pool of 379 healthy individuals of Scandinavian descent on the basis of their triallelic 5-HTTLPR polymorphism. The participants were divided into 3 groups: low (S_A/S_A, L_G/S_A, L_G/L_G), intermediate (L_G/L_A, L_A/S_A) and high expression of 5-HTT (L_A/L_A). Each group consisted of 50 participants. All participants gave informed consent upon having received detailed information on the study. The participants received monetary compensation (500 DKR) upon finishing the experiment. Participants were recruited on the basis of the triallelic 5-HTTLPR genotype from a pool of 379 healthy individuals of Scandinavian descent. The subjects in the pool had provided informed consent for DNA analysis and agreed to be contacted for an invitation to participate in further studies.

Participants were excluded from the DNA analysis and further experiments if they had any chronic pain condition, were smokers, pregnant, used medication on a regular basis (except for contraceptives), or if they had any known psychological, cardiovascular or neurological disorder.

The study was conducted according to the Declaration of Helsinki and was approved by the local ethical committee (20110165) and the Danish Data Protection Agency (2011–41–6562).

2.2. DNA-analysis

Samples for DNA extraction were obtained from saliva collected using an OC-100 kit (DNA Genotek Inc., Ontario, Canada). To determine the triallelic 5-HTTLPR genotype, PCRs were carried out in a total volume of 25 μL using the GoTaq Hot Start Polymerase (Promega, Wisconsin, USA) and 80 ng of genomic template. The forward primer sequence was 5′-CTCTGAATGCCAGCACCTAACCC-3′ and the reverse 5′-GATTCTGGTGCCACCTAGACGC-3′. Samples were amplified (Gene Amp, PCR System 9700, Applied Biosystems, California, USA) by 2-step PCR consisting of an activation step of 2 minutes at 94°C, followed by 35 cycles of 30 seconds of denaturation at 93°C, and an annealing and elongation step for 1 minute at 62°C, followed by a final elongation step of 10 minutes at 72°C. The L-allele and S-allele of the 5-HTTLPR yield a product of 529 bp and 486 bp, respectively.

Fragments were visualized with UV after 45-minute separation at 80 V on a 2.5% agarose gel. In order to ensure that the primers amplified the correct DNA region, 2 random samples were selected, and the PCR product was purified (Jet Quick PCR product purification, Genomed, Löhne, Germany) and sequenced by Eurofins MWG Operon (Ebersberg, Germany).

To determine the rs25531 polymorphism, 10 μL of the PCR product was digested for 2 hours at 37°C with 1 μL of MSP1 at 20,000 units/mL (New England Biolabs, Ipswich, MA, USA) and 1 μL of buffer per sample. The enzyme cuts at a 5′-C/CGC-3′ sequence, resulting in fragments of different length which determined the triallelic genotype (Table 1). The digested fragments were visualized by UV light after 2-hour separation at 100 V on a 4% agarose gel. This protocol resulted in a genotyping consistency rate of 98%.

2.3. Study design

Participants were asked to come to one session where 3 blocks of visual stimuli were applied in order to promote positive, negative and neutral emotions. The order of the 3 conditioning stimuli was randomized by a computer. The study was performed during the day in a quiet room at normal room temperature. The session was divided into two parts.

The first part studied how visual conditioning influenced the participants' emotional state. First, the participants completed the Positive and Negative Affective Schedule (PANAS) [56] to test their current emotional state and then the Balanced Emotional Empathy Scale (BEES) [35] to test their level of empathy as it could influence the extent to which the participants would be involved in and affected by the pictures. The participants were exposed to 3 sets of pictures (positive, negative and neutral) for 5 minutes each, separated by breaks of 5 minutes. While watching the pictures, the participants were asked every minute to rate the last minute's pictures as pleasant, unpleasant or neutral on a –10 to +10 numerical rating scale (NRS), with –10 being "maximum unpleasant," 0 being "neutral" and +10 being "maximum pleasant." During each break the participants were asked to fill in the PANAS in order to assess if the pictures influenced their emotional states.

The second part was an examination of the participants' pain perception during the visual conditioning stimuli. The participants received a total of 3 injections of sterile 5% hypertonic saline (HS) with two on one side of the face and one on the other side. Each injection was accompanied by a 5-minute exposure to one set of the visual conditioning stimuli in random order. As in the first part, the participants were asked to rate the pleasantness/unpleasantness of the visual stimuli every minute on the NRS, and moreover

Table 1
Base pairs of each band after digesting the PCR product for 2 hours at 37°C with MSP1 to identify the rs25531 polymorphism.

Genotype	L_A/L_A	L_A/L_G	L_G/L_G	S_A/L_A	S_A/L_G	S_A/S_A
Bands	330	156 174 330	156 174	287 330	156 174 287	287
Protein expression	High	Intermediate	Low	Intermediate	Low	Low