

## References

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## Introduction

COMT is an enzyme which is involved in a number of physiological functions, including the degradation of catecholamine neurotransmitters after their release in the synaptic cleft (Mannisto and Kaakkola, 1999).

Despite earlier studies suggested that COMT polymorphisms affect pain processing (Zubieta et al., 2001), the existence of an effect of COMT variation on pain sensitivity is still strongly debated (e.g., Kim et al., 2004)

In order to understand the sources of this inconsistency in the literature, here we investigated the effects of the COMT val158met polymorphism (which leads to a four-fold decrease in enzyme activity in met homozygotes) on pain processing using fMRI. We hypothesized that:

- fMRI might be more sensitive to potential genotype differences in pain processing than verbal ratings, since brain activity measures are more proximal ('intermediate') phenotypes to perception than subjective reports
- the effect of COMT polymorphism on pain processing would be apparent only in the setting of repeated pain challenge, as proposed by Jensen and colleagues (Jensen et al., 2009).

## Study Design and Methods

### Subjects

In the present study, we pooled data from the identical 'baseline runs' of three experiments to obtain a total of 54 healthy normal right handed subjects.

### Experimental Design

During the fMRI session, subjects received on the right volar forearm two identical pseudorandom sequences of calibrated heat pain stimuli: 4 LOW (~5/20 on a 0-20 scale), and 4 HIGH (~15/20) per run. See Figure 1 and Figure 2 caption for more details.



Figure 1. Structure of the pain trials

### Data Acquisition and Analysis

Brain imaging was performed with a 3 Tesla Siemens MRI System (Allegra/Trio) equipped for echo planar imaging. Thirty axial interleaved slices (4 mm thick with 1 mm skip) were acquired with TR=2000 ms, TE=40 ms, flip angle= 90°, and a 3.13 × 3.13 mm in-plane spatial resolution.

fMRI data processing was carried out using FSL's FEAT (fMRI Expert Analysis Tool), and included fieldmap unwarping, motion correction, non-brain removal, spatial smoothing (FWHM=5mm).

For each subject, the following contrasts were computed using the GLM: 'LOW pain vs. baseline' and 'HIGH pain vs. baseline'.

Based on the consistently reported observation that the effect of the COMT val158met polymorphism on pain sensitivity is generally not observed for the initial pain provocations, but rather becomes apparent in later phases of a testing session, we performed these analyses separately for each of the two runs. Group level analyses were carried out to compare brain responses to low and high pain stimuli across genotypes using FLAME stage 1. In accordance with the observation that val/val and met/met homozygotes are characterized by the strongest and weakest COMT enzymatic activity respectively (while val/met exhibit intermediate activity) a direct comparison was performed between the two homozygote groups. Z (Gaussianised T/F) statistic images were thresholded using clusters determined by Z>1.96 and a (corrected) cluster significance threshold of P=0.05.

Gene sequencing was performed according to standard methods. Genomic deoxyribonucleic acid (DNA) was extracted and quantified using well established methods. The DNA was used to determine the individual COMT genotypes of the subjects by direct resequencing using ABI capillary platform (ABI 3730xl). The polymerase chain reaction (PCR) was used to amplify the region of the COMT gene that contains the valine to methionine polymorphism at nucleotide 158. Both strands of PCR products were sequenced using the standard resequencing protocol (Current Protocols in Human Genetics v.2, unit 7.9, October 2008). Sequence reads were analyzed by PolyPhred. Mutations were scored on both strands

## Results

### Subjects

The genetic analyses revealed that 12 (~22.2%) subjects were homozygous for the 158met allele, 20 (~37%) homozygous for the 158val allele, and 22 (~40.7%) were heterozygous. No significant age difference was observed among the three groups, F(2,51)=1.493, p>0.05.

### Psychophysical results

As expected based on our experimental design, we did not detect any group differences in the evoked pain ratings (main effect of GENOTYPE: F(2, 52)= 0.67, p=0.51, n.s.; GENOTYPE \* STIMULUS RATING interaction: F(2,52)=2.15, p=0.13, n.s.). No differences were observed even when separate analyses were performed for each run independently.

Further, we did not observe any group differences in the temperatures individually calibrated to evoke the target pain levels either (main effect of GENOTYPE: F(2, 52)=1.71, p=.19, n.s.; GENOTYPE \* STIMULUS LEVEL interaction: F(2,52)=0.10, p=0.90, n.s.).

### fMRI results

In all groups, pain stimuli evoked intensity dependent (HIGH pain > LOW pain) fMRI signal increases in many of the areas commonly seen active in pain imaging studies

In the second run, the met/met subjects, compared to val/val subjects, exhibited higher BOLD signal in response to HIGH pain in a number of cortical and subcortical structures

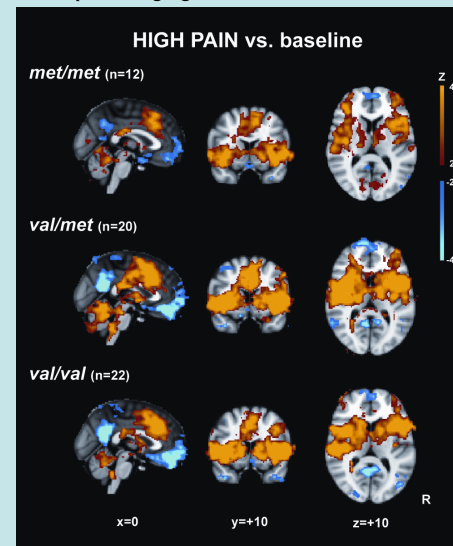


Figure 2. HIGH-pain related brain activations, overlaid on the MNI152 standard brain.

Areas activated during heat pain included primary somatosensory cortex (S1), anterior/middle cingulate cortex, insula, superior and inferior parietal lobules, secondary somatosensory cortex (S2), frontal poles, occipital cortex, thalamus, putamen, periaqueductal gray (PAG), medulla and cerebellum, which is consistent with published reports (Apkarian et al., 2005; Kong et al., 2006a; Kong et al., 2006b).

Stimulus intensities were individually calibrated in a pre-imaging training session, and possibly readjusted before the imaging session. Heat stimuli were delivered using a TSA-2001 Thermal Sensory Analyzer with a 3 cm × 3 cm probe (Medoc Advanced Medical Systems, Rimat Yishai, Israel) running the COVAS software.

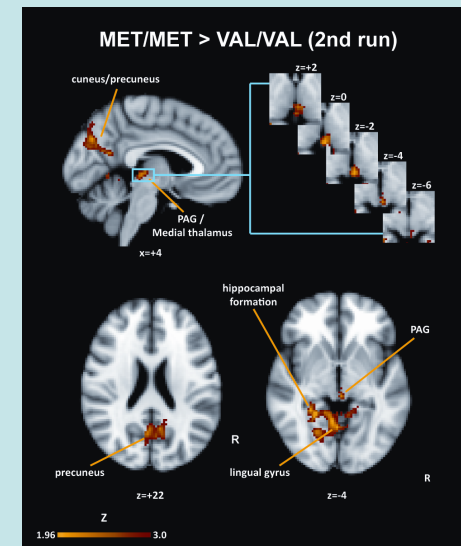


Figure 3. fMRI activations observed for the 'met/met vs val/val' contrast (2nd run, HI pain)

Areas with stronger HI-pain related BOLD signal in the met/met subjects in the second run including the periaqueductal gray matter (PAG), medial thalamus, hippocampal formation, lingual gyrus, calcarine cortex, precuneus, cuneus, superior and middle occipital gyri and cerebellum (Table 2 and Fig. 2). No brain regions showed higher pain-related BOLD signal in the val/val.

## Conclusions

We conclude that COMT does appear to affect pain processing. Our data suggest several possible explanations to account for the inconsistency in the literature including: 1) the need for a sufficiently robust challenge to the pain system to detect a genotype effect, 2) the recruitment of pain-dampening compensatory mechanisms by the putatively more pain sensitive met homozygotes and/or 3) the greater sensitivity of the fMRI signals as compared to verbal ratings to COMT genotype as a more proximal phenotype to perception.

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