Hypnotizability and Catechol-O-Methyltransferase (COMT) polymorphisms in Italians

Silvano Presciutti1, Alessandro Gialluisi1,4, Serena Barburti1, Michele Curcio1, Fabrizio Scatena3, Giancarlo Carli1,4 and Enrica L. Santarcangelo1,4 *

1 Laboratory of Cognitive and Behavioral Neurosciences, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy
2 Language and Genetics Department, Max Planck Institute for Psycholinguistics, Nijmegen, Netherlands
3 Immunohematology Unit, Azienda Ospedaliera–Università Pisana, Pisa, Italy
4 Department of Medicine, Surgery and Neuroscience, University of Siena, Siena, Italy
5 Laboratory of Neurology, University of Rome, Italy
6 Hospital of Liege, Belgium
7 Department of Neurosciences, University of Geneva, Switzerland
8 Center and University Hospital of Nice, France
9 *Correspondence: Enrica L. Santarcangelo; Laboratory of Cognitive and Behavioral Neurosciences, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Via San Zeno 31, 56127 Pisa, Italy
email: enricals@dfb.unipi.it

Higher brain dopamine content depending on lower activity of Catechol-O-Methyltransferase (COMT) in subjects with high hypnotizability scores (highs) has been considered responsible for their attentional characteristics. However, the results of the previous genetic studies on association between hypnotizability and the COMT single nucleotide polymorphism (SNP) rs4680 (Val158Met) were inconsistent. Here, we used a selective genotyping approach to re-evaluate the association between hypnotizability and COMT in the context of a two-SNP haplotype analysis, considering not only the Val158Met polymorphism, but also the closely located rs4818 SNP. An Italian sample of 53 high, 49 low hypnotizable subjects (lows), and 57 controls, were genotyped for a segment of 805 bp of the COMT gene, including Val158Met and the closely located rs4818 SNP. Our selective genotyping approach had 97.1% power to detect the previously reported strongest association at the significance level of 5%. We found no evidence of association at the SNP, haplotype, and diplotype levels. Thus, our results challenge the dopamine-based theory of hypnotizability and indirectly support recent neuropsychological and neurophysiological findings reporting the lack of any association between hypnotizability and focused attention abilities.

Keywords: hypnotizability, attention, COMT, absorption, selective genotyping, haplotype analysis

INTRODUCTION
The cognitive trait of hypnotizability (Green et al., 2005) – the ability to accept hypnotic suggestions – has been classically attributed to peculiar characteristics of the supervisory attentional system (Norifman and Shallice, 1986; Posner and Fan, 2004) allowing a more flexible attentional control in the subjects scoring high (highs) at hypnotizability scales. In fact, a few neuropsychological (Telelegen and Atkinson, 1974; Zachariae et al., 2000) and genetic studies (Lichtenberg et al., 2010; Raz, 2005; Raz et al., 2006; Szeszko et al., 2010) have suggested greater abilities of focused attention in highs with respect to low hypnotizable individuals (lows), based on higher dopaminergic activity. In the general population, attention seems to be more efficiently controlled in subjects with the Met/Met or Val/Val variant of the single nucleotide polymorphism (SNP) rs4680 at the catechol-O-methytransferase (COMT) gene than in the homozygous Val/Val individuals (Seamans and Yang, 2004). In fact, the Met/Met variant shows 40% less enzymatic activity than the Val/Val and, thus, is associated with higher dopamine levels in the prefrontal (Rousos et al., 2008) and anterior cingulate cortex (Bläs et al., 2005).

The three association studies conducted so far on the relation between the COMT Val158Met polymorphism and hypnotizability have provided inconsistent results. Two of them (Lichtenberg et al., 2000; Raz, 2005) applied analysis of variance on the hypnotizability scores in subjects stratified by the COMT genotype. In this approach, a sample of subjects not selected for hypnotizability (thus representing the distribution of this trait in the general population) is genotyped, and ANOVA is used to test the differences of the mean hypnotizability scores among the genotypes. Both studies reported a higher mean score of hypnotizability in heterozygotes (Met/Val) than in both homozygotes (Val/Val, Met/Met), but in one of them the association between hypnotizability and COMT polymorphism was significant in females only (Lichtenberg et al., 2000). On the contrary, the third study (Szészko et al., 2010) using the same approach reported intermediate hypnotizability scores in heterozygotes; these authors also contrasted the highs and lows recruited in the sample for genotype frequencies, and found a significantly higher frequency of the Val allele among highs.

Thus, the first aim of the present study was to re-evaluate the relationship between the rs4680 (Val158Met) COMT variant and hypnotizability through a selective genotyping approach. It should be noticed also that the COMT locus is polymorphic for many other SNPs that may interact in a complex way to determine phenotypic differences among individuals (Duttenko et al., 2008; Nuckley et al., 2006; Rousos et al., 2008). This occurs, for instance, for the coding regions rs4653...
As a control group representative of the general population, 57 students of the Universities of Pisa and Siena (410 M, 633 F). Consensus on the employment of umbilical cords (Controls) from the Immuno-hemathology Unit Bank at the Azienda Ospedaliera–Universitaria Pisana, were genotyped anonymously (Controls). Consensus on the employment of the umbilical cords for research had been obtained from mothers at the Azienda Ospedaliera–Universitaria Pisana soon after delivery.

DNA EXTRACTION, AMPLIFICATION AND ANALYSIS

Genomic DNA was isolated by the QIAamp DNA Blood kit (QIAGEN GmbH, Hilden, Germany) according to manufacturer’s instructions from highs and lows’ peripheral blood leukocytes. The same was done with umbilical cords samples from (Controls). For privacy requirements, blood samples were coded anonymously. The DNA extracted from 200 μl of blood was diluted with 200 μl of H2O, quantified by UV measurement at OD 260 nm and stored at −20°C until further processing. Later, the DNA sample was restored at a normal temperature and underwent a polymerase chain reaction (PCR) aimed at amplifying the target region in the COMT gene, i.e., a portion of 805 bp containing the exon 4, in which the SNP rs4818 (Leu136Leu) and rs4680 (Val158Met) were present at low frequency (0.02) in the control sample. The EM algorithm produced the haplotype frequency estimates shown in Figure 1. One of the four possible haplotypes (G-A, in the order rs4818–rs4680) was absent from both highs and lows, meaning complete linkage disequilibrium, whereas it was present at low frequency (0.02) in the control sample.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>MetMet</th>
<th>MetVal</th>
<th>ValVal</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>highs</td>
<td>11</td>
<td>25</td>
<td>17</td>
<td>53</td>
<td>0.443</td>
</tr>
<tr>
<td>lows</td>
<td>20.8</td>
<td>472</td>
<td>32.1</td>
<td>49</td>
<td>0.367</td>
</tr>
<tr>
<td>Controls</td>
<td>20.4</td>
<td>32.7</td>
<td>46.9</td>
<td>57</td>
<td>0.569</td>
</tr>
</tbody>
</table>

1 Haplotype frequency.
Table 2 | Joint genotype distribution of rs4818 (C/G, or Leu136Leu) and rs4680 (G/A, or Val158Met), in three population samples.

<table>
<thead>
<tr>
<th></th>
<th>Highs</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4680</td>
<td>AA</td>
<td>GA</td>
<td>GG</td>
<td>Total</td>
<td>AA</td>
<td>GA</td>
<td>GG</td>
</tr>
<tr>
<td>CC</td>
<td>11</td>
<td>8</td>
<td>3</td>
<td>22</td>
<td>11</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>CG</td>
<td>0</td>
<td>17</td>
<td>4</td>
<td>21</td>
<td>0</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>GG</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>25</td>
<td>17</td>
<td>53</td>
<td>10</td>
<td>16</td>
<td>23</td>
</tr>
</tbody>
</table>

1AA, (MetMet); GA, (MetVal); GG, (ValVal).

The large overlap of the 95% confidence intervals of the three samples makes it clear that there is no association between hypnotizability and these COMT haplotypes. Indeed, there was no evidence of heterogeneity ($\chi^2 = 3.78$, d.f. = 5, $p = 0.582$) in highs and lows also for the absolute frequencies of the two-SNP diplotypes.

DISCUSSION

The present study does not show any association between COMT polymorphisms and hypnotizability at the SNP, haplotype, and diplotype levels.

GENETIC FINDINGS AND NEUROPSYCHOLOGICAL EVIDENCE ON HYPNOTIZABILITY-RELATED ATTENTIONAL ABILITIES

Previous studies (Lichtenberg et al., 2000; Raz, 2005; Raz et al., 2006; Szekely et al., 2010) presented some evidence of association between the Val158Met polymorphism and hypnotizability (Table 3).

The most important discrepancy concerns our results and those reported by Szekely et al. (2010). That work found a significant difference in allele frequencies between highs and lows, but its power was limited by the necessarily small proportion of highs in the samples ($N = 19$), which is due to the distribution of hypnotizability scores in the general population (Balthazard and Woody, 1989; De Pascalis et al., 2000; Carvalho et al., 2008). Our alternative approach of selective genotyping, in which individuals are sampled from the opposite tails of a quantitative trait, can substantially increase the power of population-based associations studies (Schork et al., 2000; Van Gestel et al., 2000).

It should be noted that the odds ratio of the Val allele (recalculated from published data) is 3.0 in the work by Szekely and coll. (Szekely et al., 2010), whereas it is 0.7 in our data, and the 95% CI do not overlap. The power of our study to detect significant heterogeneity of allele frequency between highs and lows, if their frequency were as in (Szekely et al., 2010), was 97.1% at the significance level of 5%, and it was 89.8% at the significance level of 1%.

Theoretically, the different methods of hypnotic assessment between studies might account for the different results, but we consider this unlikely, as the methods used in the present and in other works provide highly correlated results (Sheehan and McConkey, 1982). Another factor possibly accounting for the discrepancy is a different level of association in different populations; this can happen if the association is caused by a nearby locus that shows variable levels of linkage disequilibrium among populations.

The present results are in line with the findings showing the absence of any hypnotizability-related difference in attentional tests (Varga et al., 2011), and also the absence of significant correlation between COMT polymorphism and executive attention performance as measured by Posner Attentional Network Test (Fossella et al., 2002). Moreover, recent neuropsychological studies contrast the classical view of hypnotizability based on high abilities of focused attention and attribute the hypnotizability-related cognitive characteristics to impaired frontal executive functions inducing a lower capacity to disengage attention from its current focus (Jameson and Sheehan, 2004; Egner et al., 2005).

Finally, the assumption that the larger content of the homovanillic acid (HA) found in highs (Spiegel and King, 1992) depends on reduced DOPA catabolism (responsible for high abilities of focused attention) is weak, as HA is a catabolite of both dopamine and norepinephrine and its content in the cerebrospinal fluid derives from their catabolism in several neural circuits (Gu,
Table 3 | Genetic association studies between the COMT Val158Met polymorphism and hypnotizability.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>MetMet</th>
<th>MetVal</th>
<th>ValVal</th>
<th>Total</th>
<th>p(A)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>77</td>
<td>41</td>
<td>19</td>
<td>137</td>
<td>0.712</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>29.2</td>
<td>28.9</td>
<td>13.9</td>
<td></td>
<td></td>
<td>highest hypnotizability score in heterozygotes</td>
</tr>
<tr>
<td>Mean HS</td>
<td>5.2</td>
<td>6.6</td>
<td>4.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANOVA based approaches</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lichtenberg et al. (2000) Unstratified</td>
<td>18</td>
<td>33</td>
<td>25</td>
<td>76</td>
<td>0.454</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>24.7</td>
<td>43.4</td>
<td>32.9</td>
<td></td>
<td></td>
<td>Highest hypnotizability score in heterozygotes; no significance test provided</td>
</tr>
<tr>
<td>Mean HS</td>
<td>6.1</td>
<td>7.6</td>
<td>5.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raz (2005)</td>
<td>Unstratified</td>
<td>30</td>
<td>66</td>
<td>31</td>
<td>127</td>
<td>0.496 ANOVA significant for genotype effect (p = 0.016); medium score in heterozygotes</td>
</tr>
<tr>
<td>%</td>
<td>23.6</td>
<td>52.0</td>
<td>24.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean HS</td>
<td>4.1</td>
<td>4.7</td>
<td>5.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Categorical data analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Szekely et al. (2010) highs (mean HS 9.3 ± 1)</td>
<td>1</td>
<td>9</td>
<td>9</td>
<td>19</td>
<td>0.289</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>5.2</td>
<td>47.4</td>
<td>47.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean HS 2</td>
<td>4.2</td>
<td>4.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lows (mean HS 2.6 ± 0.7)</td>
<td>15</td>
<td>34</td>
<td>9</td>
<td>58</td>
<td>0.552</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>25.9</td>
<td>58.6</td>
<td>15.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Met allele frequency; 2 Hypnotizability Score; 3 Confidence Interval; 4 The corresponding value of our data was 0.7; 95% CI: 0.4–1.3

MECHANISMS INDEPENDENT OF COMT POLYMORPHISMS POTENTIALLY INVOLVED IN HYPNOTIZABILITY-RELATED ATTENTIONAL CHARACTERISTICS

The highs’ attention seems to be stable rather than flexible. A few authors suggest that the carriers of the Met allele might be comparatively high in cognitive stability, but low in cognitive flexibility (Cools, 2008; Cools and D’Esposito, 2009; Colzato et al., 2010). High flexibility would be associated with great distractibility, while high stability may be related to scarce distractibility (Goschke, 2000), as suggested for highs’ (Tellegen and Atkinson, 1974; Lichtenberg et al., 2000, 2004; Zachariae et al., 2000; Raz, 2005; Raz et al., 2006). The balance between cognitive flexibility and stability (Cools and D’Esposito, 2009; Durvas and Palmiter, 2011) could depend on the interaction between the dopaminergic circuits of the prefrontal cortex (where the catecholamines metabolism relies mainly on the activity of the COMT) and of the striatum, where the catecholamines metabolism depends mostly on the mono amino oxidase (MAO) enzymatic system (Durvas and Palmiter, 2011).

Actually, polymorphisms in MAO have also been found associated with executive attention and with alerting efficiency (Fossella et al., 2002). Thus, different attentional performance could be accounted for by a peculiar balance between the catecholamines degradation occurring in different brain structures.

However, the existence of multiple subtypes of highs and lows (Balthazard and Woody, 1989; Pekala and Forbes, 1997; Green and Lynn, 2011; Terhune et al., 2011) suggests that it is unlikely that one biological determinant may account for such a complex trait like the susceptibility to hypnosis, and we may expect that several neurotransmitters and neuromodulators influence hypnotizability (Ott et al., 2005; Klinkenberg et al., 2011). Recent evidence suggests a role for nitric oxide (NO) because the hypnotizability-related vascular responses to cognitive and physical stimulation indicate greater NO availability in the highs’ vessels (Jambrik et al., 2004; Jambrik et al., 2005). In the brain, endothelial NO is responsible for basal vascular tone, interacts with other mediators in its modulation, and acts as a neurotransmitter after diffusion to the extracellular compartment (Andresen et al., 2006). Using an in vivo brain microdialysis technique, it has been demonstrated that NO significantly increases the release of acetylcholine and decreases the
release of dopamine in the rat striatum (Guarva-Guzman et al., 1994), while increasing its metabolism (Nabishima et al., 1987; Loscher et al., 1991). Thus, a greater NO availability modulating both dopamine and acetylcholine production may account for the observed higher HA content in the cerebrospinal fluid (Spiegel and King, 1992), higher arousal (Castellani and Sebastiani, 2008) and greater attentional stability (Colato et al., 2010) of highs with respect to lows.

Concluding remarks

The observed absence of any association between hypnotizability and COMT polymorphisms/haplotypes prompts reconsideration of the theory indicating a generally reduced brain DOPA catalysema as responsible for the attentional abilities of the subjects with high hypnotizability. The findings on the nitric oxide vascular availability open frontier research on possible alternative bases.

Author Contributions

Silvano Presciutti, Giancarlo Carli, and Enrica L. Santarcangelo have designed the study and written the paper; Serena Barbuti, Michele Carli, and Fabrizio Scatena have performed the DNA analysis; Alessandro Galli and Silvano Presciutti have performed statistical analyses. We are grateful to E. Castellani, M. Menzocchi, G. Paoletti, E. Scattina for their technical assistance; Alessandro Gialluisi and Silvano Presciutti have done the electrical recording; Serena Barbuti, Silvano Presciutti, Giancarlo Carli, and Enrica L. Santarcangelo have designed the study and written the paper.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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