Genetic variants of the NMDA receptor influence cortical excitability and plasticity in humans

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1Clinica Neurologica, Dipartimento di Neuroscienze, Università Tor Vergata, Rome; 2Fondazione Santa Lucia/Centro Europeo per la Ricerca sul Cervello (CERC), Rome; 3Clinica Psichiatrica, Dipartimento di Neuroscienze, Università Tor Vergata, Rome; and 4Centro Ricerca Biomedica Applicata, Policlinico S. Orsola-Malpighi, Bologna, Italy

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Mori F, Ribolsi M, Kusayanagi H, Siracusano A, Mantovani V, Marasco E, Bernardi G, Centonze D. Genetic variants of the NMDA receptor influence cortical excitability and plasticity in humans. J Neurophysiol 106: 1637–1643, 2011. First published July 13, 2011; doi:10.1152/jn.00318.2011. —N-methyl-D-aspartate (NMDA) receptors play crucial roles in glutamate-mediated synaptic transmission and plasticity and are involved in a variety of brain functions. Specific single nucleotide polymorphisms (SNPs) in the genes encoding NMDA receptor subunits have been associated with some neuropsychiatric disorders involving altered glutamate transmission, but how these polymorphisms impact on synaptic function in humans is unknown. Here, the role of NMDA receptors in the control of cortical excitability and plasticity was explored by comparing the response to single, paired, and repetitive transcranial magnetic stimulations of the motor cortex in 77 healthy subjects carrying specific allelic variants of the NR1 subunit gene (GRIN1 rs4880213 and rs6293) or of the NR2B subunit gene (GRIN2B rs7301328, rs3764028, and rs1805247). Our results showed that individuals homozygous for the T allele in the rs4880213 GRIN1 SNP had reduced intracortical inhibition, as expected for enhanced glutamatergic excitation in these subjects. Furthermore, individuals carrying the G allele in the rs1805247 GRIN2B SNP showed greater intracortical facilitation and greater long-term potentiation-like cortical plasticity after intermittent θ-burst stimulation. Our results provide novel insights into the function of NMDA receptors in the human brain and might contribute to the clarification of the synaptic bases of severe neuropsychiatric disorders associated with defective glutamate transmission.

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METHODS

The study was approved by the Ethics Committee of the University Hospital Tor Vergata (Rome, Italy). Subjects and SNP genotyping. We studied 77 drug-free, healthy volunteers (31 males; mean age, 38.3 ± 10.2 years), submitted to SNP genotyping. All subjects gave written, informed consent and were right handed (Oldfield 1971).

MassARRAY Assay Design 3.1 software was used to design a single, 20-multiplex reaction, in which the two SNPs, rs4880213 and rs6293, of the GRIN1 gene and the three SNPs, rs7301328,
The hand motor area of right primary motor cortex (M1) was defined according to international standards (Rossini et al. 1994). Active motor threshold (AMT) was defined as the lowest intensity that evoked five small responses (200 μV) in the contralateral FDI muscle in a series of 10 stimuli when the subject kept the FDI muscles relaxed in both hands, according to international standards (Rossini et al. 1994). Active motor threshold (AMT) was defined as the lowest intensity that evoked five small responses (200 μV) in a series of 10 stimulations when the subject made a 10% maximal voluntary contraction. Measurements were made on each individual trial, and the mean peak-to-peak amplitude in the relaxed right FDI at baseline and the subsequent reduction or potentiation in contralateral MEP amplitude compared with the nonconditioned MEP provided a measure of SICI or ICF, respectively. The CS was delivered at 80% of RMT (Ziemann et al. 1998b) following the test stimulus (TS) and remained unchanged until the end of recordings. MEP amplitudes were then averaged at each time point and normalized to the mean baseline amplitude (Huang et al. 2005; Mori et al. 2011).

Investigators performing TMS experiments and subjects were all blinded to genotyping during the study. TMS experiments were all performed between 2:30 p.m. and 6:00 p.m. to minimize cortisol-negative effects on cortical plasticity.

Data analysis. Repeated measures ANOVA with within-subject factor ISI and between-subjects factor GENOTYPE was used on normalized peak-peak amplitudes of the mean MEPs of each subject to compare variables before and after each experimental intervention. Post hoc paired t-tests were applied when necessary. Duncan’s test was used to correct for multiple comparisons. In all figures, error bars refer to the SE.

RESULTS

Allele frequencies. The TMS procedure was well tolerated by all subjects. Allele frequencies of the five SNPs of our sample are shown in Table 1. The allele frequencies found are comparable with Haplotype Mapping Project studies in European-Americans (CEU population; http://hapmap.ncbi.nlm.nih.gov/index.html.en).

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Our Sample</th>
<th>HapMap</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4880213</td>
<td>T/T</td>
<td>21.4</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>C/T</td>
<td>50.0</td>
<td>48.7</td>
</tr>
<tr>
<td></td>
<td>C/C</td>
<td>28.5</td>
<td>39.8</td>
</tr>
<tr>
<td>rs1805247</td>
<td>A/A</td>
<td>87.6</td>
<td>88.5</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>12.3</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>0</td>
<td>1.8</td>
</tr>
<tr>
<td>rs6293</td>
<td>A/A</td>
<td>44.0</td>
<td>39.8</td>
</tr>
<tr>
<td></td>
<td>A/G</td>
<td>46.4</td>
<td>43.4</td>
</tr>
<tr>
<td></td>
<td>G/G</td>
<td>7.5</td>
<td>16.8</td>
</tr>
<tr>
<td>rs7301328</td>
<td>G/G</td>
<td>40.2</td>
<td>37.1</td>
</tr>
<tr>
<td></td>
<td>C/G</td>
<td>48.5</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>C/C</td>
<td>11.3</td>
<td>12.9</td>
</tr>
<tr>
<td>rs3764028</td>
<td>C/C</td>
<td>34.9</td>
<td>35.9*</td>
</tr>
<tr>
<td></td>
<td>C/A</td>
<td>5.1</td>
<td>50.9*</td>
</tr>
<tr>
<td></td>
<td>A/A</td>
<td>0</td>
<td>13.2*</td>
</tr>
</tbody>
</table>

The frequency genotype data for single nucleotide polymorphisms (SNPs) rs4880213, rs1805247, rs6293, and rs7301328 are taken from the public database based on Haplotype Mapping Project (HapMap) data for European-Americans (CEU; http://www.hapmap.org). *SNP frequency data for rs3764028 are not available for the CEU population. Data shown in the table are taken from Jiang and Jia (2009), based on a Han Chinese population.
Fig. 1. GRIN1 rs4880213 polymorphism influences short-interval intracortical inhibition (SICI). With respect to the polymorphism rs4880213, post hoc analysis revealed that in “TT” carriers, SICI at 2 and 3 ms interstimulus intervals (ISI) was reduced significantly. No significant effect was found on long-interval intracortical inhibition (LICI; B), short-interval intracortical facilitation (SICF; C), and intermittent theta-burst stimulation (iTBS; D). A–C: the x-axis indicates the ISI in ms, and the y-axis represents the normalized motor-evoked potential (MEP) mean amplitudes elicited by paired-pulse stimulations. In the x-axis of D, “pre” refers to the normalized MEP before the iTBS stimulation; *P < 0.05.

Fig. 2. GRIN2B rs1805247 polymorphism influences ICF and long-term potentiation-like plasticity. With respect to the polymorphism rs1805247, post hoc analysis revealed that in the “GA” genotype, ICF at ISI 15 ms was increased significantly compared with the “AA” subjects (A). No significant effect was found on LICI (B) and on SICF (C). D: in the G carriers, the iTBS effect was increased significantly compared with AA carriers. A–C: the x-axis indicates the ISI in ms, and the y-axis represents the normalized MEP mean amplitudes elicited by paired-pulse stimulations, as in Fig. 1; *P < 0.05.
analysis showed a significant main effect of ISI (F = 3.50, P < 0.05) and GENOTYPE (F = 2.42, P < 0.05) and a significant GENOTYPE × ISI interaction (F = 3.15, P < 0.05) on SICI values. Post hoc contrasts revealed that the TT group had less SICI than the other two groups at ISIs, 2 ms and 3 ms. ICF, LICI, and SICF showed a significant effect of ISI (F = 12.13, P < 0.01 for ICF; F = 5.21, P < 0.01 for SICF; and F = 7.18, P < 0.01 for LICI) but no significant effect of GENOTYPE and GENOTYPE × ISI interaction (Fig. 1, A–C).

In iTBS experiments, we tested whether the rs4880213 polymorphism affected long-term potentiation (LTP)-like synaptic plasticity. ANOVA on the normalized data revealed a significant effect of TIME (F = 3.10, P < 0.05). Conversely, GENOTYPE and GENOTYPE × TIME interaction was not significant (Fig. 1D).

Additional analysis was performed by stratifying the population according to age z-scores and gender. No significant differences emerged from this analysis.

**GRIN2B rs1805247.** Mean RMT, AMT, and stimulus intensity required to elicit a MEP of 1 mV amplitude were not significantly different between “AA” and “AG” subjects. In our sample, there were no “GG” subjects. The analysis showed a significant main effect of ISI (F = 15.10, P < 0.01) and GENOTYPE (F = 4.55, P < 0.05) and a significant GENOTYPE × ISI interaction (F = 2.48, P < 0.05) on ICF values. Post hoc contrasts revealed that ICF elicited in the GA group was significantly larger than in the AA group at ISI 15 ms. SICI, LICI, and SICF showed a significant effect of ISI (F = 22.48, P < 0.01 for SICI; F = 30.90, P < 0.01 for LICI; and F = 6.65, P < 0.01 for SICF) but no significant effect of GENOTYPE and GENOTYPE × ISI interaction (Fig. 2, A–C).

In iTBS experiments, we found a significant effect of TIME (F = 6.45, P < 0.05) and a significant effect of GENOTYPE (F = 5.2, P < 0.05) and GENOTYPE × TIME interaction (F = 3.2, P < 0.05). Post hoc contrasts revealed that MEP amplitudes in response to iTBS elicited in the GA group were significantly larger than in the AA group at all times (Fig. 2D).

No significant differences emerged by stratifying the population according to age z-scores and gender (not shown).

**GRIN1 rs6293, GRIN2B rs7301328, GRIN2B rs3764028.** Unlike SNP rs4880213 and rs1805247, no differences were found on SICF, SICI/ICF, and LICI among the allele groups in the SNP rs6293, SNP rs7301328, and SNP rs3764028. Also, in iTBS experiments, the normalized data did not reveal any statistical difference among the allele groups of these SNPs (Table 2).

**DISCUSSION**

The present study provides the first indication that NR1 and NR2B subunits of NMDA receptors are involved in the regulation of cortical excitability and plasticity in the human cortex. Individuals homozygous for the T allele in the GRIN1 rs4880213 SNP, in fact, showed less intracortical inhibition, whereas individuals carrying the G allele of the GRIN2B rs1805247 SNP showed greater synaptic facilitation and greater LTP-like synaptic plasticity after iTBS.

We propose that the TT genotype of GRIN1 rs4880213 SNP affects SICI at 2 and 3 ms by favoring glutamate transmission. In fact, SICI has been investigated extensively and is widely regarded as a main inhibitory system in the M1 (Kujirai et al. 1993). There are two phases of SICI with maximum inhibition at ISI of −1 and 2.5 ms (Fisher et al. 2002; Roshan et al. 2003; Vucic et al. 2009). Inhibition at ISI of 1 ms may partly be due to neuronal refractoriness (Fisher et al. 2002), but synaptic inhibition may also contribute (Ilic et al. 2002; Roshan et al. 2003). On the contrary, SICI at 2–3 ms ISI is mainly due to GABAA receptor-mediated synaptic inhibition, as suggested by several pharmacological studies (Ziemann et al. 1996). However, at this ISI, SICI does not result from a pure inhibition. Instead, it reflects a balance between GABAA receptor-mediated inhibition and glutamate-mediated facilitation. The resultant inhibition or facilitation represents a complex interplay between stimulation parameters such as CS intensity and the ISI used (Ilic et al. 2002; Roshan et al. 2003). Indeed, it has been well established that the relationship between the degree of SICI and the intensity of the CS is a U-shaped curve (Ilic et al. 2002; Kujirai et al. 1993; Peurala et al. 2008). At low levels, an increment of CS intensity leads to greater SICI, likely due to the recruitment of inhibitory interneurons. Conversely, further increase in CS intensity leads to reduced inhibition and eventually causes facilitation. With an increase in CS intensity above 90% AMT, SICI, at the first peak, remains stable, whereas the peak at 2–3 ms ISI decreases. This reduction correlates with the degree of facilitation, strongly suggesting contamination by glutamate-mediated facilitation (Peurala et al. 2008). Accordingly, drugs that reduce facilitation, such as glutamate-mediated inhibition, may be useful in reducing synaptic facilitation.
GRIN2B rs1805247 SNP has been associated with mood disturbances (Zhao et al. 2011). In this respect, there is growing evidence for glutamatergic abnormalities and defective synaptic plasticity at the basis of depression and bipolar disorders (Diazgranados et al. 2010; Yüksel et al. 2010), and our data demonstrating altered ICF and plasticity associated with the GRIN2B rs1805247 SNP variant might contribute to the understanding of the pathogenesis of these disorders.

A limitation of the present work may be that the data have been analyzed as if the influence of each of the SNPs was independent. Indeed, it may be argued that the response pattern to TMS may also be heavily influenced by specific combinations of SNPs within individual subjects. Unfortunately, this important issue could not be properly addressed due to the small size of our sample, limiting the conclusions that can be drawn from our study. In this respect, it is also interesting to note that also, the brain-derived neurotrophic factor (BDNF) has been implicated in the control of NMDA receptor-dependent synaptic plasticity and its homeostatic regulation (Figurov et al. 1996). Animal studies have shown that mature BDNF plays an important role in all stages of LTP, whereas the precursor peptide (pro-BDNF) has been associated with long-term depression (Lu et al. 2005). Recent work suggests that the BDNF val66met genotype influences the direction and magnitude of TMS-induced corticospinal excitability, even if the results are contradictory (Cheeran et al. 2009). It is therefore conceivable that the BDNF polymorphism may interfere with GRIN1 and GRIN2B SNPs in the regulation of cortical excitability.

In conclusion, in this investigation, we have matched information on NMDA receptor genes with an extensive neurophysiologic evaluation of synaptic transmission and plasticity in the human cortex and found that some NMDA receptor SNP variants affect cortical excitability. Our results might be helpful for a more comprehensive understanding of NMDA receptor function in the human brain and for the clarification of the synaptic bases of severe neuropsychiatric disorders associated with defective glutamate transmission.

REFERENCES


DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).
NMDA RECEPTORS AND CORTICAL EXCITABILITY


