Repetitive transcranial magnetic stimulation induced analgesia depends on N-methyl-D-aspartate glutamate receptors

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ABSTRACT

We investigated the role of glutamate N-methyl-D-aspartate (NMDA) receptors in the analgesic effects induced by repetitive transcranial magnetic stimulation (rTMS). In a randomized, double-blind, crossover study, we compared the effects of ketamine and placebo on the analgesic effects of motor cortex (M1) or dorsolateral prefrontal cortex/premotor cortex (DLPFC/PMC) stimulation. Three groups of 12 healthy volunteers underwent active rTMS (10 Hz, 80% resting motor threshold, 1,500 pulses per session) of the right M1, active stimulation of the right DLPFC/PMC, or sham stimulation during 2 experimental sessions 2 weeks apart. Cold pain thresholds were measured on the left thenar eminence before and 1 hour after cortical stimulation, to evaluate the analgesic effects of rTMS. Ketamine (0.15 mg/kg in a 10-minute bolus followed by continuous infusion of 6 l/g/kg per minute until the end of rTMS) or placebo (saline) were administered intravenously during cortical stimulation. We also systematically measured cortical excitability parameters (resting motor threshold, suprathreshold motor-evoked potentials, short intracortical inhibition, and intracortical facilitation) before and after treatment, to investigate the possible relationship between changes in cortical excitability and rTMS-induced analgesia. Ketamine injection significantly decreased the analgesic effects of both M1 and DLPFC/PMC stimulation. The decrease in the analgesic effect of rTMS was not associated with changes in cortical excitability parameters, which were not influenced by rTMS following the administration of either saline or ketamine. Thus, rTMS-induced analgesia depends on glutamate NMDA receptors and may involve long-term potentiation-like mechanisms.

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1. Introduction

Repetitive transcranial magnetic stimulation (rTMS) of the primary motor cortex (M1) or dorsolateral prefrontal/premotor cortex (DLPFC/PMC) has repeatedly been shown to reduce clinical and experimental pain in humans and animals [2,5,18,48,51,68,70]. In addition, high-frequency rTMS (10–20 Hz) of M1 has analgesic effects in various chronic pain conditions, including neuropathic pain [3,13,22,28,31–34,45], complex regional pain syndrome [59,60], and fibromyalgia [43,55]. The mechanisms underlying rTMS-induced analgesia remain unclear. We recently showed that they involve endogenous opioids [12], but these effects probably also depend on changes in other pain modulating systems. It has been suggested that the effects of rTMS, which are initiated by changes in cortical excitability induced by the magnetic field [33,37,43,71], may reflect the plastic synaptic changes induced by the stimulation, and that these effects may have mechanisms in common with classical long-term potentiation and long-term depression phenomena [58,61]. These phenomena are characterized by a strong dependence on the frequency of the stimulation used to induce synaptic plasticity and a duration exceeding that of the stimulation period, typically by several hours to a few weeks [35,42,73]. Similarly, rTMS-induced analgesia is highly dependent on the frequency of stimulation and can last several hours after a single stimulation [12,28,48,53,55] and up to several weeks after a series of stimulations [43]. Such long-lasting effects are
consistent with the involvement of long-term potentiation- or long-term depression-like phenomena in rTMS-induced analgesia. The mechanisms of long-term potentiation and long-term depression are complex and involve multiple neurotransmitters. However, the N-methyl-D-aspartate (NMDA) receptor, a major excitatory ligand-gated ion channel in the central nervous system, has long been known to be one of the predominant molecular gateways controlling synaptic plasticity [35,42]. Unlike the \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) glutamate receptor, which requires only glutamate for opening, NMDA receptors require concurrent depolarization for opening and functionality [73]. As rTMS induces rapid depolarization of cortical neurons and long-term analgesia extending beyond the stimulation period, we hypothesized that glutamate NMDA receptors might be involved in its analgesic effects. Consistent with this hypothesis, recent experimental data for rats have shown that the antinociceptive effects of TMS are blocked by NMDA-receptor antagonists [2].

We have shown that the application of rTMS to either M1 or the DLPFC/PMC effectively attenuates experimental thermal pain in healthy volunteers, and that stimulation of both sites is more effective in reducing cold pain than heat pain [48], suggesting that the analgesia induced by the stimulation of these 2 cortical sites might involve similar mechanisms. However, we also showed that only the analgesic effects (assessed by the changes in cold pain threshold) induced by M1 stimulation were significantly decreased after the administration of naloxone [12]. Thus, the neuropharmacological bases of rTMS-induced analgesia seem to depend on the site of stimulation.

Using a similar protocol, in the present study we compared the effects of ketamine, a noncompetitive NMDA antagonist, and saline on PFC/PMC and M1 rTMS-induced analgesia in a randomized, double-blind, crossover study. We also systematically measured cortical excitability parameters before and after treatment, to determine the relationship between changes in these parameters and rTMS-induced analgesia.

2. Methods

The study was approved by the appropriate local institutional review board and was carried out on 36 paid, healthy volunteers with no clinical history, clinical symptoms or signs of peripheral or central nervous system disorders, no acute pain, and not on medication at the time of testing or during the preceding month. Volunteers were carefully briefed about the experimental procedures of the study. All participants gave written informed consent for participation.

2.1. Study design

The study comprised 3 parallel arms corresponding to 3 types of stimulation: active stimulation of M1 (M1-rTMS arm), active stimulation of the DLPFC/PMC (DLPFC/PMC-rTMS arm), and sham stimulation (of M1 or the DLPFC/PMC; Sham-rTMS arm). Volunteers were randomly assigned to 1 of the 3 arms (12 volunteers per arm). Each volunteer participated in 2 experimental sessions 2 weeks apart, in which we compared the effects of intravenous ketamine and placebo (saline) on rTMS-induced analgesia administered according to a randomized, double-blind, crossover design. Thus, 12 volunteers received active DLPFC/PMC-rTMS during both experimental sessions, 12 volunteers received active M1-rTMS during both experimental sessions, and 12 volunteers received Sham-rTMS during both experimental sessions (Fig. 1). In the Sham-rTMS arm, the coil was placed on either the right DLPFC/PMC or the right M1 cortex, selected at random in a 1:1 ratio.

2.2. Experimental procedures

Each experimental session started with the determination of baseline cold pain threshold on the left thenar eminence and measurements of cortical excitability parameters for the right motor cortex. Ten minutes before rTMS (administered to the right M1 or right DLPFC/PMC), the volunteer received an intravenous bolus followed by a continuous infusion of either placebo (ie, saline) or ketamine, which was continued throughout the rTMS session. Immediately after the rTMS session, cortical excitability parameters were measured for a third time and cold pain thresholds were determined by the same procedure used at baseline. This was based on our previous results showing that rTMS-induced analgesic effects lasted at least 1 hour under our experimental conditions [12,48]. A nurse prepared the infusions and played no further role in the experiment. For ketamine, subjects received a bolus of 0.15 mg/kg over 10 minutes, followed by a continuous infusion of 6 \( \mu \)g/kg per minute throughout the rTMS session (15 minutes). This dose of ketamine was chosen on the basis of previous studies in which this drug was used in various experimental and clinical pain models [6,21,26,30]. The volume of placebo (saline) administered and its rate of infusion were similar to those for the active drug. Blood pressure, heart rate, and oxygen saturation were monitored during each session and side effects were systematically recorded.

2.3. Quantitative sensory testing

Cold pain thresholds were measured with a Somedic thermotest (Somedic AB, Stockholm, Sweden), in a quiet room, at 22°C. Individuals were seated in a comfortable armchair. A contact thermode of Peltier elements measuring 25 × 50 mm was applied to the skin over the left thenar eminence. The baseline temperature of the thermode was adjusted to the patient’s skin temperature (32.2 ± 0.4°C). Thresholds were measured with the method of limits, as previously described [12]: stimuli of decreasing temperatures were applied and, for each stimulus, the subject was instructed to press a button that reversed the thermal stimulation as soon as the stimulation became painful. The cold pain threshold (expressed in °C) was calculated as the mean of 3 successive determinations, with an interval of 20–30 seconds between consecutive stimuli.

2.4. Transcranial magnetic stimulation

Subjects were seated in a comfortable reclining chair and asked to keep their hands as relaxed as possible. Magnetic stimulation was applied with a MagPROX100 machine (MagVenture Tonika Elektronic, Farum, Denmark), using a figure-of-eight coil oriented at a tangent to the scalp, with the main phase of the induced current in the anterior–posterior direction. The patients were fitted with earplugs during TMS.

2.4.1. Assessment of cortical excitability

Motor cortical excitability testing included the determination of resting motor threshold (RMT), suprathreshold motor-evoked potentials (MEPs), short intracortical inhibition (SICI), and intracortical facilitation (ICF) for the right hemisphere. MEPs were recorded for the first interosseous muscle of the contralateral hand, with an electromyelography amplifier module (MagVenture Tonika Elektronic) and surface electrodes (Alpine Biom, Skovlunde, Denmark). The RMT was defined as the lowest intensity eliciting a motor-evoked potential of at least 50 μV in 50% of trials. The relationship between stimulus intensity and MEP amplitude (ie, the stimulus–response curve) was assessed as previously described.
[40], by measuring the MEP evoked by stimulation at 120% and 140% of the RMT and calculating the ratio of the MEP amplitude obtained at 140% of the RMT to that at 120% of the RMT (RMT 140/120). Intracortical modulation was investigated according to a previously described paired-pulse protocol [33,43,44]. Paired pulses were delivered, with the intensity of the conditioning stimulus set at 80% of the RMT and the intensity of the test stimulus at 120% of the RMT. Interstimulus intervals of 2 and 4 ms were used for SICI, and interstimulus intervals of 10 and 15 ms were used for ICF. For each interstimulus interval, the results of 4 trials were averaged, and the changes in test MEP amplitude induced by conditioning stimuli were expressed as a percentage of the control MEP amplitude. For each series of measurements, the lowest results for intracortical inhibition (ICI) and the highest results for ICF in each interstimulus interval were retained for analysis [33].

2.4.2. Treatment sequence
The rTMS parameters were similar to those used in our previous experimental and clinical studies [12,43,48,55]. Each stimulation session consisted of 15 series of 10-second pulses with a frequency of 10 Hz and an interval of 50 seconds between each train, giving a total of 1,500 pulses per session. The stimulation intensity used was 80% the RMT. Active stimulation was applied to the right primary motor cortex (M1) or to the right DLPFC/PMC, corresponding to the area located 5 cm in front of M1 in the sagittal plane [1,5,12,18,52]. Sham stimulation was carried out with a sham coil of identical size, colour, and shape, emitting a sound similar to that emitted by the active coil, applied to either M1 or the DLPFC/PMC. During stimulation, the coil was oriented at a tangent to the scalp in the anterior–posterior direction and fixed to an arm that could be adjusted in 3 dimensions.

2.5. Monitoring of side effects and assessment of the quality of blinding
Side effects related to rTMS and/or pharmacological treatment were systematically recorded. Blinding was assessed at the end of the experimental session, by asking the participants whether they could guess which type of cortical stimulation (active or sham) and pharmacological treatment (ketamine or saline) they had received.

2.6. Statistical analysis
Results are expressed as means ± 1SD. Repeated-measures analysis of variance was performed with the factors “arm” (ie, active rTMS to M1, active rTMS to the DLPFC/PMC, or Sham rTMS), “experimental session” (ie, ketamine or saline) and “time” (before, immediately after rTMS, and 1 hour after rTMS) and the interactions “arm” by “experimental session” and “experimental session” by “time” were performed to compare the effects of active or sham rTMS after saline or ketamine administration on cold pain thresholds (before and 1 hour after rTMS) and cortical excitability parameters (before, immediately after, and 1 hour after rTMS). We used Fisher’s least significant difference test for post hoc analysis. Paired Student’s t tests were used to analyse the effects (baseline vs after treatment) of each drug infusion. Fisher’s exact significance test was used to compare the prevalence of side effects related to rTMS.
and the intravenous infusions across the experimental sessions. Differences were considered significant if $P < 0.05$.

### 3. Results

We included 12 female and 24 male healthy volunteers. Sex ratio and mean age were similar among the 3 groups: M1-rTMS (M/F = 7/5; mean age = 30.6 ± 4.5 years), DLPFC/PMC-rTMS (M/F = 8/4; mean age = 29.0 ± 4.5 years), and Sham-rTMS (M/F = 7/5; mean age = 29.0 ± 6.4 years, $P = 0.2$). Volunteers were also matched for body mass index and educational level in 3:3:3 blocks. Baseline values for cold pain thresholds for the 2 experimental sessions (ie, administration of ketamine or placebo) were similar ($P = 0.35$) in each arm: 12.32 ± 2.23°C and 12.51 ± 1.89°C in the M1-rTMS arm, 13.60 ± 2.55°C and 13.92 ± 2.34°C in the DLPFC/PMC-rTMS arm, and 12.52 ± 2.45°C and 12.80 ± 2.56°C in the Sham-rTMS arm. Baseline values for cold pain thresholds were also similar ($P = 0.25$) for the 3 arms.

#### 3.1. Effects of ketamine and saline on the analgesia induced by rTMS

In the M1 stimulation group, the analgesic effects of rTMS, corresponding to a decrease in cold pain threshold temperature after cortical stimulation, were significantly weaker ($P = 0.007$) after the administration of ketamine ($-0.78 ± 1.5°C$) than after the administration of placebo ($-2.45 ± 3.1°C$) (Fig. 2A). Similarly, in the DLPFC/PMC stimulation group, the analgesic effects of rTMS were significantly weaker ($P = 0.039$) after ketamine ($-0.56 ± 2.1°C$) than after placebo ($-2.38 ± 2.8°C$) (Fig. 2B).

By contrast, no significant change ($P = 0.25$) in cold pain threshold after the administration of ketamine ($-0.20 ± 1.7°C$) or placebo ($0.52 ± 1.8°C$) was observed in the sham stimulation group (Fig. 2C).

#### 3.2. Comparison of the effects of active and sham rTMS

Comparison (analysis of variance) of the changes between the 3 groups showed a significant effect for “arm” and “stimulation session” ($F = 8.54; P < 0.001$), indicating that both types of active stimulation induced significant analgesic effects (ie, a decrease in cold pain threshold temperature) not observed with the sham stimulation (Fig. 3).

#### 3.3. Cortical excitability changes

Cortical excitability parameters displayed no significant change immediately or 1 hour after the administration of ketamine or placebo in any of the 3 arms (ie, M1-rTMS, DLPFC/PMC-rTMS, or Sham-rTMS) (Table 1).

#### 3.4. Side effects and assessment of the quality of blinding

##### 3.4.1. Side effects related to rTMS

M1 stimulation caused transient mild headache in 1 participant (8%) and DLPFC/PMC and sham stimulation caused similar symptoms in 2 volunteers (16%). Headaches were reported either during or within 30 minutes after the session, and they lasted <60 minutes in all cases.

##### 3.4.2. Side effects related to the pharmacological treatment

Ketamine infusion caused clouded sensorium and dizziness during the infusion in 28 participants (78%). The infusion of saline caused nausea and abdominal discomfort in 12 participants (33%) and acute anxiety symptoms in 3 (8%) (intravenous infusion side effects: saline vs ketamine $\chi^2 = 2.22; P = 0.13$). Thirty minutes after cortical stimulation, symptoms (mild in all cases) were present in 8 (22%) participants in the ketamine session and in 8 (22%) participants during the saline session. None of the participants reported symptoms 60 minutes after the rTMS session.

##### 3.4.3. Quality of blinding

Twenty-nine (81%) participants said they could not guess whether they had received active or sham stimulation, and 7 guessed correctly. Twenty-one (58%) participants said they could not tell the sequence of the infusions; 9 guessed right, and 6 guessed wrong.

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Fig. 2. Comparison of the mean (±SD) changes in cold pain threshold (CPT; obtained at 1 hour minus baseline values) in the primary motor cortex (M1; A), dorsolateral prefrontal cortex/premotor cortex (DLPFC/PMC; B), and Sham (C) arms of the study for both experimental sessions: intravenous infusion of ketamine or saline. *P < 0.05.
4. Discussion

We show here that the analgesic effects of rTMS of both M1 and the DLPFC/PMC on experimental cold pain were significantly decreased by the intravenous administration of ketamine, and that these changes were not associated with parallel changes in cortical excitability parameters. These results partially confirm our working hypothesis, but support the view that rTMS-induced analgesia involves glutamate-dependent neural networks and NMDA receptors.

NMDA receptors are abundant in the central nervous system, in both the spinal cord and the brain, and the role of excitatory amino acids as major transmitters for excitatory synapses throughout the brain is well documented [42]. In particular, the role of glutamate and NMDA receptors in spinal and supraspinal transmission, and in the integration and modulation of nociceptive somatosensory signals, has been extensively documented [15,49,64]. In our study, ketamine induced no significant changes in cold threshold in the subjects receiving sham stimulation. This result is consistent with previous data indicating that NMDA antagonists, such as ketamine, can reduce pathological pain or experimental pain induced by repetitive noxious stimuli, but are much less effective against experimental pain induced by a single noxious stimulus [21]. Thus, the significant decrease in analgesia observed after ketamine infusion was directly related to its effects on the mechanisms of rTMS-induced analgesia and was not biased by a potential direct (analgesic) effect of ketamine on pain transmission.

The mechanisms underlying rTMS-induced analgesia remain unclear. Functional neuroimaging studies have shown that the effects of rTMS of M1 are not confined to the motor system. Instead, this stimulation induces changes in the activity of cortical and subcortical structures involved in pain processing and modulation, including the thalamus and the anterior cingulate and insular cortices [9,38,71]. The role of the DLPFC/PMC in pain modulation has also long been established in experimental studies, through its connections with the limbic system and brainstem structures involved in descending modulation in particular [16,50]. Consistent with the experimental data, several functional neuroimaging studies in humans have confirmed that, like M1 stimulation, rTMS of the DLPFC/PMC induces changes in the activity of a network of structures involved in the integration and modulation of pain signals, including the thalamus, brainstem, and insular and cingulate cortices [8,41,56,66]. All these areas are rich in glutamate NMDA receptors, and the role of these receptors in descending modulatory controls originating in the brainstem area, notably in the periaqueductal gray and rostral ventromedial medulla, is well documented [46].

Unlike naloxone [12], which blocks only the analgesic effects elicited by M1 stimulation, ketamine decreased the effects of both M1 and DLPFC/PMC stimulation, suggesting that a final common pathway may underlie the analgesia induced by the stimulation of these 2 cortical sites. In particular, the analgesia induced by M1 and DLPFC/PMC stimulation may be mediated by common descending pain modulatory controls. Consistent with this hypothesis, it has been shown in animals that the antinociceptive effects of cortical (electrical) stimulation are associated with changes in neuronal activities in the periaqueductal gray [51]. However, we

Table 1

Cortical excitability parameters before and after active and sham repetitive transcranial magnetic stimulation (rTMS) with saline or ketamine infusion.

<table>
<thead>
<tr>
<th>Study arm</th>
<th>Infusion</th>
<th>Time</th>
<th>RMT (% M0)</th>
<th>MEP 120% (μV)</th>
<th>MEP 140% (μV)</th>
<th>PEM 140/120%</th>
<th>ICI_2 ms</th>
<th>ICI_4 ms</th>
<th>ICI_10 ms</th>
<th>ICI_15 ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1-rTMS</td>
<td>Saline</td>
<td>Baseline</td>
<td>58.6 ± 9.4</td>
<td>949.2 ± 809.7</td>
<td>2643.2 ± 1807.4</td>
<td>3.3 ± 2.2</td>
<td>0.5 ± 0.4</td>
<td>0.2 ± 0.2</td>
<td>0.3 ± 0.2</td>
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</tr>
<tr>
<td></td>
<td>Saline</td>
<td>M0</td>
<td>58.1 ± 7.3</td>
<td>1391.4 ± 987.4</td>
<td>2581.1 ± 1872.8</td>
<td>2.1 ± 1.0</td>
<td>0.5 ± 0.2</td>
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<td>1.1 ± 0.5</td>
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<tr>
<td></td>
<td>Saline</td>
<td>M60</td>
<td>58.1 ± 7.3</td>
<td>1391.4 ± 987.4</td>
<td>2581.1 ± 1872.8</td>
<td>2.1 ± 1.0</td>
<td>0.5 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>1.1 ± 0.5</td>
<td>1.5 ± 0.7</td>
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<td>Ketamine</td>
<td>B</td>
<td>56.6 ± 16.2</td>
<td>794.2 ± 520.0</td>
<td>2542.3 ± 2049.2</td>
<td>3.0 ± 1.5</td>
<td>0.5 ± 0.3</td>
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<td>1.5 ± 1.1</td>
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<tr>
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<td>M0</td>
<td>59.3 ± 8.7</td>
<td>1046.4 ± 819.4</td>
<td>2496.6 ± 1907.1</td>
<td>3.0 ± 2.1</td>
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<td>59.8 ± 9.5</td>
<td>1105.5 ± 742.1</td>
<td>3197.2 ± 2160.7</td>
<td>3.1 ± 1.7</td>
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<td>52.8 ± 7.4</td>
<td>759.5 ± 599.6</td>
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<td>Ketamine</td>
<td>Saline</td>
<td>Baseline</td>
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<td>1567.9 ± 973.9</td>
<td>5.6 ± 8.8</td>
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<td>M0</td>
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<td>1904.1 ± 1403.5</td>
<td>3.0 ± 2.3</td>
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<td>M60</td>
<td>61.2 ± 11.1</td>
<td>504.2 ± 394.3</td>
<td>1113.5 ± 783.0</td>
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<td>1320.4 ± 695.0</td>
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<td>2.8 ± 0.9</td>
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<tr>
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<td>M60</td>
<td>63.0 ± 11.6</td>
<td>4250.2 ± 295</td>
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<td>3.3 ± 2.7</td>
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<td>1.5 ± 1.1</td>
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</tr>
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</table>

M1, primary motor cortex; DLPFC/PMC, dorsolateral prefrontal cortex/premotor cortex; RMT, resting motor threshold; MEP, motor evoked potential at 120% and 140% of RMT; ICI, intracortical inhibition; MSO, maximum stimulator output; M0, immediately after the rTMS session (minute zero); M60, 60 minutes after the end of the rTMS session.

Fig. 3. Comparison of the mean (±SD) changes in cold pain threshold (CPT; obtained at 1 hour minus baseline values under saline infusion) in the primary motor cortex (M1), dorsolateral prefrontal cortex/premotor cortex (DLPFC/PMC), and Sham arms. *P < 0.05.
have previously shown, in healthy volunteers [48], that the analgesia induced by the rTMS of M1 or the DLPFC/PMC is not associated with changes in electrophysiological recordings of the RII spinal nociceptive reflex in healthy volunteers. This is not consistent with a direct action of rTMS on descending pain modulation. It is therefore likely that the activation of descending pain modulation is not the only mechanism involved in rTMS-induced analgesia, and more experimental studies are required to investigate further the effects of rTMS on the central integration and modulation of pain signals.

In particular, specific investigation is required into the cellular mechanisms underlying the changes in excitability of cortical neurons initially induced by stimulation, and their relationships to the changes in the activity of neurons involved in pain modulatory systems. We addressed this issue here, by measuring cortical excitability and intracortical modulation in paired-pulse stimulation paradigms, before and after the administration of ketamine or placebo. We hypothesized that the effects of ketamine on rTMS-induced analgesia were related to changes in these electrophysiological parameters, potentially reflecting the initial mechanisms of stimulation [19]. This hypothesis is supported by clinical studies showing that patients with chronic pain syndromes display a significant decrease in SICI and ICF, and that the analgesic effects of rTMS are directly related to a restoration of intracortical modulation, both in patients with neuropathic pain [32] and in those with fibromyalgia [43]. In addition, despite publication of conflicting results, previous pharmacological studies in healthy volunteers have suggested that SICI and ICF can be modulated by NMDA antagonists [14,23,36,65,72]. However, none of the electrophysiological parameters measured in our volunteers was significantly altered during rTMS of M1 or the DLPFC/PMC, during the administration of either placebo or ketamine. Our “negative” results are consistent with those of several other studies, finding no evidence of significant changes in cortical excitability after rTMS, but conflicting results have also been reported. For example, high-frequency (10–20 Hz) simulation of M1 has been reported to induce no change [11,39,63], a significant decrease [25,27,47], or a significant increase [4,10,17,37,40,54] in MEP amplitude in different studies. The effects of DLPFC/PMC stimulation on cortical excitability have been studied less frequently than those of M1 stimulation, but the results obtained are no more consistent [20,62]. Conflicting results have also been reported for the effects of rTMS on other cortical excitability parameters, such as input–output curves, ICF, and ICI. For instance, Kheder et al. [29] reported no change in input–output curves, ICF, or ICI after high-frequency rTMS of M1 with 1,500 pulses in 16 healthy subjects, a protocol similar to that used here. Using a similar rTMS protocol, Jung et al. [27] observed a decrease in ICF, ICI, and input–output curves. Wu et al. [69] reported a transient increase in ICF and a decrease in ICI only immediately after the stimulation. These discrepancies probably largely reflect methodological differences, but they may also result from true interindividual variability [17], due to genetic factors in particular [7]. In almost all studies on chronic pain, cortical excitability parameters are reported not to increase above normal values after rTMS. Indeed, the changes observed after treatment tend instead to correspond to an increase of abnormally low values, suggesting that cortical excitability displays homeostatic regulation [67]. This may account for the lack of significant changes in cortical excitability reported here and in other studies on healthy volunteers. The lack of effect of rTMS on cortical excitability in healthy subjects may also reflect a difference between clinical studies and experimental studies in healthy volunteers. By contrast to some clinical studies, all experimental studies published to date, including this one, involved only a single session of rTMS, which may be insufficient to induce sustained changes in cortical excitability.

At subanaesthetic doses, the effects of ketamine depend preferentially on the noncompetitive blockade of NMDA receptors through binding at the phencyclidine site. However, interactions with other receptors, including opioidergic, cholinergic, monoaminergic, and AMPA receptors, have also been reported, although generally only at higher concentrations [24,57]. It is not possible to rule out an effect of these receptors formally in this study; however, such an effect seems unlikely given the low dose and short duration of the infusion, and the absence of clinical effects on experimental pain under conditions of sham rTMS stimulation.

Blinding is always a concern in studies using ketamine, because of the side effects of the drug. Here, the percentage of volunteers reporting side effects did not differ significantly after ketamine or placebo, but the side effects induced by ketamine were different from those induced by placebo. However, it is unlikely that this induced a bias in our study because it did not seem to influence blinding, which was assessed systematically. Most of the volunteers could identify neither the type of stimulation nor the drugs they received.

In conclusion, our results showing that the analgesic effects induced by M1 or DLPFC/PMC stimulation depend on NMDA receptors add to our as yet limited knowledge of the neuropharmacological basis of rTMS-induced analgesia. These results are consistent with a role for long-term potentiation-like phenomena in this type of analgesia, but more studies are required to investigate the cellular mechanisms underlying these effects and also to investigate in more details the role of changes in synaptic plasticity in the long-term analgesic effects of rTMS in patients with chronic pain.

Conflict of interest statement

The authors declare no conflict of interest related to this study.

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References

Hirayama A, Saitoh Y, Kishima H, Shimokawa T, Oshino S, Hirata M, Kato A, Yoshimine T. Reduction of intractable deafferentation pain by navigation-
guided repetitive transcranial magnetic stimulation of the primary motor cortex. PAIN 2006;112:22–7.

