Masking the Auditory Evoked Potential in TMS–EEG: A Comparison of Various Methods

Esther M. ter Braack · Cecile C. de Vos ·
Michel J. A. M. van Putten

Received: 18 March 2013 / Accepted: 15 August 2013 / Published online: 1 September 2013
© Springer Science+Business Media New York 2013

Abstract There is growing interest in combining transcranial magnetic stimulation (TMS) with electroencephalography (EEG). Because TMS pulses are accompanied by a clicking sound, it is very likely that part of the response in the EEG consists of an auditory evoked potential (AEP). Different methods have been applied to mask the sound of TMS. However, it is unclear which masking method is most effective in reducing the AEP. In this study we explore the presumed contribution of the AEP to the response and evaluate different ways to mask the TMS clicking sound. Twelve healthy subjects and one completely deaf subject participated in this study. Eight different masking conditions were evaluated in nine hearing subjects. The amplitude of the N100–P180 complex was compared between the different masking conditions. We were not able to completely suppress the N100–P180 when the coil was placed on top of the head. Using an earmuff or exposing the subjects to white or adapted noise caused a small but significant reduction in N100–P180 amplitude, but the largest reduction was achieved when combining a layer of foam, placed between coil and head, with white or adapted noise. The deaf subject also showed a N100–P180 complex. We conclude that both the TMS clicking sound

and cortical activation by the magnetic pulse contribute to the N100–P180 amplitude.

Keywords Transcranial magnetic stimulation (TMS) · Electroencephalography (EEG) · Auditory evoked potential

Introduction

Transcranial magnetic stimulation (TMS) is a widely used technique to noninvasively activate the human cortex. After the introduction in the mid-eighties (Barker et al. 1985), TMS has developed into a method that is used to study motor cortical excitation (resting motor threshold) and inhibition (short and long interval cortical inhibition) by measuring modulation of the motor evoked potential (MEP). In addition, various diagnostic and therapeutic applications have emerged, including determining the central motor conduction time for diagnosing spinal cord compression, amyotrophic lateral sclerosis or multiple sclerosis (Chen et al. 2008), and applying repetitive TMS for treating depression (Padberg and George 2009).

Recently, there is a growing interest in combining TMS with electroencephalography (EEG), because it is a more direct measurement of the brain's reaction to a pulse than the MEP. In addition, the response of other areas than the motor cortex can be studied with TMS–EEG. When TMS is applied while recording EEG, a characteristic waveform—the TMS evoked potential (TEP)—is induced in the EEG (Ilmoniemi and Kičić 2010). The methodology of measuring and analyzing the TEP is similar to other event-related potential measurements, such as the visual or auditory evoked potential (AEP). Assuming that the stimulus always induces a specific response in the EEG, and considering all other brain activity as uncorrelated, the

This is one of several papers published together in Brain Topography in the “Special Issue: Auditory Cortex”.

E. M. ter Braack (✉) · C. C. de Vos · M. J. A. M. van Putten
Department of Clinical Neurophysiology, MIRA—Institute for Biomedical Technology and Technical Medicine, University of Twente, Carré CR 3.714, P.O. Box 217, 7500 AE Enschede, The Netherlands
e-mail: e.m.terbraack@utwente.nl

C. C. de Vos · M. J. A. M. van Putten
Department of Clinical Neurophysiology, Medisch Spectrum Twente, Enschede, The Netherlands

response can be extracted by averaging over several stimuli. Combining TMS with EEG can give more insight into the connectivity between different brain areas, by evaluating the TEP at different electrode positions while stimulating a specific target.

The response shows characteristic components at different latencies. Negative components at 15, 45 and 100 ms and positive components at 30, 60 and 180 ms, which are specific for the TEP at electrode Cz after motor cortex stimulation, have been reported in several studies (Bonato et al. 2006; Esser et al. 2006; Ferreri et al. 2011; Ilmoniemi and Kičić 2010; Komssi et al. 2004; Levit-Binnun et al. 2010; Paus et al. 2001; Ter Braack et al. 2013). The TEP has shown to be reproducible within a one-week interval (Liomis et al. 2009) and changes shape when different parts of the cortex are stimulated (Casarotto et al. 2010). The physiological processes responsible for the different components are still largely unknown (Bonato et al. 2006), although some studies suggested that the N100 reflects cortical inhibitory processes (Nikulin et al. 2003; Kičić et al. 2008). The variation in the amplitude of the MEP has also been shown to correspond to variations in the amplitude of earlier TEP peaks (N15 and P30) (Mäki and Ilmoniemi 2010).

Because a TMS pulse is accompanied by a clicking sound, it is very likely that at least part of the TEP consists of an AEP (Ilmoniemi and Kičić 2010; Miniussi and Thut 2009; Nikouline et al. 1999). The AEP consists of several peaks (Picton et al. 1974), of which the long latency components P50, N100 and P180 could potentially interfere with the TEP. At present, it is not completely known which components of the TEP may be corrupted by components of the AEP. Although it has been shown that especially the N100 and P180 of the TEP are strongly correlated to auditory stimuli (Nikouline et al. 1999; Tiiainen et al. 1999), the magnitude of the contribution of the TMS click to the TEP is still unclear.

To reduce the presence of the AEP, different methods have been applied to mask the sound of the TMS pulse. In some studies, earplugs alone (Julkunen et al. 2008; Kähkönen et al. 2001, 2005a, b), or a combination of earplugs with an earmuff (Bikmullina et al. 2009; Kičić et al. 2008; Nikulin et al. 2003), have been used. Other studies report the administration of sound through (regular or inserted) headphones to make sure that the subject does not hear the TMS click. In these studies, either white noise (Fuggetta et al. 2005; Hamidi et al. 2010; Levit-Binnun et al. 2010; Paus et al. 2001; Veniero et al. 2010; Werf and Paus 2006) or noise created from the TMS click itself (adapted noise) (Ferrarelli et al. 2010) was used. It has been recognized that sound also travels via bone conduction and not via air alone (Nikouline et al. 1999). Therefore, in addition to playing white or adapted noise, different authors placed a thin layer of foam between the TMS coil and the

head of the subject (Casali et al. 2010; Casarotto et al. 2010; Esser et al. 2006; Ferrarelli et al. 2010; Mäki and Ilmoniemi 2010; Massimini et al. 2005, 2010; Rosanova et al. 2009).

Despite these various studies, it is still not clear which sound masking method is the most effective in reducing the AEP and which TEP components are possibly contaminated by it. In this study we further explore the presumed contribution of the AEP to the TEP, and evaluate different ways to mask the TMS clicking sound by comparing the presence of the P50 and the amplitude of the N100–P180 complex in both normal hearing subjects and a completely deaf person.

Materials and Methods

Subjects

Twelve healthy subjects (8 males, mean age 25 years, range 23–27 years, 11 right-handed) participated in this study after given written informed consent. We also included one subject who was born completely deaf (1 female, age 37 years, right-handed), which was confirmed with an audiogram. Whether the deafness was due to central or peripheral deficits has not been determined. The experimental protocol was approved by the local ethics committee (Medisch Spectrum Twente) and was in accordance with the declaration of Helsinki. We followed the guidelines for the use of TMS in clinical practice and research (Rossi et al. 2009).

Experimental Conditions

Subjects were seated in a chair, with their hands pronated in a relaxed position. They kept their eyes open and focussed on a marked point on the wall. There were two experiments.

In experiment 1, we evaluated the effect of 8 different masking conditions on the TEP in nine hearing subjects (subject 10 only participated in experiment 2, subjects 11 and 12 only participated in the control measurements, see below). These 8 conditions, in which earplugs, an earmuff, two types of noise and a layer of foam were combined, are listed in Table 1. The acronyms used are NM (no masking), E (earplugs), EE (earplugs/earmuff), EEf (earplugs/earmuff/foam), EW (earplugs/white noise), EWF (earplugs/white noise/foam), EA (earplugs/adapted noise) and EAF (earplugs/adapted noise/foam). The white and adapted noise were applied using external headphones. In addition, we tested the influence of various masking methods on the motor threshold, by determining the threshold in conditions NM, EA and EAF (see Table 1). This threshold was also determined without masking, but with the layer of foam between coil and head (condition F, see Table 1). The

Table 1 Experiment 1: masking conditions and motor threshold determination

Condition	Masking	Motor threshold	10 cm
NM	None	Yes	Yes
E	Earplugs	No	Yes
EE	Earplugs + earmuff	No	Yes
EEF	Earplugs + earmuff + foam	No	No
EW	Earplugs + white noise	No	Yes
EWf	Earplugs + white noise + foam	No	No
EA	Earplugs + adapted noise	Yes	Yes
EAF	Earplugs + adapted noise + foam	Yes	No
F ^a	Foam	Yes	No

^a Condition F was only used for evaluating the effect of the layer of foam on the motor threshold

motor threshold was defined as the lowest stimulus intensity which produced at least five MEPs of 50 μ V out of ten consecutive stimuli (Rossini et al. 1994), and is presented as a percentage of the maximal output of the TMS stimulator. Conditions NM, E, EE, EW, EA (see Table 1) were also evaluated when the coil was held at 10 cm from the head. At this distance, there is no brain activation induced by the magnetic pulse, which falls off with square of the distance from the coil (Griffiths 2008), but only an auditory stimulus due to the TMS clicking sound.

In experiment 2, we evaluated conditions NM (no masking) and EA (masking with earplugs/adapted noise) in the deaf subject and in a hearing subject (subject 10). The coil was now positioned at four different distances from the head; at 10 cm, at 6 cm and 2 cm with Plexiglas between coil and head to mimic bone conduction of sound, and at 0.5 cm with a layer of foam between coil and head. This resulted in 8 conditions, listed in Table 2.

We performed additional control measurements in two hearing subjects (subject 11 and 12). In these measurements, we compared (1) external headphones with inserted earphones; (2) the effect of using foam alone; (3) the response to sham stimulation, where we held the coil

sideways on top of the head, i.e. tilted by 90°. For measurement 1 we tested all conditions from the original experiment 1, using external headphones as well as inserted earphones. For measurement 2 we tested conditions NM (no masking), F (foam), E and EF (earplugs without and with foam), W and WF (white noise without and with foam), A and AF (adapted noise without and with foam), using both external headphones and inserted earphones. For measurement 3 we tested all conditions from the original experiment 1 using both external headphones and inserted earphones, with the coil tilted sideways.

Masking Strategies

The earplugs (3 M, St. Paul, Minnesota, United States) had an SNR (single number rating) of 37 dB and the earmuff (also from 3 M) had an SNR of 35 dB. The layer of foam was 0.5 cm thick, but when placed between coil and head it was pushed in substantially by the coil, with a remaining thickness of $\sim$ 2 mm. To create the adapted noise, we recorded the sound of the coil click and generated noise which contained the same frequency content as the coil click itself. The intensity of the white noise and adapted noise was set for each subject individually, by increasing the volume until the subject reported that he or she could no longer hear the coil click, or until the maximum volume of our equipment (95 dB) was reached. For the conditions with the coil placed directly on top of the head or with foam between coil and head, all subjects required this maximum volume of 95 dB.

TMS Targeting

Single biphasic TMS pulses, with pulse duration of 400 μ s and a random inter-pulse interval between 2 and 4 s, were delivered manually, using a 70 mm figure-of-eight air film coil and a Rapid² stimulator (The Magstim Company Ltd., Whitland, United Kingdom). The coil was placed tangentially over the hot-spot of the abductor digiti minimi muscle (ADM) in the left hemisphere, with the handle pointing backward and laterally at an angle 45° away from the midline. The maximum stimulator output was 1.5 T; stimulation intensity during the masking conditions was always set at 80 % of this output to ensure the same TMS clicking sound intensity for all subjects. For each masking condition we applied 50 TMS pulses.

Positioning of the coil was achieved using a robot-navigated system (Advanced Neuro Technology, Enschede, Netherlands). A headband carrying four passive reflective markers was fixed to the head of the subject and tracked by a Polaris infrared camera system (Northern Digital Inc., Waterloo, Ontario, Canada). The robot and the tracking system were registered to a common coordinate

Table 2 Experiment 2: masking conditions at various distances

Condition (cm)	Masking
10	No masking
10	Earplugs + adapted noise
6 with Plexiglas	No masking
6 with Plexiglas	Earplugs + adapted noise
2 with Plexiglas	No masking
2 with Plexiglas	Earplugs + adapted noise
0.5 with foam	No masking
0.5 with foam	Earplugs + adapted noise

system using a calibration procedure. The robot-guided TMS coil was added to the coordinate system by registration of three reference positions on the coil using a tracking pointer. A standard 1.5 T MRI scan was used to create a head model; this model was then registered to the subject's head and the coordinate system by collecting three landmarks and ~300 additional points with a tracking pointer.

Electromyography

To determine the motor threshold, surface electrodes were placed in a belly-tendon montage over the right ADM muscle. The ground electrode was placed on the dorsal side of the wrist. We recorded the EMG using an additional amplifier (TMSi, Oldenzaal, Netherlands) connected to the EEG amplifier, ensuring synchronized measurements. EMG was sampled at 2,048 Hz and low-pass filtered with an anti-aliasing filter with a cut-off frequency of 550 Hz.

EEG Recording and Analysis

The EEG was recorded continuously during TMS using a 64-channel DC amplifier (TMSi, Oldenzaal, Netherlands) and a TMS-compatible 64-electrode cap (ANT, Enschede, Netherlands). Electrode impedances were kept below 5 kOhm. The EEG-signals were low-pass filtered using an anti-aliasing filter with a cut-off frequency of 550 Hz and sampled at 2,048 Hz. The ground electrode was placed between electrode positions Fz and Fpz. We used a common average reference for the recordings. A single TMS pulse produced a stimulation artefact of 1–2 millivolts, lasting approximately 5 samples (2.5 ms). For analysis, the signal was re-referenced to the average of the left and right mastoid bone. Trials were defined from one second before to one second after every TMS pulse, resulting in 50 trials of 2 s for each masking condition per subject. Trials with eye-blinks were automatically rejected using a fixed threshold of 150 μ V for electrode channel Cz, which resulted in at least 45 accepted trials for all subjects. In the remaining trials, we removed the offset by subtracting the baseline. Then, we replaced the samples of the TMS artefact with a linear interpolation between six samples preceding and fourteen samples following the start of the TMS artefact. After this, trials were band-pass filtered between 1 and 50 Hz using a fourth order Butterworth filter. The filtered trials were then averaged to obtain the TEP, and the response measured at electrode Cz was selected for further analysis. After manually selecting a specific latency window for each subject, condition and peak, based on visual inspection of the TEP, we determined the amplitude of the N100 and P180 component automatically and compared the peak-to-peak amplitude between masking conditions

for all subjects, normalized with respect to the no masking condition. When no clear peaks or multiple peaks were visible, we used the latencies of previous selected peaks in that subject to select a data point showing the highest amplitude within that latency range. Because the P50 could not be identified in all subjects, we evaluated the presence of this component before and after masking through visual inspection.

Statistical Analysis

The motor thresholds and determined peak-to-peak amplitude of the N100–P180 complex for each masking condition were compared with the no masking condition for each subject. This was also done for the foam conditions with respect to the no-foam conditions. A paired *t* test was used to test for statistical significance. Differences with $p < 0.05$ were considered significant.

Results

There were no adverse events during the TMS measurements. All subjects showed a characteristic TEP response, although there was a variation in amplitude, caused by the difference in each subject's MT and the stimulation intensity of 80 % that was used for the experimental conditions. In all subjects, the N100–P180 complex reduced in amplitude when masking was applied. One of the other components of the AEP, the P50, could be identified in 4 subjects, and was completely suppressed in 3 of these subjects in the adapted noise (+foam) masking condition (EAF). Figure 1, presenting the TEP in a single subject, shows the reduction in amplitude of the N100–P180 complex, and the disappearance of the P50 when the earmuff (EEF) was replaced by headphones playing adapted noise (EAF).

The effect of different masking techniques on the normalized N100–P180 amplitude when the coil is held at 10 cm from the head is shown in Fig. 2 (upper panel). Earplugs alone, or in combination with an earmuff, reduced the amplitude significantly. The N100–P180 was removed completely in all subjects when either white or adapted noise was used, demonstrated by a response amplitude that resembled baseline activity.

Reducing the N100–P180 complex proved to be much more difficult when the coil was held on top of the head (Fig. 2, lower panel). Earplugs alone caused no significant reduction in N100–P180 amplitude. The earmuff did reduce the amplitude significantly, but the amount of reduction was small. Exposing the subjects to either white or adapted noise also caused a significant reduction in N100–P180, but the largest reduction was achieved when adding a layer of foam between coil and head ($p < 0.01$ for

Fig. 1 TEP response at electrode Cz in a single subject in two different masking conditions. When an earmuff with a layer of foam is used, there is a P50 visible (indicated with the arrow), which is part of the AEP. When the earmuff is replaced with earphones playing adapted noise, the P50 disappears. EEf masking condition with earplugs/earmuff/foam; EAF masking condition with earplugs/adapted noise/foam

Fig. 2 Amplitude of the 100–180 complex when different masking conditions are used, average of 9 hearing subjects. All amplitudes are shown relative to the baseline condition (NM). Results are shown for when the coil was held at 10 cm from the head (top figure) and when the coil was held on top of the head (bottom figure). Error bars represent the standard deviation. * $p < 0.05$ and ** $p < 0.001$ when compared to the baseline condition (NM). Abbreviations indicating the different masking conditions can be found in Table 1

all foam conditions compared to no foam conditions: EEf/EE, EWF/EW and EAF/EA). However, we were not able to completely suppress the N100–P180 complex when the coil was held on top of the head. The intensity of the masking sound (white or adapted noise) was 95 dB for all

subjects when the coil was held on top of the head, but four of the twelve hearing subjects (including subject 10, 11 and 12) reported still hearing the clicking sound. With the coil in the 10 cm position, a lower intensity of the masking sound sufficed; none of the subjects still heard the clicking sound.

In the nine hearing subjects, the motor threshold significantly increased when a layer of foam was placed between coil and head (NM 59 % vs. foam 70 %, $p < 0.001$, and EA 57 % vs. EAF 70 %, $p < 0.001$), which is most likely caused by the small increase in distance between coil and the target brain area. Using adapted noise did not change the motor threshold (NM 59 % vs. EA 57 %, $p = 0.08$).

In Fig. 3, we show the results from experiment 2. The hearing subject showed a clear AEP, both when the coil was placed at 10 and 6 cm from the head. The AEP components disappeared after masking was applied. The deaf subject did not show an AEP at these two distances. When the coil was kept closer to the head, the deaf subject showed a TEP with characteristic components at 30, 45, 60, 100 and 180 ms. The TEP in the hearing subject did respond to the applied masking condition: the N100–P180 amplitude decreased, and the P60 was reduced after masking. Visually, it seems that the amplitude of the P180 is reduced more easily than the N100 amplitude. The P180 increased as the coil was positioned closer to the head, ranging from 17 at 10 to 60 μV at 0.5 cm. For the 0.5 cm condition, there appears to be a small modulation in the deaf subject, comparable to the change observed in the hearing subject.

When we applied sham stimulation as a control measurement in the two hearing subjects, we found a P50–N100–P180 (see Fig. 4). These AEP components were reduced by masking. When we applied white or adapted noise during sham stimulation, the response was completely suppressed, even without using foam, as can be seen in Fig. 4. We found no differences in the response using external headphones or inserted headphones. Foam alone did reduce the N100–P180 amplitude, but not as much as using white or adapted noise alone.

Discussion

The physiological processes responsible for the different components of the TEP are still unknown (Ilmoniemi and Kicić 2010). However, it is very likely that the TEP is, at least partly, influenced by the clicking sound of the TMS coil (Nikouline et al. 1999). Therefore, an adequate masking technique may reduce the contribution of the AEP to the TMS evoked response. In this study, we compared different types of masking used in TMS–EEG experiments.

Fig. 3 TMS evoked potential in a hearing subject and a deaf subject for experiment 2. The deaf subject shows no response to only auditory stimuli (*top* figures, *red lines*), but when the coil is held close to the head (*bottom* figures, *red lines*), there is a TEP visible with characteristic components (P30–N45–P60–N100–P180). The hearing subject (*black lines*) shows an AEP when the coil is held at 10 and 6 cm from the head, which disappears when masking is applied. The P60, marked with *arrows*, reduces in amplitude when masking is applied. The amplitude of the N100–P180 complex reduces as well after masking. *Arrows* indicate the P60 in the hearing subject in the no masking condition. Note, that the amplitude of the P180 in the hearing subject after masking is of similar amplitude as the P180 in the deaf subject (*bottom right panel*)

Furthermore, we performed measurements in a deaf subject to obtain a TEP that is not at all influenced by auditory stimuli.

The AEP that is evoked by the TMS clicking sound can be identified by the N100–P180 complex, but also, in some subjects, by the presence of the P50 in the TEP. We show that white or adapted noise is able to completely remove the AEP caused by the TMS sound, when the coil is held at 10 cm from the head. At this distance the TMS magnetic pulse does not induce any activation in the brain, so the AEP is the only response visible in the EEG.

Using white or adapted noise also removed the AEP when the coil was positioned at 6 cm from the head, with a piece of Plexiglas between coil and head to mimic bone conduction of sound. This condition has been used before to prove that playing noise removes all auditory evoked activity from the response (Massimini et al. 2005). In another study, it was shown that no AEP was measured when playing white noise and positioning the coil 2 cm

from the head (Paus et al. 2001), but this study did not explore the bone conduction of sound. In our measurements, the auditory response was smaller when we removed the piece of Plexiglas (results not shown), confirming that bone conduction did increase the amplitude of the AEP. As the coil was moved closer to the head (10–6–2–0.5 cm), the intensity of the clicking sound may have also increased, adding to the gradual increase in amplitude of the N100–P180.

When the coil was touching the head, the N100–P180 amplitude is also modulated by the use of various masking methods, but it always remains present in the evoked response. This suggests that these components are also partly induced by the magnetic field of the pulse. Using white or adapted noise decreases the N100–P180 considerably, but adding a layer of foam between coil and head resulted in the largest attenuation of the AEP, reducing to half of its amplitude compared to noise alone. Using this additional layer of foam is therefore essential in

Fig. 4 Auditory evoked responses in the control measurements (coil tilted sideways on top of the head) in a hearing subject. Foam alone causes a small reduction in N100–P180 amplitude, comparable to using earplugs alone. Adding white noise (so without foam) completely suppresses the AEP, including the P50

minimizing the auditory components of the AEP, most likely because it minimizes bone-conduction. Foam alone did not reduce the AEP as much as listening to white or adapted noise alone, so the strength of successful masking lies in combining both foam and noise. It has to be noted that with foam the motor threshold increases significantly, which is most likely due to the increased distance between the coil and targeted brain area. This agrees with a previous report of an increased motor threshold when an EEG cap was placed between coil and head compared to measurements without the cap. This increase was also caused by an increase in the distance between coil and head (Julkunen et al. 2009). In addition, the focality of stimulation may also change for different stimulation distances.

The amplitude of the different TEP components may be influenced by numerous factors, such as a decrease in N100 amplitude when preparing a voluntary movement (Kičić et al. 2008; Nikulin et al. 2003). However, it is possible that observed variations in N100 or P180 partly reflect variations in auditory processing in addition to a TMS effect, and therefore N100 and P180 (and perhaps even P60) modulation should be used carefully as an outcome measure when no masking or a non-sufficient masking method is used. For example, in one of our subjects parts of the AEP remained in the TEP after masking, illustrated by the remaining presence of a P50 component. This may have been the case as well for other subjects, as the P50 latency may overlap with the P60 of the TEP. Figure 3 shows that the P60 reduces in amplitude after masking is applied, suggesting a hidden P50 auditory component. As a matter of fact, in five hearing subjects we could not even identify

the P50 before masking. Still, the P50 should not be visible after successful masking, and its presence after masking indicates that the AEP is not sufficiently reduced.

Some of our subjects reported still hearing the TMS clicking sound with a headphone playing white or adapted noise, which may have been caused by the fact that subjects were instructed to listen closely to the TMS sound while adapting the noise intensity. This possibly led them to also focus more on the TMS sound during the rest of the experiment, thereby increasing the amplitude of the AEP (Schiff et al. 2008). The presence of the AEP during protocols in which subjects focus on a specific (non-auditory) task may therefore be less prominent. On the other hand, it could also be that the subjects found it difficult to differentiate between hearing and feeling the TMS pulse: we were unable to assess this possible difference.

We performed a TMS–EEG experiment in a deaf subject to further differentiate between the TEP and AEP, as our measurements in the hearing subjects could not fully reveal what the exact contribution of the AEP is to the N100–P180 complex. The results from this additional experiment showed that the N100 and P180 were also present in the deaf subject, confirming that these components in hearing subjects are indeed largely induced by the magnetic pulse. There appears to be a small modulation in the deaf subject as well, comparable to the change observed in the hearing subject. This change may result from normal variation or noise between different measurements, and/or a minimal hearing ability in the deaf subject although she showed no response on the audiogram nor reported any awareness of the sound. In either case, the presence of this large N100–P180 component in the deaf subject strongly suggests a non-auditory contribution. In the hearing subject, the other characteristic TEP components (P30–N45) were not modulated by masking, and therefore these peaks are certainly solely TMS induced. Although this was a single case comparison, it proves that it is indeed highly unlikely that the N100 and P180 are only induced by auditory effects.

In the control measurement using sham stimulation with the coil sideways touching the head, we found no early TEP components, but only a P50, N100 and P180. When we applied masking, this response was completely suppressed. The sham stimulation aimed at mimicking the clicking sound with bone conduction. However, no foam was required in this sham condition to eliminate the response (see Fig. 4), suggesting that bone conduction did not play an important part in the auditory processing. This corresponds to the results of the Plexiglas condition at 6 cm, where the response was also already eliminated with only noise masking, so without applying foam. Conversely, when the coil is held in a normal position (directly on top of the head), foam does further reduce the amplitude of the N100 and P180 components. Because foam increases the distance

between coil and head, the amplitude reduction using foam may be partly due to a small decrease in intensity of the magnetic field. Another possible explanation for this discrepancy could be that the Plexiglas condition, as well as the sham condition, does not mimic bone conduction that well after all. However, the response did decrease in amplitude when we removed the Plexiglas layer, suggesting that there was indeed bone conduction, although possibly less pronounced than with the coil touching the head. With the Plexiglas layer, the intensity of the masking sound was probably high enough to mask this less pronounced bone conduction component so no foam was needed.

The noise masking might have been more efficient with the use of inserted headphones, as used by previous authors (Massimini et al. 2005; Paus et al. 2001; Werf and Paus 2006; Veniero et al. 2010; Fuggetta et al. 2005). Therefore, we compared external and inserted headphones in two subjects. We found no differences in the evoked potentials between these two conditions, although one of the subjects reported that the inserted earphones provided better masking. The intensity of the masking sound in the external headphone and inserted headphone was the same, which may be the most important feature to successfully mask the TMS clicking sound. A disadvantage of regular headphones is that they may interfere with the coil positioning when stimulating the temporal regions.

There are limitations in our study. We applied TMS at the same stimulus intensity for all subjects, independent of their motor threshold. This was done to evaluate the performance of the masking conditions in a more standardized way. However, for every individual subject this stimulation intensity related differently to the motor threshold, which could be higher or lower than the used settings. This introduces variations in TEP amplitudes, including the TMS induced part of the N100–P180 complex.

We did not evaluate the amplitude change of the N100 and P180 separately, but it appeared that the N100 was less influenced by masking than the P180. This could indicate that the N100 is largely induced by TMS activation, meaning that the auditory components only account for a small part of the N100, making the N100 a more stable output measure than the P180 when evaluating TEP modulation.

With a relatively small number of averaged responses we obtained well-defined TEPs, which showed large differences in N100–P180 amplitude. However, using more trials may further improve the signal-to-noise ratio, which could be necessary to evaluate very small changes in the TEP between groups or experimental conditions.

As Nikouline et al. suggested (Nikouline et al. 1999), there could be some sensory effects caused by activation of the skin by the magnetic pulse, so called somatosensory evoked potentials (SEP). When the coil is held directly on top of the head, there may still be a SEP present, adding to

the TEP in both the hearing subjects and the deaf subject. Our measurements did not enable us to differentiate between the SEP and magnetic activation of the cortex.

To summarize, masking strongly diminishes the AEP components in the TMS evoked responses. Using white or adapted noise combined with a layer of foam between coil and head results in the largest reduction of these auditory responses. Although the TMS clicking sound contributes to the N100–P180 amplitude, our results confirm that this response is for the most part induced by the magnetic pulse itself.

Acknowledgments The authors wish to thank Benjamin de Jonge, MSc, for his assistance during the measurements. This study is financed by the Dutch PIDON Grant, in which Advanced Neuro Technology (Enschede, Netherlands) participates as an industrial partner.

Conflict of interest E. ter Braack, C. de Vos and M. van Putten reported no financial interests or potential conflicts of interest.

References

- Barker AT, Jalinous R, Freeston IL (1985) Non-invasive magnetic stimulation of human motor cortex. *Lancet* 1(8437):1106–1107
- Bikmullina R, Kičić D, Carlson S, Nikulin VV (2009) Electrophysiological correlates of short-latency afferent inhibition: a combined EEG and TMS study. *Exp Brain Res* 194(4):517–526
- Bonato C, Miniussi C, Rossini PM (2006) Transcranial magnetic stimulation and cortical evoked potentials: a TMS/EEG co-registration study. *Clin Neurophysiol* 117(8):1699–1707
- Casali AG, Casarotto S, Rosanova M, Mariotti M, Massimini M (2010) General indices to characterize the electrical response of the cerebral cortex to TMS. *Neuroimage* 49(2):1459–1468
- Casarotto S, Lauro LJR, Bellina V, Casali AG, Rosanova M, Pigorini A, Defendi S, Mariotti M, Massimini M (2010) EEG responses to TMS are sensitive to changes in the perturbation parameters and repeatable over time. *PLoS One* 5(4):e10281
- Chen R, Cros D, Curra A, Lazzaro VD, Lefaucheur J-P, Magistris MR, Mills K, Rösler KM, Triggs WJ, Ugawa Y, Ziemann U (2008) The clinical diagnostic utility of transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol* 119(3):504–532
- Esser SK, Huber R, Massimini M, Peterson MJ, Ferrarelli F, Tononi G (2006) A direct demonstration of cortical LTP in humans: a combined TMS/EEG study. *Brain Res Bull* 69(1):86–94
- Ferrarelli F, Massimini M, Sarasso S, Casali A, Riedner BA, Angelini G, Tononi G, Pearce RA (2010) Breakdown in cortical effective connectivity during midazolam-induced loss of consciousness. *Proc Natl Acad Sci USA* 107(6):2681–2686
- Ferreri F, Pasqualetti P, Määttä S, Ponzio D, Ferrarelli F, Tononi G, Mervaala E, Miniussi C, Rossini PM (2011) Human brain connectivity during single and paired pulse transcranial magnetic stimulation. *Neuroimage* 54(1):90–102
- Fuggetta G, Fiaschi A, Manganotti P (2005) Modulation of cortical oscillatory activities induced by varying single-pulse transcranial magnetic stimulation intensity over the left primary motor area: a combined EEG and TMS study. *Neuroimage* 27(4):896–908
- Griffiths DJ (2008) Introduction to electrodynamics, 3rd edn. Pearson Benjamin Cummings, San Francisco

- Hamidi M, Slagter HA, Tononi G, Postle BR (2010) Brain responses evoked by high-frequency repetitive transcranial magnetic stimulation: an event-related potential study. *Brain Stimul* 3(1):2–14
- Ilmoniemi RJ, Kičić D (2010) Methodology for combined TMS and EEG. *Brain Topogr* 22(4):233–248
- Julkunen P, Jauhainen AM, Westerén-Punnonen S, Pirinen E, Soininen H, Könönen M, Pääkkönen A, Määttä S, Karhu J (2008) Navigated TMS combined with EEG in mild cognitive impairment and Alzheimer's disease: a pilot study. *J Neurosci Methods* 172(2):270–276
- Julkunen P, Säisänen L, Sarasti M, Könönen M (2009) Effect of electrode cap on measured cortical motor threshold. *J Neurosci Methods* 176(2):225–229
- Kähkönen S, Kesäniemi M, Nikouline VV, Karhu J, Ollikainen M, Holli M, Ilmoniemi RJ (2001) Ethanol modulates cortical activity: direct evidence with combined TMS and EEG. *Neuroimage* 14(2):322–328
- Kähkönen S, Komssi S, Wilenius J, Ilmoniemi RJ (2005a) Prefrontal TMS produces smaller EEG responses than motor-cortex TMS: implications for rTMS treatment in depression. *Psychopharmacology* 181(1):16–20
- Kähkönen S, Komssi S, Wilenius J, Ilmoniemi RJ (2005b) Prefrontal transcranial magnetic stimulation produces intensity-dependent EEG responses in humans. *Neuroimage* 24(4):955–960
- Kičić D, Lioumis P, Ilmoniemi RJ, Nikulin VV (2008) Bilateral changes in excitability of sensorimotor cortices during unilateral movement: combined electroencephalographic and transcranial magnetic stimulation study. *Neuroscience* 152(4):1119–1129
- Komssi S, Kähkönen S, Ilmoniemi RJ (2004) The effect of stimulus intensity on brain responses evoked by transcranial magnetic stimulation. *Hum Brain Mapp* 21(3):154–164
- Levit-Binnun N, Litvak V, Pratt H, Moses E, Zaroor M, Peled A (2010) Differences in TMS-evoked responses between schizophrenia patients and healthy controls can be observed without a dedicated EEG system. *Clin Neurophysiol* 121(3):332–339
- Lioumis P, Kičić D, Savolainen P, Mäkelä JP, Kähkönen S (2009) Reproducibility of TMS-evoked EEG responses. *Hum Brain Mapp* 30(4):1387–1396
- Mäki H, Ilmoniemi RJ (2010) The relationship between peripheral and early cortical activation induced by transcranial magnetic stimulation. *Neurosci Lett* 478(1):24–28
- Massimini M, Ferrarelli F, Huber R, Esser SK, Singh H, Tononi G (2005) Breakdown of cortical effective connectivity during sleep. *Science* 309(5744):2228–2232
- Massimini M, Ferrarelli F, Murphy M, Huber R, Riedner B, Casarotto S, Tononi G (2010) Cortical reactivity and effective connectivity during REM sleep in humans. *Cogn Neurosci* 1(3):176–183
- Miniussi C, Thut G (2009) Combining TMS and EEG offers new prospects in cognitive neuroscience. *Brain Topogr* 22(4):249–256
- Nikouline V, Ruohonen J, Ilmoniemi RJ (1999) The role of the coil click in TMS assessed with simultaneous EEG. *Clin Neurophysiol* 110(8):1325–1328
- Nikulin VV, Kičić D, Kähkönen S, Ilmoniemi RJ (2003) Modulation of electroencephalographic responses to transcranial magnetic stimulation: evidence for changes in cortical excitability related to movement. *Eur J Neurosci* 18(5):1206–1212
- Padberg F, George MS (2009) Repetitive transcranial magnetic stimulation of the prefrontal cortex in depression. *Exp Neurol* 219(1):2–13
- Paus T, Sipila PK, Strafella AP (2001) Synchronization of neuronal activity in the human primary motor cortex by transcranial magnetic stimulation: an EEG study. *J Neurophysiol* 86(4):1983–1990
- Picton TW, Hillyard SA, Krausz HI, Galambos R (1974) Human auditory evoked potentials. I. Evaluation of components. *Electroencephalogr Clin Neurophysiol* 36(2):179–190
- Rosanova M, Casali A, Bellina V, Resta F, Mariotti M, Massimini M (2009) Natural frequencies of human corticothalamic circuits. *J Neurosci* 29(24):7679–7685
- Rossi S, Hallett M, Rossini PM, Pascual-Leone A, TMS Consensus Group TS (2009) Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol* 120:2008–2039
- Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, Dimitrijević MR, Hallett M, Katayama Y, Lücking CH (1994) Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr Clin Neurophysiol* 91(2):79–92
- Schiff S, Valenti P, Andrea P, Lot M, Bisiacchi P, Gatta A, Amodio P (2008) The effect of aging on auditory components of event-related brain potentials. *Clin Neurophysiol* 119(8):1795–1802
- Ter Braack E, de Jonge B, van Putten MJ (2013) Reduction of TMS Induced Artifacts in EEG Using Principal Component Analysis. *IEEE Trans Neural Syst Rehabil Eng* 21(3):376–382 (in press)
- Tiitinen H, Virtanen J, Ilmoniemi RJ, Kamppuri J, Ollikainen M, Ruohonen J, Näätänen R (1999) Separation of contamination caused by coil clicks from responses elicited by transcranial magnetic stimulation. *Clin Neurophysiol* 110(5):982–985
- Veniero D, Maioli C, Miniussi C (2010) Potentiation of short-latency cortical responses by high-frequency repetitive transcranial magnetic stimulation. *J Neurophysiol* 104(3):1578–1588
- Werf YDVD, Paus T (2006) The neural response to transcranial magnetic stimulation of the human motor cortex. I. Intracortical and cortico-cortical contributions. *Exp Brain Res* 175(2):231–245