Cortical inhibition in attention deficit hyperactivity disorder: new insights from the electroencephalographic response to transcranial magnetic stimulation

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Attention deficit hyperactivity disorder is one of the most frequent neuropsychiatric disorders in childhood. Transcranial magnetic stimulation studies based on muscle responses (motor-evoked potentials) suggested that reduced motor inhibition contributes to hyperactivity, a core symptom of the disease. Here we employed the N100 component of the electroencephalographic response to transcranial magnetic stimulation as a novel marker for a direct assessment of cortical inhibitory processes, which has not been examined in attention deficit hyperactivity disorder so far. We further investigated to what extent affected children were able to regulate motor cortical inhibition, and whether effects of age on the electroencephalographic response to transcranial magnetic stimulation were compatible with either a delay in brain maturation or a qualitatively different development.

N100 amplitude evoked by transcranial magnetic stimulation and its age-dependent development were assessed in 20 children with attention deficit hyperactivity disorder and 19 healthy control children (8–14 years) by 64-channel electroencephalography. Amplitude and latency of the N100 component were compared at rest, during response preparation in a forewarned motor reaction time task and during movement execution. The amplitude of the N100 component at rest was significantly lower and its latency tended to be shorter in children with attention deficit hyperactivity disorder. Only in controls, N100 amplitude to transcranial magnetic stimulation was reduced by response preparation. During movement execution, N100 amplitude decreased while motor evoked potential amplitudes showed facilitation, indicating that the electroencephalographic response to transcranial magnetic stimulation provides further information on cortical excitability independent of motor evoked potential amplitudes.
and spinal influences. Children with attention deficit hyperactivity disorder showed a smaller N100 amplitude reduction during movement execution compared with control children. The N100 amplitude evoked by transcranial magnetic stimulation decreased with increasing age in both groups. The N100 reduction in children with attention deficit hyperactivity disorder at all ages suggests a qualitative difference rather than delayed development of cortical inhibition in this disease. Findings further suggest that top-down control of motor cortical inhibition is reduced in children with attention deficit hyperactivity disorder. We conclude that evoked potentials in response to transcranial magnetic stimulation are a promising new marker of cortical inhibition in attention deficit hyperactivity disorder during childhood.

**Keywords:** ADHD; TMS-evoked EEG potential; contingent negative variation; motor cortex inhibition; development

**Abbreviations:** ADHD = attention deficit hyperactivity disorder; CNV = contingent negative variation; MEP = motor-evoked potential; TMS = transcranial magnetic stimulation

### Introduction

Attention deficit hyperactivity disorder (ADHD) is one of the most frequently diagnosed neuropsychiatric disorders in childhood. A core symptom is motor hyperactivity (Biederman, 2005; Spencer et al., 2007). Previous studies using transcranial magnetic stimulation (TMS) in combination with motor-evoked potentials (MEPs; muscle contraction in response to TMS) have reported deficits in specific inhibitory functions of the motor loop in ADHD (Moll et al., 2000, 2001a, b; Gilbert et al., 2005; Gilbert, 2006; Buchmann et al., 2007). Therefore, a disturbed balance between motor facilitation and motor inhibition has been proposed to underlie motor hyperactivity in children with ADHD (Moll et al., 2000, 2001a, b; Buchmann et al., 2007). Still it remains unclear to what extent these inhibitory deficits can be influenced by cognitive control. Impaired cortical inhibitory function due to inefficient top-down regulation of executive control systems may play an important role in the pathophysiology of ADHD (Sergeant, 2000).

The electroencephalographic response following TMS (Komssi et al., 2004; Komssi and Kahkonen, 2006) after 100 ms (TMS-evoked N100) is of special interest for the investigation of cortical inhibitory deficits in ADHD. The highly reproducible (Lioumis et al., 2009; Ilmoniemi and Kicic, 2010) TMS-evoked N100 allows a non-invasive investigation of cortical excitability and is thought to reflect cortical inhibition (Nikulin et al., 2003; Bender et al., 2005a). In contrast to MEPs, which are potentially affected by a combination of cortical, subcortical and spinal mechanisms (Kiers et al., 1993), the TMS-evoked N100 directly assesses cortical responses to TMS without influences of spinal inhibitory mechanisms (Nikulin et al., 2003; Bender et al., 2005a; Ilmoniemi and Kicic, 2010). In healthy subjects, the TMS-evoked N100 component decreases during motor cortex disinhibition related to movement execution (Nikulin et al., 2003) and motor response preparation (Bender et al., 2005a) and increases during response inhibition (Bonnard et al., 2009). Therefore, the TMS-evoked N100 is considered to be a marker of motor cortex inhibition influenced by cortico–striato–thalamo–cortical loops (Bender et al., 2005a). The exact molecular mechanisms underlying TMS-evoked N100 are still unclear and have yet to be disentangled by pharmacological studies.

Investigation of the TMS-evoked N100 in children with ADHD at rest and during response preparation might provide evidence to what extent top-down control contributes to reduced motor cortex inhibition in ADHD. To the best of our knowledge, the TMS-evoked N100 has not yet been investigated in ADHD.

Another important question is whether the deficiency of motor cortex inhibition in ADHD reflects a true qualitative deficit or just a developmental delay. MEP-based inhibition measures such as short interval intracortical inhibition to paired pulses, which have been examined in ADHD previously, show an increase during development in childhood and early adolescence (Mall et al., 2004; Walther et al., 2009). Thus, both a delayed development as well as inhibitory deficits could explain short interval intracortical inhibition. The idea of a cortical developmental delay underlying ADHD symptoms (Kinsbourne, 1973; Denckla and Rudel, 1978; Mostofsky et al., 2003) was supported by neuroimaging studies that demonstrated immature prefrontal brain regions responsible for motor control in children with ADHD (Rubia et al., 2000). Furthermore, there seems to be a general delay in cortical maturation with differing regional maturational trajectories, most prominent in prefrontal regions, in volumetric MRI studies (Shaw et al., 2007).

Measurements of the TMS-evoked N100 will allow a distinction between a general developmental delay and a persisting inhibitory deficit because its amplitude and latency correlate negatively with age (Bender et al., 2005a). Thus, a TMS-evoked N100 amplitude reduction in ADHD would point towards a qualitatively reduced inhibition while a TMS-evoked N100 amplitude increase would point towards a developmental delay. The different effects of age on both parameters in cross-sectional studies underline that TMS-evoked N100 and paired pulse short interval intracortical inhibition reflect different forms of cortical inhibition.

We hypothesize an impaired cortical inhibitory function due to an intrinsic inhibitory deficit in children with ADHD reflected by smaller TMS-evoked N100 amplitudes compared with healthy children. Moreover, the influence of selective attention and motor preparation in a forewarned reaction time task (contingent negative variation; CNV) is investigated, presuming that an ineffective executive control function in children with ADHD results in a smaller effect of motor preparation on TMS-evoked N100 compared with healthy controls. According to previous results (Nikulin et al., 2003), we further hypothesize that during movement execution TMS-evoked N100 amplitudes should be even more strongly reduced than during motor preparation in the
CNV task. A clear dissociation between a facilitation of MEP amplitudes and a TMS-evoked N100 reduction during movement execution is expected. Such dissociation between MEP- and TMS-evoked N100 amplitudes would suggest that the analysis of TMS-evoked EEG potentials provides additional information independent of the conventional measurement of MEP amplitudes.

Materials and methods

Subjects

A group of 20 right-handed (assessed by the Edinburgh Handedness Inventory; Oldfield, 1971) children with ADHD and a gender- and age-matched group of 19 right-handed healthy controls were examined. Due to different effects of TMS applied to the motor cortex of the dominant and the non-dominant hemisphere (Semmler and Nordstrom, 1998; De Gennaro et al., 2004), only right-handed children were included. Control children were recruited from primary and high schools in Heidelberg, whereas children with ADHD were recruited on an outpatient basis. Detailed characteristics of the study samples can be found in Table 1.

The diagnosis of combined-type ADHD was based on the research criteria of the DSM-IV. Therefore, we applied the Kiddie-Schedule for Affective Disorders and Schizophrenia (Ambrosini et al., 1989) and a validated German ADHD-rating scale (Fremdbeurteilungsbogen Hyperkinetisches Syndrom; Döpfner and Lehmkuhl, 2000; Erhart et al., 2008) measuring DSM-IV-based ADHD symptoms. The Kiddie-Schedule for Affective Disorders and Schizophrenia was also used to exclude any other psychiatric disorders within both groups. Each subject received a general clinical and neurological examination. All children with ADHD included in the study suffered from the combined type of ADHD according to DSM-IV criteria. The ADHD rating scale scores are given in Table 1. Predominantly inattentive children were not included, since the study focused on the motor system. Children with ADHD did not fulfil the criteria of any other neurological or psychiatric disorder such as dyslexia, dyscalculia, autism spectrum disorder, tic disorder/Gilles de la Tourette syndrome or obsessive-compulsive disorder (exclusion criteria). Four children with ADHD had a comorbid oppositional defiant disorder.

A corrected visual acuity $\geq 0.8$ and a normal intelligence level (IQ $\geq 80$) were required for all participants (Table 1). IQ was evaluated with a short form of the German version of the Wechsler intelligence test for children (WISC/HAWIK III; Tewes and Wechsler, 2000) including four subtests (Schallberger, 2005). Subjects with a positive personal or family history of epilepsy or any kind of seizures in their individual history were excluded. Furthermore, medication with any kind of psychoactive drug except stimulant medication (methylphenidate) served as exclusion criterion. Fourteen subjects with ADHD received medical treatment (four with immediate release methylphenidate, seven with long acting methylphenidate, three with a combination of extended and immediate release methylphenidate) whereas six subjects were medication-naïve. The medication was stopped 60 h prior to the recordings (Moll et al., 2001b). Whenever necessary, children were examined on a Monday morning with medication being withdrawn over the weekend.

All children showed a reliable resting motor threshold $\leq 100\%$ of maximum stimulator output (Magstim 200; Magstim Ltd.). Similar to previous studies, the TMS procedure was well tolerated by all participants (Garvey and Gilbert, 2004; Gilbert et al., 2004).

The study was approved by the local ethics committee and the purpose and methods of the study were explained in an age-related way to the children. Informed written consent was obtained by all participants and their parents in accordance with the Declaration of Helsinki.

Contingent negative variation task

Forty CNV trials were recorded using a visual warning stimulus S1 [a white exclamation mark on 34 $\times$ 27 cm (width $\times$ height) black background that was presented for 150 ms] and a visual imperative stimulus S2 (a white line drawing of a hand on a 34 $\times$ 27 cm black background) on a computer screen at 1 m distance. Interttrial intervals varied randomly from 7 s to 15 s. Stimulus onset asynchrony between S1 and S2 was 3 s. Subjects were instructed to respond as fast as possible when S2 occurred by pressing a red button with the index finger of their dominant right hand.

The CNV paradigm was implemented by Presentation (Version 14.2., Neurobehavioural Systems Inc.). Two different combinations of CNV and TMS were assessed: TMS during the intertrial intervals of a CNV task. A clear dissociation between a facilitation of MEP amplitudes and a TMS-evoked N100 reduction during movement execution is expected. Such dissociation between MEP- and TMS-evoked N100 amplitudes would suggest that the analysis of TMS-evoked EEG potentials provides additional information independent of the conventional measurement of MEP amplitudes.

Table 1  Detailed characteristics of the study samples

<table>
<thead>
<tr>
<th>Diagnostic group</th>
<th>ADHD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size (n)</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>Age (mean $\pm$ SD)</td>
<td>11.4 $\pm$ 1.7 years</td>
<td>12.2 $\pm$ 2.0 years</td>
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<tr>
<td>Age range</td>
<td>8.8–14.0 years</td>
<td>8.2–14.8 years</td>
</tr>
<tr>
<td>Gender</td>
<td>18 males, 2 females</td>
<td>17 males, 2 females</td>
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<tr>
<td>Handedness (EHI; mean $\pm$ SD)</td>
<td>91 $\pm$ 11</td>
<td>87 $\pm$ 16</td>
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<tr>
<td>IQ (mean $\pm$ SD)</td>
<td>106.9 $\pm$ 10.6</td>
<td>116.6 $\pm$ 10.4</td>
</tr>
<tr>
<td>IQ range</td>
<td>81–130</td>
<td>97–132</td>
</tr>
<tr>
<td>FBB-HKS total (mean $\pm$ SD)</td>
<td>2.2 $\pm$ 0.4</td>
<td>–</td>
</tr>
<tr>
<td>FBB-HKS ‘hyperactivity and impulsivity’ (mean $\pm$ SD)</td>
<td>2.1 $\pm$ 0.4</td>
<td>–</td>
</tr>
<tr>
<td>Resting motor threshold (mean $\pm$ SD)</td>
<td>79 $\pm$ 14%</td>
<td>76 $\pm$ 12%</td>
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</tbody>
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EHI = Edinburgh Handedness Inventory; FBB-HKS = validated German version of the ADHD rating scale (20 items according DSM IV and ICD-10 ADHD criteria, scored by the parents of the child: 0 = not present, 1 = mildly present, criterion not fulfilled, 2 = clearly present, criterion fulfilled, 3 = extremely severe). The FBB-HKS total score is the mean of all 20 items, while the hyperactive-impulsive subscore excludes the nine DSM IV inattention items. Norms indicate for example that for 7 to 10-year-old boys an FBB-HKS total score $\geq 2.15$ corresponds to a Conners parent rating scale $t$-score of $\sim 70$ (about two standard deviations above the mean). The group mean value of 2.2 indicates that either about all hyperactivity-impulsivity items were clearly fulfilled with clinically relevant impairment or some items were even coded as ‘extremely severe’.
(while subjects were resting) or TMS during the late part of the CNV (late CNV), which is supposed to reflect sensory attention and motor preparation. During the CNV task, 20 TMS pulses were applied during the intertrial intervals and 20 pulses during late CNV. In addition, 20 CNV trials and 20 intertrial intervals were recorded without TMS. The different conditions varied randomly. In trials with TMS during late CNV (2.8 s after onset of S1), S2 was delayed until 0.5 s after TMS (3.3 s after onset of S1) in order to avoid interference between processes related to TMS and to presentation of S2. The CNV task always preceded the self-paced movement task, where subjects pressed the button that triggered TMS on the stimulator at irregular intervals varying between ~5 and 20 s. TMS-evoked N100 responses to stimulation during response preparation (late CNV) and during movement execution were compared with TMS-evoked N100 responses to randomly intercalated stimulations during the intertrial intervals.

**Transcranial magnetic stimulation**

Monophasic single-pulse TMS was applied using a Magstim 200 stimulator with a 13 cm diameter circular coil that was positioned over the vertex and oriented tangentially to the skull on the left hemisphere. The current flow within the coil was oriented counter-clockwise when viewed from above to stimulate preferentially the left motor cortex (Di Lazzaro et al., 1998, 2001). Stimulation was performed over the hand area of the motor cortex and the coil position resulting in maximum MEP amplitude in the right first dorsal interosseous muscle was marked on the electrode cap. The weight of the coil was carried by a tripod stand while an assistant assured a constant positioning of the coil during recordings.

Resting motor threshold was determined as the lowest intensity that reliably produced MEP amplitude of at least 50 μV in the relaxed right first dorsal interosseous muscle in at least 5 of 10 trials (Rossini et al., 1994; Paus et al., 2001). Relaxation of the first dorsal interosseous muscle was controlled by acoustic feedback. Children were hearing-protected against the clicks of the TMS coil by earplugs. We refrained from using a masking noise against bone conduction because of its possible detrimental effects on the subject’s vigilance level (Bender et al., 2005). Instead, we performed spatiotemporal source analysis to separate motor cortex responses to TMS from possible confounding influences by volume conduction of auditory evoked potentials (see below).

For TMS during the CNV task, intensity was set to 105% resting motor threshold to obtain reliable MEPs. In cases where 105% resting motor threshold exceeded the maximum stimulator output, intensity was set to 100% (one control subject, resting motor threshold: 96% maximum stimulator output; one subject with ADHD, resting motor threshold: 100% maximum stimulator output). Resting motor threshold is higher in children than in adults (Nezu et al., 1997), and the additional distance between the coil and the scalp caused by the EEG electrode cap further increases resting motor threshold despite the use of flat electrodes. The results were not affected by the single subject with ADHD with the slightly lower relative stimulus intensity. All children with ADHD were stimulated with slightly higher absolute stimulation intensities than the control group (resting motor threshold was 79 ± 14% maximum stimulator output in children with ADHD and 76 ± 12% maximum stimulator output in healthy controls). As TMS-evoked N100 amplitude increases with increasing TMS-intensity (Komiss et al., 2004; Bender et al., 2005a), this means that, if anything, decreases of TMS-evoked N100 amplitude in ADHD would be underestimated.

In order to confirm the inhibitory nature of TMS-evoked N100, a subsample of 17 healthy controls and 12 children with ADHD participated in a second task, in which the subjects themselves pressed the button that triggered the magnetic stimulation by an abduction movement of their right index finger. TMS intensity was again 105% of the resting motor threshold. This way we could compare TMS-evoked N100 as well as MEP amplitudes during movement execution with the respective measurements at rest (Nikulin et al., 2003).

**Electroencephalographic recordings**

Continuous direct current EEG from 64 channels at a sampling rate of 500 Hz was recorded (BrainAmps MR plus, BrainProducts) against a recording reference near the vertex. Sixty-four sintered silver/silver chloride electrodes (impedances < 10 kΩ) were fixed by using different sized equidistant electrode caps (Easycap GmbH). Vertical electrooculogram was recorded from an electrode attached 1 cm below the left eye, whereas horizontal electrooculogram was recorded from electrodes 1 cm lateral to the outer canthi. TMS and EEG were synchronized by transistor–transistor logic triggers in the CNV task (Presentation, Version 14.2., Neurobehavioural Systems Inc.). The stimulation software sent triggers to both the EEG recording system and to the magnetic stimulator. In trials with self-paced movements, the EEG was segmented on the onset of the TMS artefact in the EEG. We checked that the steep rise of the TMS artefact assured that this procedure introduced no jitter exceeding the jitter of the trigger timing in the CNV task, i.e. the broad TMS-evoked N100 component did not differ in amplitude regardless of whether it was calculated by segmentation on TMS triggers or the onset of the TMS artefact.

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**Figure 1** Timing of the contingent negative variation task and TMS. In trials without TMS, stimulus onset asynchrony between S1 and S2 was 3 s. In trials with TMS during late CNV (2.8 s after onset of S1, red flash), S2 was delayed until 0.5 s after TMS (3.3 s after onset of S1) in order to avoid interference between processes related to TMS and to presentation of S2. The CNV task always preceded the self-paced movement task, where subjects pressed the button that triggered TMS on the stimulator at irregular intervals varying between ~5 and 20 s. TMS-evoked N100 responses to stimulation during response preparation (late CNV) and during movement execution were compared with TMS-evoked N100 responses to randomly intercalated stimulations during the intertrial intervals.
Motor-evoked potential recordings

Surface EMG of the right first dorsal interosseous muscle was recorded from silver/silver chloride self-adhesive electrodes in a belly tendon montage by a Jaeger-Toennies Neuroscreen. Epochs lasted from 180 ms before the TMS pulse to 420 ms afterwards in EMG recordings. Electrode impedances were kept below 10 kΩ. Data were sampled at 2560 Hz and a bandpass filter of 20–1000 Hz was employed. MEP peak-to-peak amplitudes were determined for the different experimental conditions. Due to technical problems for \( n = 2 \) (CNV paradigm) or \( n = 3 \) (self-paced movement paradigm) healthy controls, no MEP measurements could be obtained. We made sure that the reported effects on TMS-evoked N100 amplitudes were not produced by extreme values in these subjects for which no MEP data were available.

Signal preprocessing

EEG-data analysis was processed via Brain Vision Analyzer (Brain Products GmbH) and BESA (BESA GmbH). Offline, recordings were transformed to an average reference.

For the investigation of TMS-evoked N100 at rest (TMS during intertrial intervals in the CNV task), EEG data were segmented based on TMS triggers into epochs of 2 s (0.5 s before TMS and 1.5 s after TMS). For analysis of TMS-evoked N100 during response preparation (TMS during late CNV), EEG data were segmented based on the warning stimulus into epochs of 5.3 s (3.8 s before and 1.5 s after TMS-stimulus).

Recordings 500–300 ms before TMS stimulus were chosen as baseline to avoid contamination of the EEG response by the TMS artefact. When TMS was applied during response preparation (late CNV), the baseline referred to the 1000 ms before the warning stimulus, in order to avoid effects of a baseline shift by late CNV amplitude.

A visual artefact rejection was applied as we did not want to rely upon automatic rejection procedures in the presence of TMS-induced artefacts. Far less than one-third of all trials had to be removed because of artefacts. Artefact rejection was conducted by a person who was blind to the study hypotheses. While artefact rejection reduced signal variance, it did not introduce systematic changes compared with averages of the uncorrected data. Leads with electrode polarization artefacts due to the position of the stimulation coil (most often F1) were excluded and interpolated by nearest neighbours. Only trials with correct response within 1 s after the presentation of 52 were included for further analysis.

The TMS-evoked N100 component could be clearly separated from the TMS-induced artefact (Bender et al., 2005a). The TMS-induced artefact was further reduced by a principal component analysis-based correction algorithm (BESA GmbH) described by Litvak et al. (2007). Additionally, a low-pass filter for the EEG signal (high cut-off 20 Hz; slope 48 dB/octave) was applied.

Data analysis

The TMS-evoked N100 was determined as the highest negative peak at C3, i.e. ipsilateral to TMS, in the interval 80–200 ms after TMS. This broad time window was chosen to take into account the long TMS-evoked N100 latencies of children (Bender et al., 2005a). C3 electrode was chosen according to previous reports that indicated a maximum of TMS-evoked EEG response of motor cortex and TMS-evoked N100 amplitude slightly posterior and ventral to C3 for left-hemispheric TMS or C4 for right-hemispheric TMS (Paus et al., 2001; Nikulin et al., 2003; Bender et al., 2005a; Bonato et al., 2006).

By visual inspection, it was confirmed for every participant that there was only one distinct negative peak in this latency range. The TMS-evoked N100 was calculated as the mean amplitude in the time window ±20 ms around the TMS-evoked N100 peak. TMS-evoked N100 latency was determined as the time from TMS to the N100 peak at C3.

The amplitude of late CNV was determined at electrode C3 as the mean potential amplitude from 500 ms to 30 ms before TMS in order to check for possible confounding effects of baseline shifts produced by late CNV amplitude on TMS-evoked N100 amplitude.

Source modelling

Time-variant, spatially stagnant, current dipoles were used to model electrically active brain areas minimizing residual variance and dipole energy (Picton et al., 1995). A four-shell spherical head model was applied (BESA research 5.3; BESA GmbH). The model was fitted on the grand average of the control group at rest in order to obtain a model that describes the most reliable characteristics of the spatiotemporal data matrix (Böcker et al., 1994; Bender et al., 2005b). Scalp topography during TMS-evoked N100 showed two pronounced maxima (Fig. 2), left centroparietal negativity and a less lateralized prefrontal positivity. The time courses of these two peaks differed slightly from each other (cf. source waveforms of dipoles 1 and 2 in Fig. 3). Thus, a single dipolar source was insufficient to explain the surface topography, and a careful assessment of ocular artefacts was necessary.

We applied the ocular artefact correction algorithm according to Gratton et al. (1983) as implemented in Brain Vision Analyzer. Since the TMS-artefact can considerably distort the calculated propagation factors, we ‘cut the TMS artefact out’ (Taylor et al., 2010) up to the start of the rise of the TMS-evoked N100 component (0–60 ms). However, this procedure could not remove time-locked blink responses to TMS. Blink potentials are mainly caused by currents flowing from the positively charged cornea to the anterior forehead when the eyelids slide down (Matsuo et al., 1975; Anterov et al., 1985). Thus, blinks produce a slightly lower negativity below and a stronger positivity above the eye (Picton et al., 2000; Bender et al., 2008). During early TMS-evoked N100 (60–110 ms), the frontopolar positivity was accompanied by a smaller infraorbital negativity, a hint towards time-locked blinks in response to the TMS (single trial example in Supplementary Fig. 1). Therefore, we modelled an additional dipole source. The effects on the scalp EEG electrodes can be modelled by a radially oriented equivalent dipole near the eyes (Lins et al., 1993), influences on central and more posterior electrodes are minor (Picton et al., 2000).

We obtained a stable two dipole solution, regardless of the fitting procedure. In order to minimize the overlap between the two generators of the frontopolar positive and the centroparietal negative peaks, we started by fitting a dipole on the descending flank of the TMS-evoked N100 peak in C3 (130–200 ms), after the end of the negative peak in the infraorbital channel (Supplementary Fig. 1). A second dipole source was fitted on the ascending flank of the TMS-evoked N100 peak in Fp1 (60–110 ms) with the first dipole still active. Finally, the two sources were refit together in order to avoid distortions in the modelling of the left central dipole due to any remaining blink influences. The genetic algorithm implemented in BESA applied to the ascending TMS-evoked N100 (60–110 ms) also yielded the same reliable two-dipole solution with a stable source in the left precentral region and one frontopolar source from different starting points. Also, when starting with temporal tangential dipoles, the dipole was fitted to the left precentral area. Remaining residual variance did
Figure 2  Mean TMS-evoked N100 amplitude in healthy controls and children with ADHD. (A) Mean TMS-evoked N100 amplitude recorded at C3 in control children. Black line = at rest; red line = during response preparation. (B) Mean TMS-evoked N100 amplitude recorded at C3 in children with ADHD. Black line = at rest; red line = during response preparation. (C) Effect of response preparation on the TMS-evoked N100 amplitude in controls (blue line) and children with ADHD (red line). Values were calculated for the mean of the covariate Age and thus slightly differ from the values in Table 2. Note that the effect of movement preparation on TMS-evoked N100 amplitude was only significant within the control group [interaction Group × Condition; F(1,36) = 4.0; P = 0.05]. Error bars indicate 95% confidence intervals. (D) Topography of the TMS-evoked N100 recorded 120 ms after TMS. Response preparation corresponds to the late part of the contingent negative variation (late CNV). Frontopolar positivity is marked in red and the centroparietal negativity ipsilateral to the TMS side is marked in blue.
not point towards an additional generator and consisted of low amplitude widespread potentials.

The model was applied to all individual averages, and the mean dipole moments in the TMS-evoked N100 time interval ±20 ms around the TMS-evoked N100 peak on dipole 2 was exported for statistical analysis. These source waveforms indicate the level of activity in the respective dipoles, and thus allow a determination of the extent to which the surface potential is explained by activity in the

Figure 3  Source analysis of TMS-evoked N100 in subjects with ADHD and typically-developing control children. (A) Dipole source localization and orientation in the healthy control group (grand average). Dipole 1 (red) was located near the eyes with a rather radial orientation that has been described for blink-related potentials. Dipole 2 (blue) was located near the stimulated primary motor cortex hand area and showed a mostly radial orientation. There is also a smaller tangential component with an orientation perpendicular to the posterior wall of the precentral gyrus. (B) Timecourse of the respective dipole moments in healthy control children. Dipole 1 peaked slightly earlier than dipole 2. On the single subject level, there was no constant temporal relationship between the two peaks, pointing towards two independent sources. The black bar marks the ‘cut out’ time interval around the TMS artefact. The cutting out procedure is established in the literature and was used to avoid distortions of the ocular correction factors by the TMS artefact. (C) Surface topography of TMS-evoked N100 and surface topographies explained by dipole 1 and dipole 2. While dipole 1 accounts for a large part of the frontopolar positivity, dipole 2 explains the ipsilateral centroparietal negativity together with a small part of the frontopolar positivity. Note that there was no deep temporal positivity in TMS-evoked N100 surface topography, indicating no prominent contribution of temporal sources to centroparietal TMS-evoked N100 by a tangentially oriented source in the temporal auditory cortex. (D) Localization of dipole 2 in a standard brain. (E) Dipole model for the ADHD group (grand average, dipole orientations refitted). (F) Time course of the respective dipole moments in children with ADHD (compare with Fig. 3B).
respective equivalent dipole. Dipole source localizations were kept constant when applied to individual averages, given the considerably lower signal-to-noise ratio of the individual data sets, however, dipole orientation was refit in order to adjust for individual differences in the anatomy of gyri and sulci (Picton et al., 1995; Bender et al., 2006). We made sure that refitting dipole orientations only resulted in minor adjustments and the spatial filter characteristics of the dipoles were maintained.

The localization of the left central dipole was confirmed by a source analysis of TMS-evoked N100 following subthreshold TMS, which evoked no EMG response. In this condition, TMS-induced blinks were much less common or absent. The result of this control experiment evoked no EMG response. In this condition, TMS-induced blinks were much less common or absent. The result of this control experiment illustrated in a representative healthy control child.

Statistical analyses

Experiment 1: contingent negative variation task

In two separate analyses, the main target parameters TMS-evoked N100 amplitude and latency were tested in parallel general linear models with the categorical between subject predictor diagnostic Group (ADHD versus healthy controls), the repeated measurement factor Condition (at rest versus response preparation) and the linear predictor Age. MEP amplitudes were examined in the same way, in order to assess whether TMS-evoked EEG potentials were independent of changes in MEP amplitude. Significant main effects and interactions were further examined by Tukey’s honestly significant difference post hoc tests. In order to test for maturational differences between the groups, we tested for significantly different age-related regression slopes for TMS-evoked N100 (interaction Age * Group) in two separate multifactorial regression analyses for TMS-evoked N100 at rest and during response preparation (Statistica, StatSoft Inc.).

The same analyses were repeated with the dipole moment of dipole 2 (Fig. 3) during the TMS-evoked N100 peak in order to exclude any influences of artificial sources (auditory responses to the coil click, TMS-related blinks) on our results. Dipole 2 served as a spatial filter assessing specifically potentials that are in agreement with primary and premotor cortex activation. Two-sided t-tests were used when appropriate for comparison of two means only. The significance level was 0.05.

Experiment 2: self-paced movements

Group differences and influences of movement execution on TMS-evoked N100 amplitude were assessed in a general linear model with the repeated measurement factor Condition (TMS-evoked N100 to a single TMS pulse at rest versus TMS-evoked N100 to TMS during movement execution), the categorical predictor Group (ADHD versus healthy controls) and the linear predictor Age. Again, the influences of the factors Condition and Group were examined for MEP amplitudes in order to exclude that findings with respect to TMS-evoked N100 amplitude were a consequence of differences in cortical excitation reflected by MEP amplitudes.

Surface electrode TMS-evoked N100 analyses were confirmed by parallel analyses on the TMS-evoked N100 peak in the source waveform of dipole 2 (Fig. 3) as a spatial filter to isolate the potential topography that is compatible with (pre/primary) motor cortex activation.

Results

Experiment 1

Behavioural data

Reaction time in the CNV-task (mean ± SD) was 275 ± 58 ms in children with ADHD and 301 ± 54 ms in healthy control children. It did not differ significantly between the two groups (t = 1.5; P = 0.15).

Resting motor threshold of ADHD and control children (Table 1) did not differ (t = 0.8; P = 0.42).

Electrophysiological data

TMS-evoked N100 amplitude

Time-course and topography of the TMS-evoked potential are illustrated in Fig. 2A, B and D. For mean values and standard deviations see Table 2. The topography of TMS-evoked N100 at rest showed an ipsilateral centrotoparietal negativity and a frontopolar positivity with both potentials decreasing during response preparation.

The factors Age and diagnostic Group explained 28% of the variance of the TMS-evoked N100 amplitudes at rest in the respective linear model [F(2,36) = 7.0; P = 0.003].

The TMS-evoked N100 amplitude was smaller in children with ADHD than in healthy control children [main effect Group F(1,36) = 4.8; P = 0.04; Fig. 2 and Table 2]. Response preparation and sensory attention during the CNV task resulted in a decrease of TMS-evoked N100 amplitude when compared with the rest condition [main effect Condition F(1,36) = 5.0; P = 0.03]. TMS-evoked N100 amplitudes declined with increasing Age [F(1,36) = 10.5; P = 0.003; standardized regression coefficient β±standard error at rest 0.47 ± 0.14; during response preparation 0.41 ± 0.15; Fig. 4A and B]. The positive regression coefficients indicate a decrease of the negative TMS-evoked N100 amplitude with increasing age.

Table 2 TMS-evoked N100 amplitude recorded at C3 at rest and during response preparation (lateral CNV) in healthy controls and children with ADHD

<table>
<thead>
<tr>
<th>Diagnostic group</th>
<th>n</th>
<th>CNV-task</th>
<th>Mean amplitude ± standard deviation (μV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>19</td>
<td>TMS-evoked N100 at rest</td>
<td>–58.0 ± 13.7</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>TMS-evoked N100 during response</td>
<td>–32.1 ± 8.3</td>
</tr>
<tr>
<td>ADHD</td>
<td>20</td>
<td>TMS-evoked N100 at rest</td>
<td>–32.4 ± 7.9</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>TMS-evoked N100 during response</td>
<td>–23.2 ± 7.8</td>
</tr>
</tbody>
</table>
In children with ADHD, the response preparation related TMS-evoked N100 decrease was smaller than in control children (interaction Group × Condition; \(F(1,36) = 4.0; P = 0.05\)). Tukey’s post hoc tests revealed a difference of the TMS-evoked N100 amplitude at rest and during motor preparation/sensory attention only for the control group (\(P = 0.005\)), whereas the TMS-evoked N100 amplitude in children with ADHD at rest and during response preparation did not differ significantly (\(P = 0.55\)).

The age-related regression slopes on TMS-evoked N100 amplitude for children with ADHD and healthy controls did not differ from each other (Fig. 4A and B) as indicated by the non-significant interaction between Age × Group at rest \(F(1,35) = 0.1; P = 0.74\) and during response preparation \(F(1,35) = 0.7; P = 0.41\).

Medication naïve children with ADHD and children with ADHD who discontinued their stimulant medication did not differ systematically in the overall age-dependent development (Fig. 4A).

Including IQ together with Age as predictor variables into an ANCOVA model with the factors Group and Condition revealed no effect of IQ on TMS-evoked N100 at rest or during response preparation during late CNV [main effect IQ \(F(1,35) = 0.4; P = 0.51\)]. The explained variance (\(R^2\)) of the total model including IQ was still 28% as in the model without IQ. The effects of Age \(F(1,35) = 9.9; P = 0.003\) as well as the interaction Group × Condition \(F(1,35) = 4.2; P = 0.048\) were confirmed. The results of Tukey’s post hoc tests remained nearly unchanged (\(P = 0.004\) instead of \(P = 0.005\) for the decrease of TMS-evoked N100 amplitude at rest).

Figure 4 Scatter plots illustrating the effects of Age on TMS-evoked N100 amplitude at rest and N100 latency in ADHD and healthy children. TMS-evoked N100 amplitude at C3 and mean TMS-evoked N100 latency (N100 latency at rest + N100 latency during response preparation/2) decreased with age in both groups. Mean TMS-evoked N100 latency was used for calculations since response preparation had no effect on TMS-evoked N100 latency. On the abscissa the age of children is plotted, while the ordinate shows the TMS-evoked N100 amplitude in μV and mean TMS-evoked N100 latency in ms, respectively. Positive values of TMS-evoked N100 amplitude within the scatter plot resulted when negative TMS-evoked N100 amplitudes were small because we analysed a broad reliable time window of 40 ms around the TMS-evoked N100 peak, so that adjacent positive potentials could lead to positive values in the case of small TMS-evoked N100 peaks. TMS-evoked N100 amplitudes in control children were in good agreement with previous results (Bender et al., 2005a). (A) TMS-evoked N100 amplitude at C3 in ADHD (six medication naïve subjects with ADHD are marked in green). (B) TMS-evoked N100 amplitude at C3 in healthy controls. (C) TMS-evoked N100 latency in ADHD (six medication naïve subjects with ADHD are marked in green). (D) TMS-evoked N100 latency in healthy controls.
N100 during response preparation in the control group) compared with the model without IQ. In contrast to the interaction Group × Condition, the main effects of Group [F(1,35) = 2.8; P = 0.10] and Condition [F(1,35) = 2.8; P = 0.10] were not significant in this model.

**TMS-evoked N100 latency**

Age and diagnostic Group explained 36% of the variance of mean TMS-evoked N100 latency at rest in a linear model [F(2,36) = 10.3; P = 0.0004]. The TMS-evoked N100 latency showed a trend towards shorter latencies in children with ADHD than in healthy controls [main effect Group F(1,36) = 3.7; P = 0.06; Table 3]. Response preparation during late CNV did not affect TMS-evoked N100 latency [main effect Condition F(1,36) = 0.4; P = 0.55]. A main effect of Age on TMS-evoked N100 latency was observed [F(1,36) = 19.2; P = 0.0001; standardized regression coefficient β ± standard error at rest −0.59 ± 0.14; during response preparation −0.58 ± 0.14], indicating shorter latencies for older children (Fig. 4C and D).

Again, there was no significant difference between the ADHD and the control group with respect to the age-related regression slopes at rest [interaction Age × Group F(1,35) = 0.2; P = 0.67] or during response preparation [F(1,35) = 0.1; P = 0.78].

**MEP and CNV amplitudes**

Group identity and experimental condition affected TMS-evoked N100 and MEP amplitudes in different ways. There were no group differences in MEP amplitudes at rest [main effect Group F(1,34) = 2.3; P = 0.13; means ± standard error: ADHD (n = 20) 244 ± 42 μV; healthy controls (n = 17) 203 ± 45 μV]. MEP amplitudes during response preparation during late CNV did not differ either [main effect Condition F(1,34) = 0.4; P = 0.53; means ± standard error: ADHD 279 ± 41 μV; healthy controls 209 ± 48 μV]. There was no interaction between the factors Group and Condition [F(1,34) = 0.9; P = 0.34]. MEP amplitudes at 105% resting motor threshold increased with increasing age [main effect of linear predictor Age F(1,34) = 7.0; P = 0.01].

Late CNV amplitude became more negative with increasing age [effect of the linear predictor Age F(1,36) = 5.8; P = 0.02], with children < 12 years of age often showing still positive late CNV amplitudes. There were no group differences in late CNV amplitude at the electrodes where TMS-evoked N100 was measured [effect of Group F(1,36) = 1.0; P = 0.33; mean amplitudes ± standard deviations: healthy controls +0.7 ± 4.6 μV and children with ADHD +0.2 ± 3.5 μV]. The TMS-evoked N100 results did not change by correction for late CNV amplitude (calculating the baseline during late CNV directly before the TMS stimulus compared with a baseline before the warning stimulus) due to the low CNV amplitudes.

**Source analysis**

Source analysis yielded two stable dipole localizations shown in Fig. 3. Dipole 2 was located near the hand area of the primary motor cortex and showed an orientation that was rather perpendicular to crown and the posterior wall of the precentral gyrus, i.e. primary motor cortex. The tangential part of this dipole did not explain the prefrontal positivity fully, because the prefrontal positivity had almost an equal strength as the centroparietal negativity, despite a much more distant localization from the dipole. As a result, most of the prefrontal positivity was explained by a second dipole located near the eyes. The time course of the infra- and supraorbital vertical electrooculogram suggested that this was due to time-locked blinks in response to TMS, which occurred in some but not all subjects (Supplementary Fig. 1). As these blinks were time-locked to TMS, they were not removed by the Gratton and Coles ocular correction algorithm, and due to the overlap with TMS-evoked N100 they were difficult to reject in manual artefact rejection. Instead, remaining influences of TMS-related blinks could well be modelled by an equivalent dipole source. About 91% of the variance of the potential topography could be explained in the TMS-evoked N100 time interval 80–140 ms by this two dipole solution in healthy control children at rest compared with 90% in children with ADHD.

In subthreshold TMS, when less blinks occurred, the prefrontal positivity was strongly reduced (Fig. 5), and again, no hints for volume conduction effects from the temporal auditory cortex could be found.

All results from the surface potential analysis were confirmed in dipole source analysis due to stable correlations between the dipole moment of dipole 2 during the TMS-evoked N100 peak and TMS-evoked N100 amplitude at C3 (r = 0.80; t = 8.2; P < 0.0001 at rest; r = 0.54; t = 3.9; P = 0.0004 during late CNV). There was a decrease in dipole 2 activation during TMS-evoked N100 with increasing age [main effect Age F(1,36) = 16.5; P = 0.0002]. Healthy control children had greater dipole 2 activation than children with ADHD [main effect Group F(1,36) = 5.5; P = 0.02]. However, this effect was modulated by an interaction Group × Condition [F(1,36) = 4.2; P = 0.047]. Tukey’s post hoc tests showed differences in TMS-evoked N100 amplitude between rest and response preparation for the healthy control group (P = 0.006) but not for the ADHD group (P = 0.99). There was no main effect of Condition [rest versus response preparation F(1,36) = 0.2; P = 0.64].

### Table 3 TMS-evoked N100 latency at rest and during response preparation in healthy controls and children with ADHD

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>CNV task</th>
<th>Mean latency ± standard deviation (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>19</td>
<td>TMS-evoked N100 latency at rest</td>
<td>116.0 ± 5.0</td>
</tr>
<tr>
<td>ADHD</td>
<td>19</td>
<td>TMS-evoked N100 latency during response preparation</td>
<td>111.3 ± 4.7</td>
</tr>
<tr>
<td>Controls</td>
<td>20</td>
<td>TMS-evoked N100 latency at rest</td>
<td>109.1 ± 3.7</td>
</tr>
<tr>
<td>ADHD</td>
<td>20</td>
<td>TMS-evoked N100 latency during response preparation</td>
<td>108.2 ± 4.1</td>
</tr>
</tbody>
</table>
Peak latencies of the moment of dipole 2 during the TMS-evoked N100 time interval were almost the same as the latencies for TMS-evoked N100 at C3 (data not shown to avoid redundancies).

**Experiment 2: self-paced movements**

We found a strong reduction of the TMS-evoked N100 amplitude during movement execution [main effect Condition; \( F(1,26) = 10.6; P = 0.003 \)]. TMS-evoked N100 amplitude during movement execution was \(-15.2 \pm 30.4\mu V\) in healthy control children and \(-18.0 \pm 53.6\mu V\) in children with ADHD. The interaction between the factors Condition and Group \( [F(1,26) = 7.1; P = 0.01] \) was explained by less reduction of the TMS-evoked N100 during overt movement execution in children with ADHD compared with healthy control children.

In contrast, MEP amplitudes (available for 14 healthy controls and 12 children with ADHD) were facilitated during movement [main effect Condition \( F(1,24) = 167.7; P < 0.00001 \)] with MEP amplitudes exceeding 1 mV (mean ± standard error: healthy controls 1264 ± 122 mV; ADHD 1303 ± 130 mV). There were no significant main effects or interactions involving the factor Group when MEP amplitude was analysed. Thus, there was a clear dissociation between MEP amplitudes and TMS-evoked N100 amplitudes with opposite effects of the movement condition on both parameters.

Again, source analysis confirmed the surface potential results. There was a significant interaction Group × Condition \([F(1,26) = 9.5; P = 0.005]\) for the moment of dipole 2 during the TMS-evoked N100 while there were no main effects for Group

\[
[F(1,26) = 1.5; P = 0.23] \quad \text{or Condition} \quad [F(1,26) = 0.4; P = 0.52].
\]

Tukey’s post hoc tests indicated a decrease of dipole 2 activation during TMS-evoked N100 during movement execution in healthy children \( (P = 0.003) \) but not children with ADHD \( (P = 0.90) \).

### Correlations between ADHD rating scale scores and TMS-evoked N100

There was a negative correlation between the ADHD rating scale’s hyperactivity score and age (i.e. declining motor hyperactivity with increasing age; \( r = -0.48; t = 2.2; P = 0.04 \)) and a similar negative correlation between TMS-evoked N100 amplitude and age \( (r = -0.40; t = 2.7; P = 0.01) \). Controlling for age-effects, there was no partial correlation between the hyperactivity score and TMS-evoked N100 amplitude at C3 \( (r = -0.14; t = 0.6; P = 0.58) \). Results were nearly identical when the motor cortex dipole moment during TMS-evoked N100 was used instead of TMS-evoked N100 amplitude at electrode C3.

### Discussion

There were five main findings in this study.

1. We found a significantly smaller TMS-evoked N100 amplitude in children with ADHD compared with healthy subjects. The TMS-evoked N100 is thought to reflect cortical inhibition (Nikulin et al., 2003; Bender et al., 2005a; Kicic et al., 2008; Bikmullina et al., 2009; Bonnard et al., 2012).

![Figure 5](https://academic.oup.com/brain/article-abstract/135/7/2215/351439/568772/1)
Its reduced amplitude provides further evidence of an inhibitory deficit in children with ADHD.

(2) Consistent with previous results (Nikulin et al., 2003), we found that TMS-evoked N100 amplitude was strongly suppressed during movement execution, while MEP amplitudes were facilitated. TMS-evoked N100 occurred already at sub-threshold TMS intensities with highly reliable amplitudes (Bender et al., 2005a). This supports previous evidence that TMS-evoked N100 adds additional and independent information to measures based on MEP amplitudes and may be a marker of cortical inhibition (Bender et al., 2005a; Bonnard et al., 2009; Ilmoniemi et al., 2010). We found that less TMS-evoked N100 reduction (i.e. less decrease of inhibition) occurred during overt movement performance in ADHD compared with healthy controls.

(3) Response preparation during late CNV resulted in a significant decrease of the TMS-evoked N100 amplitude within the control group, whereas there was no significant effect on the TMS-evoked N100 amplitude in children with ADHD. The interaction between Group and Condition indicated that response preparation affected TMS-evoked N100 amplitudes of healthy controls and children with ADHD in a significantly different way, i.e. there was less top-down modulation in children with ADHD.

(4) Our results clearly contradict the hypothesis that all inhibitory deficits in children with ADHD could be explained by delayed cortical maturation. We found a decrease in TMS-evoked N100 amplitude and latency with age in both groups, with decreased TMS-evoked N100 amplitude and latency for ADHD at all ages tested (Fig. 4).

(5) Source analysis: a dipole source was reliably fitted near the stimulated hand area of the primary motor cortex with an orientation that is in good agreement with activation of the crown (radial part) and the posterior wall (tangential part) of the precentral gyrus. It explained the strongly lateralized centroparietal negative TMS-evoked N100 peak and a small part of the frontopolar positivity. In contrast, most of the frontopolar positivity that slightly preceded the centroparietal TMS-evoked N100 in time was explained by an equivalent dipole source near the eyes, most likely reflecting TMS-induced blinks.

TMS-evoked N100 as a marker of inhibitory deficits and mechanisms of top-down control of motor inhibition

TMS-evoked N100 is increased when actively resisting a movement (Bonnard et al., 2009), but decreased during movement execution (Nikulin et al., 2003) and response preparation (Bender et al., 2005a) during late CNV.

With respect to the molecular mechanisms behind TMS-evoked N100, further studies with pharmacological manipulations seem necessary (Daskalakis et al., 2008; Bikmullina et al., 2009). Here, we discuss only what can be inferred from the TMS-evoked N100 amplitude decrease during movement preparation and execution. The late part of the CNV is supposed to reflect sensory attention and motor preparation necessary for a fast response to the imperative stimulus S2 (Rockstroh, 1989). In this context, the lower TMS-evoked N100 amplitude at rest in children with ADHD as well as the reduced decrease of TMS-evoked N100 during response preparation (late CNV) point towards an impairment of cortico–thalamo–cortical or cortico–basal ganglia–thalamo–cortical inhibitory loops. This perspective is supported by structural brain imaging studies postulating abnormalities in executive control functions in ADHD involving the prefrontal cortex as well as basal ganglia (Seidman et al., 2005). The apparent contradiction that alcohol, which enhances GABAergic inhibition, reduces TMS-evoked N100 (Kähkönen et al., 2007) may be accounted for by dopaminergic alcohol effects on top-down control loops (Cott et al., 1976). This explanation would be in line with the behavioural disinhibition under alcohol (Ostling and Fillmore, 2010).

Another important point is that the different age-dependent development of TMS-evoked N100 (decrease with increasing age) and paired pulse short interval intracortical inhibition (increase with increasing age; Mall et al., 2004) suggest that both parameters depend on different mechanisms. The TMS-evoked N100 development could be linked to a decrease of synaptic density or cortical thickness of the primary motor cortex. This would be consistent with a volumetric study showing a ‘preterm’ development of the primary motor cortex in children with ADHD, reaching peak motor cortical thickness ~7 years of age (Shaw et al., 2007). In contrast, the maturation of paired pulse short interval intracortical inhibition might be linked to increasing GABA-A receptor density with increasing age (Galanopoulou, 2008; Duncan et al., 2010).

Based on the latency of TMS-evoked N100, GABA-B receptors have been suggested to contribute to its generation (Bikmullina et al., 2009). GABA-B receptor density peaks after 3 weeks or continuously declines to adult values in rats (Malitschek et al., 1998; Bianchi et al., 2005). This developmental trajectory could contribute to age effects on TMS-evoked N100 amplitude. However, findings about maturation of the cortical silent period, which has also been associated with GABA-B neurotransmission, do not support this hypothesis. Some experiments found no maturation changes in cortical silent period while others found an age-dependent increase (Moll et al., 1999; Garvey et al., 2003). In sum, TMS-evoked N100 age-dependent development seems to differ from all MEP-based inhibition parameters.

Influence of top-down cognitive control on motor cortex inhibition in attention deficit hyperactivity disorder

Two different explanations may be proposed for less TMS-evoked N100 modulation during the CNV task in children with ADHD. On one hand, a smaller reduction of inhibition may be sufficient to trigger an actual movement in children with ADHD due to already reduced inhibition levels at rest (Moll et al., 2000, 2001a, b; Buchmann et al., 2007). Since the TMS-evoked N100 during response preparation in healthy controls approached the TMS-evoked N100 in children with ADHD at rest, children with...
ADHD may present with lower levels of motor cortex inhibition already under resting conditions.

On the other hand, these findings might be explained by an inefficient regulation of executive control systems resulting in an impaired regulation of motor preparation in children with ADHD. An efficient flexible top-down control could enable healthy children to increase motor cortex inhibition at ‘rest’ and to disinhibit their motor system in a well-timed manner during response preparation and execution. Both explanations, reduced inhibition in the motor cortex as well as reduced cognitive top-down control could contribute to an understanding of our findings.

**Age effects on motor cortex inhibition in attention deficit hyperactivity disorder**

Both the lower TMS-evoked N100 amplitudes and shorter TMS-evoked N100 latencies in children with ADHD clearly contradicted a quantitative developmental delay and pointed towards qualitative differences between the groups. Although we cannot completely discard that higher amplitudes may need more time to develop and, therefore, may have resulted in longer TMS-evoked N100 latencies, usually amplitudes and latencies of event-related potential components are regarded as independent parameters and have not always been found to show a similar developmental course (Johnstone et al., 1996; Onofri et al., 2001; Geneva et al., 2002; Wunderlich et al., 2006).

There was no significant difference in the regression slopes between healthy children and children with ADHD. The developmental curve in ADHD was rather parallel-shifted towards lower TMS-evoked N100 amplitudes though subtle slope differences may have been missed due to insufficient statistical power to detect them.

Motor hyperactivity and impulsivity decrease with increasing age in patients with ADHD (Biederman et al., 2000; Kooij et al., 2005) and the decreased paired pulse short and long interval intracortical inhibition in children with ADHD (Buchmann et al., 2007) were not unanimously replicated in adults with ADHD (Hoepfner et al., 2008). Accordingly, it was suggested that motor cortical inhibition levels show a development-dependent (partial) normalization with increasing age. The age effect on TMS-evoked N100 fits these findings because during adolescence, the TMS-evoked N100 amplitudes of the ADHD and the healthy group seemed to converge, most likely due to a floor effect.

**Methodological issues**

Reaction times in the CNV task did not differ significantly between the two groups so that an influence of reaction time seems unlikely to have caused the observed differences in the cognitive control of the TMS-evoked N100 amplitude. In any case, reaction time was found to have no effect on TMS-evoked N100 amplitude (Nikulin et al., 2003). Normal, or even reduced, mean reaction times have been found in hyperactive-impulsive children (Klein et al., 2006) and the relatively low number of trials in our CNV task may have limited the occurrence of attentional lapses but emphasized an impulsive reaction style.

Furthermore, the TMS-evoked N100 amplitude is positively correlated with the stimulation intensity applied (Bender et al., 2005a). Since both groups were stimulated with similar intensities, differences in TMS intensity cannot account for the observed differences in the TMS-evoked N100 amplitude. If anything, the slightly higher stimulation intensity used in the ADHD group would have led to an underestimation of the observed lower TMS-evoked N100 amplitude in this group. On average, control children were a little older than children with ADHD. However, this fact is unlikely to account for our results because older children show smaller TMS-evoked N100 amplitudes (Bender et al., 2005a).

In a recent study, methylphenidate was found to increase CNV-amplitudes (Linsen et al., 2011). Most of the children with ADHD in the present study received treatment with methylphenidate. Although methylphenidate was paused 60 h before recordings according to previous studies (Moll et al., 2001b), it cannot be entirely excluded that a long-term effect of medication might have affected our results. This is, however, very unlikely, because the CNV-amplitudes in our sample were very small and not different between ADHD and healthy control children. Furthermore, previous TMS studies showed similar effects in previously medicated and medication naïve groups (Moll et al., 2001b; Buchmann et al., 2007). Future studies will need to assess the effects of stimulant treatment on TMS-evoked N100.

The topography of the TMS-evoked N100 potential (Fig. 2D) shows a similar distribution over the stimulated dominant motor cortex at left central leads in both groups, while in ADHD, in addition, a stronger negativity located over the mid-frontocentral leads can be seen. This could indicate a stronger propagation of TMS-evoked activity in the motor system of children with ADHD to the supplementary motor area or even to the contralateral motor cortex. In this respect, more mirror movements have been described in children with ADHD and may point towards deficits in transcallosal inhibition in ADHD (Macneil et al., 2011). Though MEP data were not available for all subjects, the results showed a clear dissociation between TMS-evoked N100 and MEP amplitudes.

As the TMS-evoked N100 reduction in healthy subjects far exceeded the known amplitudes of the Bereitschaftspotential (readiness potential) even in adult subjects, we do not report details about the Bereitschaftspotential analysis, as major confounding influences on TMS-evoked N100 amplitude suppression seem impossible. Late CNV amplitudes showed that the amplitudes of premovement potentials are low in the examined age-range.

EEG registration implies that the electrodes increase the distance between the TMS coil and the cortex. Thus, higher TMS intensities are necessary to yield the same effects as without the EEG cap. As in previous studies, careful coil positioning (the weight of the coil was carried by a tripod stand and an assistant of the experiment assured the correct and constant position with respect to the subject’s head) avoided electrode artefacts, especially in the target electrode C3.

Source analysis indicated no sources in the temporal auditory cortices. TMS-evoked N100 was strongly lateralized ipsilateral to the side of stimulation. A trigeminal response to scalp sensation
can be ruled out, as it would show a contra- or bilateral topography (Paus et al., 2001; Nikulin et al., 2003).

Limitations

Our results need to be replicated in larger samples of children and adolescents with ADHD. Further, this study was cross-sectional and any conclusions with respect to maturation need to be proven in prospective longitudinal studies.

Conclusion

TMS-evoked EEG potentials proved to represent a promising new marker of cortical inhibition in children with ADHD. Reduced cognitive control of the motor system could contribute to diminished motor cortex inhibition in children with ADHD, and is likely to be responsible for the observed deficient TMS-evoked N100 amplitude reduction during motor response preparation and motor execution compared with rest in ADHD. Effects of age on TMS-evoked N100 were not compatible with a delayed development accounting for the reduced TMS-evoked N100 in ADHD, pointing towards intrinsic inhibition deficits in the motor system of ADHD.

Acknowledgements

We highly appreciate the helpful comments of Professor H. C. Steinhausen during the composition of this manuscript. We kindly thank S. Walther and Dr. J. J. Simon for implementing the paradigms and K. Herwig, D. Gmehlin and A. Stiefel for their assistance during the recordings. Moreover, we gratefully acknowledge the time and effort which the participating children and their families devoted to this study.

Funding

This study was supported by the Riese Foundation of the Goethe University, Frankfurt/Main, Germany.

Supplementary material

Supplementary material is available at Brain online.

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