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ORIGINAL ARTICLE

Development of corticospinal motor excitability and cortical silent period from mid-childhood to adulthood – a navigated TMS study

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Abstract

OBJECTIVES We characterized the maturation of the excitability of the motor cortex and corticospinal tract from childhood to adulthood using electric field (EF) navigated TMS and correlated the results with manual dexterity.

METHODS Both hemispheres of healthy right-handed children (6–9 years, n=10), preadolescents (10–12 years, n=13), adolescents (14–17 years, n=12) and young adults (22–34 years, n=12) of both genders were examined. The optimal cortical representation site and resting motor threshold (rMT) were determined for the abductor pollicis brevis muscle. Motor evoked potential (MEP) latencies and amplitudes in relaxed and active states, input–output curves and silent period (SP) durations were determined. Manual dexterity was assessed with the Box and Block Test.

RESULTS rMT (in terms of maximal stimulator output or EF strength) decreased with age ($p<0.001$) and stabilized when reaching adolescence. The MEP amplitude ($p=0.037$) and latency increased ($p<0.001$) with age. Input-output curves showed age-dependent changes in several parameters. SP duration decreased with age ($p < 0.001$), and demonstrated hemispheric asymmetry in the children ($p=0.030$). Manual dexterity correlated negatively with rMT ($p<0.001$).

DISCUSSION The excitation/inhibition balance develops with age and correlates with manual dexterity. Strong corticospinal inhibition was observed in the children and this was found to decrease with age. Interhemispheric asymmetry was only observed for SP duration in the children. Knowledge of normal development is crucial for the understanding of developmental disabilities, and using estimates of effective EF may be advantageous in future pediatric studies.

Key words

Transcranial magnetic stimulation; human maturation; development; motor cortex; excitability; silent period

Running title: Development of motor cortex excitability

INTRODUCTION

Neuromotor function plays an essential role in normal cognitive development, and is frequently impaired in children with developmental disabilities. Fine motor skills appear in a rudimentary fashion during the first year of life. Noticeable gains are then made through the early school years, and there is continued improvement in quality and speed of motor skills until adolescence, or even until the age of 30 years [17]. The status of motor function may act as a 'biomarker' for neighboring systems and circuits, which are responsible for the behavioral anomalies in developmental disabilities [13, 61].

Neuroimaging studies have demonstrated age-related increases in white matter that are thought to reflect progressive myelination, whereas age-related decreases in grey matter are thought to reflect both synaptic pruning and myelination [21]. The maturation of the corpus callosum continues into young adulthood, but the growth of callosal regions containing motor fibers may be already complete before the age of 10 years [7]. Myelination of the corticospinal tract is completed morphologically by early childhood [79]. Neuromotor development and its pathological functional changes can be readily examined with transcranial magnetic stimulation (TMS) [43]. The motor threshold (MT) that reflects the developmental stage of myelination of the corticospinal tracts is high in children and then decreases approximately linearly until mid-adolescence [16, 17, 48] or even until early adulthood [51]. It is also known that the motor-evoked potential (MEP) amplitudes are smaller and even polyphasic in early childhood and the motor conduction velocities are slower in children compared with adults, mainly attributed to immature myelination [43, 48]. The central motor conduction time gradually shortens from 2 to about 13 years and then plateaus [16, 48]. The conduction time in peripheral components also initially decreases, but then from the age of 5 years progressively increases in proportion to the height [16] jointly resulting in the progressive prolongation of MEP latency. However, the height-adjusted MEP latency (suggested to parallel the complex rearrangement of the corticospinal tract during acquisition of complex motor capabilities) decreases, whereas the latency during muscle contraction increases with age. This difference, called the latency jump, has been suggested as a specific TMS-derived indicator of maturation [8]. Late muscular MEP responses involving reticulospinal tract are more prevalent in the proximal than distal muscles, and suggested to be of diagnostic value in children for detection of unilateral dysfunction of the central nervous system (CNS) [38, 42].

With TMS, it is also possible to assess inhibitory functioning and its deviations during neuromotor development. This information cannot be obtained with any other neuroimaging methodologies [29]. Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the CNS and has a central role in a wide variety of physiological and biochemical processes regulation of cognition [45], memory and learning [27], circadian rhythms [1], neural development [60], adult neurogenesis [52], and motor function [23] including motor learning [75]. GABA has an elementary and homeostatic, possibly also compensatory role for intrinsic motor excitability [22]. $GABA_B$ has been suggested to have a more important role than $GABA_A$ for motor functioning [73] and its dysfunction may be important in behavioral anomalies in developmental disabilities such as autism spectrum disorders [15, 53, 58], complex motor stereotypies [26], in evaluating the cortical excitability in mild traumatic brain injury [71], epilepsy [6, 56], and Tourette's syndrome [57]. Understanding the role of $GABA_B$ in motor plasticity could have clinical relevance in terms of therapeutic rehabilitation. There are already several ongoing clinical trials in pediatric patients [18, 24, 59].

Paired-pulse paradigms assess corticospinal inhibition reflecting $GABA_A$ and $GABA_B$ activity, and $GABA_B$ neurotransmitter activity can be assessed by silent period (SP) measurements [31, 82]. In children, results from early paired-pulse studies have suggested that there is less net intracortical inhibition through $GABA_A$ receptor activity [37, 83]. However, this was questioned in a recent study, which individually took into account the contaminating effect of concurrent facilitation that instead was enhanced in young children [69]. The maturational trajectories of TMS-evoked inhibitory parameters reflecting especially $GABA_B$ activity have been poorly defined (SP) or lacking (LICI). Previous studies on maturation using SP measurements have shown somewhat contradictory results, with either no age-related changes [20, 28], or an increase in duration with age [41]. Furthermore, transcallosal excitability/inhibition can be evaluated by other useful TMS parameters such as ipsilateral SP and paired-pulse interhemispheric inhibition (IHI) that have been recently characterized during development [10] and may be related with motor performance [20]. These may have clinical relevance in rehabilitation applications for disabled children such as of perinatal stroke hemiparesis or cerebral palsy [9, 35].

There is some evidence of hemispheric asymmetry in TMS measures in healthy children mirroring the asynchronous cortical development. Higher MTs in the right (non-dominant) hemisphere compared to the left hemisphere have been found, and this asymmetry gradually levels off with age [20]. There is also indication of hemispheric asymmetry in SP (longer SP durations in the right

hemisphere) [20] as well as asymmetry in long- interval cortical inhibition (LICI) in young adults [81]. The asymmetry in the excitability of the motor cortex has also been shown to relate to manual dexterity [81], and could theoretically mirror the development of hemispheric dominance and leftward lateralization, which is so far quite unresolved for its neural basis and timing [80]. Abnormal development of hemispheric asymmetry is observed in mental illnesses that exhibit language symptoms, such as schizophrenia and autism [80]. A clear hand preference, related to hemispheric lateralization is observed by 6 years of age [67]. In the current study, we correlated the neurophysiological TMS data with the functional assessment of the gross motor dexterity using the Box and Block Test.

There are a few studies on healthy adult aging and corticospinal excitability [11, 78], but publications on healthy children are scarce. Furthermore, the pivotal developmental TMS studies were performed with a round coil and measures of cortical excitability were assessed as the percentage of maximum stimulator output (MSO). None of the previous studies used neuronavigation [30, 66], and the availability of this more accurate methodology therefore warrants revisiting these topics. Brain-scalp distances are lower in children compared to adults [4], and the physiological distance affects the MT when quantified as the percentage of MSO [33]. The electric field (EF) provides a measure of the actual strength of stimulation induced on the cortex, and takes into account the geometry of the individual head including the conductivity (e.g. air-tissue, skull-intracranial) and the differences in distance and thickness [25]. Therefore, using EF strength instead of MSO when comparing subject groups with notable differences in scalp-to-cortex distance, as is the case in developmental studies, might open up more accurate estimation of cortical excitability and lead to more predictable dosing of for example therapeutic repetitive TMS.

In this study, we used EF navigated TMS (nTMS) to assess corticospinal excitability and the long-interval intracortical inhibition measured with SP and hemispheric asymmetry during different phases of maturation in healthy right-handed children, preadolescents, adolescents and adults, aged from 7 to 33 years. The EF estimations were assessed in addition to percentage of MSO. The use of EF navigation results in more stable MEPs with significantly higher amplitudes and shorter latencies [34]. We accurately targeted the stimulations to the anatomical hand knob area in the precentral gyrus, functionally verified as the site producing repeatable MEPs of the highest amplitude. TMS results were correlated with gross motor function to identify neurophysiological markers of motor development.

METHODS

Participants

The study participants were 47 healthy subjects (**Table 1**), equally distributed in terms of gender in each categorical group: children 6–9 years (n=10), preadolescents 10–12 years (n=13), adolescents 14–17 years (n=12) and adults 22–34 years (n=12). All subjects were right-handed, except for one ambidextrous (predominantly right-handed) boy in the youngest group. Handedness was determined by the Waterloo Handedness Questionnaire (in revised and reduced form with 20 items) [76]. Children and adolescents were recruited from the nearby schools or via an ongoing study at the Institute of Biomedicine, University of Eastern Finland [84]. The adult subjects were recruited from the faculty personnel. Exclusion criteria were contraindications to magnetic resonance imaging (MRI) or TMS [65]. After informing about the nature of the study, written informed consent was received from subjects, as well as from the guardian, in the case of participants younger than 15 years of age. The birth data was accessible in 25 out of 35 subjects, and out of these, two children born late preterm (weeks 35 and 36). The study was approved by the Research Ethics Committee of the Hospital District of Northern Savo (48/2010). The experiments were carried out in accordance with the latest version of the Helsinki declaration.

Manual dexterity

Manual dexterity was assessed with the Box and Block Test evaluating motor speed and skill [39]. The test score was the number of small wooden cubes moved one at a time from one side of a box to the other side over a wall for one minute, when performed separately for both hands. This test is an easy, feasible, valid, and reliable measurement for gross manual dexterity in young children [32].

Neuronavigated transcranial magnetic stimulation

Subjects were scanned with a 3T scanner (Philips Achieva TX, Philips Healthcare, Eindhoven, The Netherlands). Structural three-dimensional T1-weighted MR-images were acquired (TR 8.07 ms, TE 3.7 ms, flip angle 8°, 1x1x1 mm^3 resolution) for navigation. A neuroradiologist screened the MRI before nTMS examination.

The hemispheres were examined in a randomized order. NTMS was performed with an eXimia stimulator (version 3.2.2, Nexstim Plc., Helsinki, Finland) and a figure-of-eight coil with a biphasic wave-form combined with a navigation system to enable continuous visualization of the stimulation coil in relation to the individual cortical anatomical structures and ensuring optimal tilting, *i.e.*

placing the coil tangentially to the head. Throughout the measurement, muscle activity was monitored on-line and by stimulus-locked electromyography (EMG) to record TMS-induced MEPs using disposable Ag-AgCl surface electrodes on the abductor pollicis brevis (APB) muscle. The optimal cortical representation site and coil orientation to produce repeatable MEPs of the highest amplitude from a resting APB muscle were determined [78]. Following measurements were targeted at that site:

1) The resting motor threshold (rMT) was assessed using a threshold hunting paradigm using Motor Threshold Assessment Tool 2.0 [2, 3] with an amplitude limit of ≥50 μV.

2) Eleven MEPs were collected at stimulus intensity of 120% of rMT in two conditions: at rest and during slight muscle contraction (about 200 μV in amplitude), using an interstimulus interval of 4–6 s.

3) An input–output curve was constructed using stimulation intensities between 90% and 150% of rMT in steps of 10 in a randomized order (10 pulses per intensity, resulting in 70 stimuli).

4) For SP measurements, seven EMG samples were collected using a stimulus intensity of 120% of rMT with moderate muscle contraction. The subjects were provided with the online EMG, and asked to used muscle contraction about half of the maximum force (about 500 μV in amplitude). The task was to squeeze the balls simultaneously with both hands [77, 78]. SP measurement was not performed in adults.

In addition to the percentage value of the MSO, the corresponding effective induced electric field was assessed on the exterior of the cortex using the navigation software [12]. The calculation of the intracranial electric field is based on a spherical individual head model (over 40,000 spheres that are adjusted locally to the shape and size of the individual head) relative to the physical parameters of the TMS coil in 3D space [66]. The method is explained in more detail in [68].

Analysis

Scalp-to-cortex distance was measured as a peeling depth from the scalp to the surface of gray matter at the optimal representation site of the APB muscle using the navigation software [44]. MEP latencies and peak-to-peak amplitudes were determined from each trial (excluding the first one, since its amplitude may be significantly higher than that of the following ones [36], and the mean values for each subject were calculated. Latencies were determined in both relaxed and active conditions, and the latency jump was calculated as a difference of the mean values. SP duration was assessed as absolute durations [77]; the mean for each subject was calculated after excluding trials with the shortest and longest duration. The input–output curves as a function of the EF were

analyzed by fitting the Boltzmann sigmoidal function to determine maximal value (EMR_{max}), the slope of the curve and the mid-point of the curve (S50) [14]. This parameter was optimized to fit the MEP data using a multidimensional nonlinear least-mean-square algorithm in Matlab (Mathworks Inc, Natick, MA, USA).

Statistical analysis

Statistical analyses were performed with SPSS version 22 (IBM Corporation, Somers, NY, USA). A linear mixed model was used to test the Box and Block Test result, MT (as percentage of the stimulator output and EF strength), MEP (latency and amplitude), SP duration, and input-output curve (slope). Main effects were tested with group and hemisphere as fixed factors, and the subject as a random factor. Post-hoc comparisons for significant between-group differences were performed with Sidak adjustment for multiple comparisons. When significant interaction was observed between group and hemisphere, a within-group pairwise comparison was performed. For input-output curves, post-hoc comparisons were examined between consecutive age groups. For correlation analysis, we used the nonparametric Spearman's rho, since not all parameters were normally distributed (Kolmogorov-Smirnov and Levene's test). In the correlation analysis, we used the individual combined (mean) value of left and right hemisphere for each parameter. Similarly, the mean score for left and right hand in the Box and Block Test was used. Partial correlation was used to test the effect of age and height on the MEP latency, and the effect of age on the dependence between excitability measures and manual dexterity. A P-value of <0.05 was considered to be statistically significant.

RESULTS

The groups differed in manual dexterity, scalp-to-cortex distance, and height (**Table 1**). Manual dexterity improved with age ($F = 28.95$, $p < 0.001$, post-hoc analysis revealed that all groups differed from each other except for adolescents and adults, **Table 1**). The dexterity of the right hand was better than the left hand ($F = 5.11$, $p = 0.029$), and pairwise comparison revealed this to be significant in adolescents ($F = 4.413$, $p = 0.042$). No mirror movements were observed in any subject during the test. In one subject in the youngest group, the rMT of the APB exceeded the maximum stimulator output, and no further measurements were performed.

Resting motor threshold

rMT decreased across the groups as a function of increasing age expressed as %-MSO ($F = 18.20$, $p < 0.001$, post hoc analysis revealed that all groups differed from each other except adolescents and adults, **Table 2, Figure 1**). rMT decreased also as EF value ($F = 13.33$, $p < 0.001$, post hoc displaying the difference between children and adolescents, children and adults, and preadolescents and adults (**Table 2)**.

Motor evoked potential

MEP amplitude increased as a function of age ($F = 3.11$, $p = 0.037$, post hoc the difference was found significant between children and adults, **Table 2**). Moreover, both the relaxed and active MEP latency increased with age (relaxed $F = 12.28$, $p < 0.001$, active $F = 16.69$, $p < 0.001$, post hoc significant differences were between children and adolescents, children and adults, preadolescents and adolescents, and preadolescents and adults, **Table 2)**. Correlation was stronger between latency and height ($rho = 0.842$, $p < 0.001$) than latency and age ($rho = 0.509$, $p = 0.001$). When corrected for age, the correlation between height and latency remained ($r = 0.799$, $p < 0.001$), but age and latency no longer correlated after correcting for height. There was no effect of age, hemisphere, or interaction between age and hemisphere on the latency jump.

Input–output curves

Since the maximal stimulation intensity used for IO-curve was 150% MT, only subjects whose rMT was <67% of the MSO were included in the IO-curve measurements. This resulted in data for 42 subjects (9 children, 13 preadolescents, 8 adolescents, 12 adults). The curve did not reach plateau for preadolescents. Age had a significant effect on the slope ($F = 3.76$, $p = 0.019$, **Figure 2**). In the post hoc comparison, the difference was significant between preadolescents and adults ($p = 0.026$). We found that there was an overall effect in the EMR_{max}-value, which refers to the plateau value (F=3.01, p=0.044), which in the post-hoc comparison of the consecutive age-groups was limited between children and preadolescents (p=0.022), i.e. children demonstrating 1840µV smaller EMR_{max}-value compared to preadolescents. In the S50 parameter, we observed strong age-dependent effect (F=20.40, p<0.001). In the post-hoc comparison of the consecutive age-groups, the finding was limited between children and preadolescents (p<0.001), i.e., children demonstrating on average 42V/m higher S50 values than preadolescents. The fitted Boltzmann sigmoidal functions provided a good fit to all applied data, indicated by excellent fit within each age-group (bias-corrected 95% CIs for R^2 between measured data and fitted function in adults: 0.94–0.97, adolescents: 0.95–0.97, preadolescents: 0.91–0.96 and children: 0.88–0.94).

Silent period

SP duration decreased with increasing age ($F = 8.56$, $p < 0.001$, **Table 2**, the group difference being significant between children and adolescents). Representative examples of raw data from each age group are shown in Figure 3. There was an interaction between hemisphere and group ($F = 4.73$, $p = 0.017$), and pairwise comparison indicated a significant interhemispheric difference in the children ($F = 5.23$, $p = 0.030$).

Correlations between manual dexterity, age, rMT and SP

Box and Block Test score correlated negatively with rMT as percentage of MSO (*rho* = -0.638, $p < 0.001$) and SP duration (*rho* = -0.484, $p = 0.006$). Since rMT and SP duration also correlated with age, we performed partial correlation analysis controlled for age, and the statistical significance was no longer reached: rMT ($r = -0.301$, $p = 0.106$) and SP ($r = 0.158$, $p = 0.406$).

DISCUSSION

This study investigated the neurophysiological development of the corticospinal motor tract from childhood to adulthood. Age-dependency was observed for rMT, SP duration, and MEP characteristics including input-output curves. The excitability profile of the motor cortex in the children (6 to 9 years of age) deviated from other groups, whereas that of adolescents highly resembled that of adults, although progress further continued both in the input-output curves and in manual dexterity. By using the EF estimation in MT measures, similar result was found reflecting actual differences in excitability between the age groups. An age-dependent increase was found in both relaxed and active MEP latencies, and this was due to height of the subjects. According to our results, the variability in the MEP latencies was not more marked in children than in adults [43]. Similarly, the decrease in latency jump with age was attributable to the height of the subjects. Hemispheric asymmetry was only observed in SP duration in children.

MT reflects the developmental stage of myelination of the corticospinal tracts as well as the membrane characteristics and synaptic efficacy of the cortical and spinal motoneurons [20]. In accordance with previous studies, the rMT was highest in the children and then decreased as a function of age, reaching a plateau in adolescence [16, 20]. Previously, it has been reported that it may be difficult to elicit MEPs in relaxed muscles in children using a focal coil and rMT has not always been determinable [40]. In our study, the rMT exceeded the MSO only in the youngest child examined (age 6 years 10 months). There is also recent evidence that gestational age at birth and

birth weight significantly influences rMT [50]. In our study population, the MTs of the subjects born preterm were not significantly different from others, though the number of subjects does not allow for assessing effects of preterm age on excitability.

We also assessed the rMTs in terms of the EF strength on the surface of the cortex and achieved an approximation of an actual measure of cortical excitability. The required induced EF was considerably higher than that of healthy adults [12]. Our results were in line also with those achieved motor mappings in children with neurological disorders [46, 47]. The use of the effective induced EF may present a methodological departure that may be preferable for developmental studies, since it diminishes the anatomical differences due to the development (the scalp-to-cortex distance increase with age, mainly attributable to increases in the cerebrospinal fluid and inner table distance) [4, 25] and may thus to give a more realistic picture of the excitability of the cortex and corticospinal tract.

The use of input-output curves in children is encouraged as they provide the most accurate measure of cortical and corticospinal excitability [50] and could possibly improve the diagnostic value of TMS in children with motor disturbances [43]. We observed a shift towards greater excitability with increasing age and an overall effect of age on the slope, EMR_{max} and S50. The maximum amplitude (EMR_{max}) was much lower in children than those of older age groups, meaning that not even high stimulus intensities could elicit high MEP amplitudes. The development of a steeper slope with increasing age may be indicative of an enhanced synaptic connectivity, favoring rapid recruitment of corticospinal output during recruitment of the motor cortex in volitional movement, suggesting a more sensitive (higher gain) regulation of cortical output and wider modification range of synaptic plasticity [62-64]. The pattern of recruitment of motoneurons may be different between the age groups, and this might result from changes in the synaptic spinal motoneuron density [49]. Alternatively, the number of motoneurons being activated may be the same, but it might occur in less synchronous manner in the younger subjects, presumably as the result of more temporally dispersed I-waves [49], which would lead to phase cancellation of the action potentials of individual motor units and hence a smaller peak-to-peak MEP amplitude [50]. The curve of preadolescents did not reach plateau, i.e. MEP maximum, suggesting that there remains capacity for further neuronal excitation. S50 also differed significantly between children and preadolescents, which demonstrates an overall development of excitability.

Our observation of increased SP duration suggests stronger $GABA_B$-ergic inhibition in children. This finding is supported by the evidence acquired from studies combining TMS with electroencephalography (TMS-EEG), where increased inhibitory N100 responses reflecting $GABA_B$-ergic inhibition [54], have been found in children [5, 44]. In this study, we did not perform SP in adults, but in our previous study using an identical setup [78], mean values of 91.6 (24.3) ms are reported for adults aged 20–29 years, which suggests a further decrease from adolescence to adulthood. Prior studies have found no effect of age on SP duration [20, 28], or by contrast, an increase in duration with age [41]. However, Garvey and coworkers reported the longest SP durations in the subjects less than ten years of age [20].

An interesting question is how our finding of increased inhibition in children relates to the phenomenon of surround inhibition [74], which has not been studied during the development. Theoretically, more efficient surrounding inhibition of the motor system in children would allow better discrimination of the hand muscles. The following reduction of the inhibition with age, observed as shortening duration of SPs, would then be paralleled with improvement in manual dexterity. Surround inhibition has also been used to detect the neurophysiological hemispheric asymmetry related to handedness, and possibly greater dexterity [72]. Studying the surround inhibition specifically in children is a potential direction for future studies. The complex interaction of different inhibitory systems as well as their relation to excitability [22], motor practice and handedness in the developmental context however needs further clarification.

We found that the dexterity of the right hand was better than that of the left hand in adolescents, and the variation in the scores of manual dexterity test in this age group was also higher. There is previous finding that the most dynamic developmental period for skillful movement is around 12 years of age and lasts well into the third decade of life [17]. Gender differences in asymmetry of motor functions in adolescence possibly related to testosterone levels have been reported [29]. The nature of the motor tasks performed may also influence the result [17, 29]. The interaction between handedness, motor skills and maturation of the corticospinal tract is complex [29] and warrants further elucidation.

No differences were found between the hemispheres in rMT, which is in line with previous data on adults [11, 78]. However, in 7 out of 9 children in the youngest group the rMT was lower in the left, dominant hemisphere, which is in accordance with a large-scale cross-sectional study on preadolescents [50]. Also, another previous study on neuromotor development displayed similar

asymmetry and the interhemispheric difference decreased with age [20]. The rMTs were highly variable between subjects and exhibited large interhemispheric differences (from -13 to 19), especially in the youngest group. The interindividual variation in rMTs leveled off at adolescence. Our input-output curve results did not confirm the previous observation of the dominant hemisphere maturating faster in adolescence [19]. Previous developmental TMS study reported asymmetric SP durations in all age groups [19] whereas we only observed it in the youngest group. The results regarding the SP asymmetry on adults have been contradictory [55, 78].

Manual dexterity correlated negatively with the rMT and SP duration. When controlled for age, the significance disappeared, indicating mainly an age–dependent correlation between manual dexterity and TMS measures. Lower rMT might reflect well-organized cortical output. Earlier, better manual dexterity has been shown to associate with lower motor threshold [50, 70], but not for the contralateral SP duration [20].

Strengths and limitations

To date, this is the first developmental study on the motor cortex using nTMS. As usual for TMS studies, the inter-individual variability was large in all measures. SP measurements are simple to perform, but the large inter-individual variance has hampered its clinical use. The inter-individual variation in SPs was significantly smaller than previously reported in developmental [20, 41], or clinical pediatric studies [71], which may partly be due to use of controlled and standardized measurement as well as neuronavigation [34, 78]. Neuronavigation may have also contributed to that we were able to construct the input-output curves in most subjects and the measurements were performed in relaxed muscles. A strength in our study is that the groups were equally large and even the younger age groups had more than 10 subjects. However, more detailed elucidation of the development of lateralization would require a larger sample of subjects. Despite a clear limitation of the lack of SP measurements in adults, our previously collected data should be equivalent to suit well for this purpose. The TMS variables used in this study reflect the excitability of both cortical and spinal excitability changes from both upper and lower motoneurons, and does not differentiate the respective influence of these. On the other hand, TMS may be useful to differentiate the relative contribution of the corticospinal tract in comparison to extrapyramidal pathways in the generation of motor disorders [43]. The diagnostic value of TMS in children with neurological deficits with central motor disturbances as well as problems and pitfalls of the method have been reported earlier [43].

To conclude, this study has demonstrated the neurophysiological maturation and simultaneous development in motor function in neurologically healthy subjects aged 7 years upward. Even though neurophysiologic measures of adolescents highly resemble those of adults, they further continue until adulthood. We assessed the variability of MEP amplitudes and latencies in children and adolescents of different ages using nTMS. The results may be beneficial as an adjunct to neuroimaging in the assessment of children with unilateral motor disturbances. Obtaining input-output curves was feasible in children and showed age-dependent changes. The age-dependent development was found in the input-output curves and decreasing SP durations with age. Hemispheric asymmetry was only observed for SP duration in children, showing stronger inhibition in the non-dominant hemisphere. Cortical excitability assessed in terms of effective EF strength on the cortex was similar to that expressed as percentage of stimulator output, and provide a method of estimation for planning future clinical trials and for therapeutic applications. Our results support the strong potential of TMS in experimental and diagnostic applications.

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CONFLICT OF INTEREST

Petro Julkunen has received an unrelated consultancy fee from Nexstim Plc., manufacturer of nTMS devices. Other authors report no conflict of interest to be disclosed.

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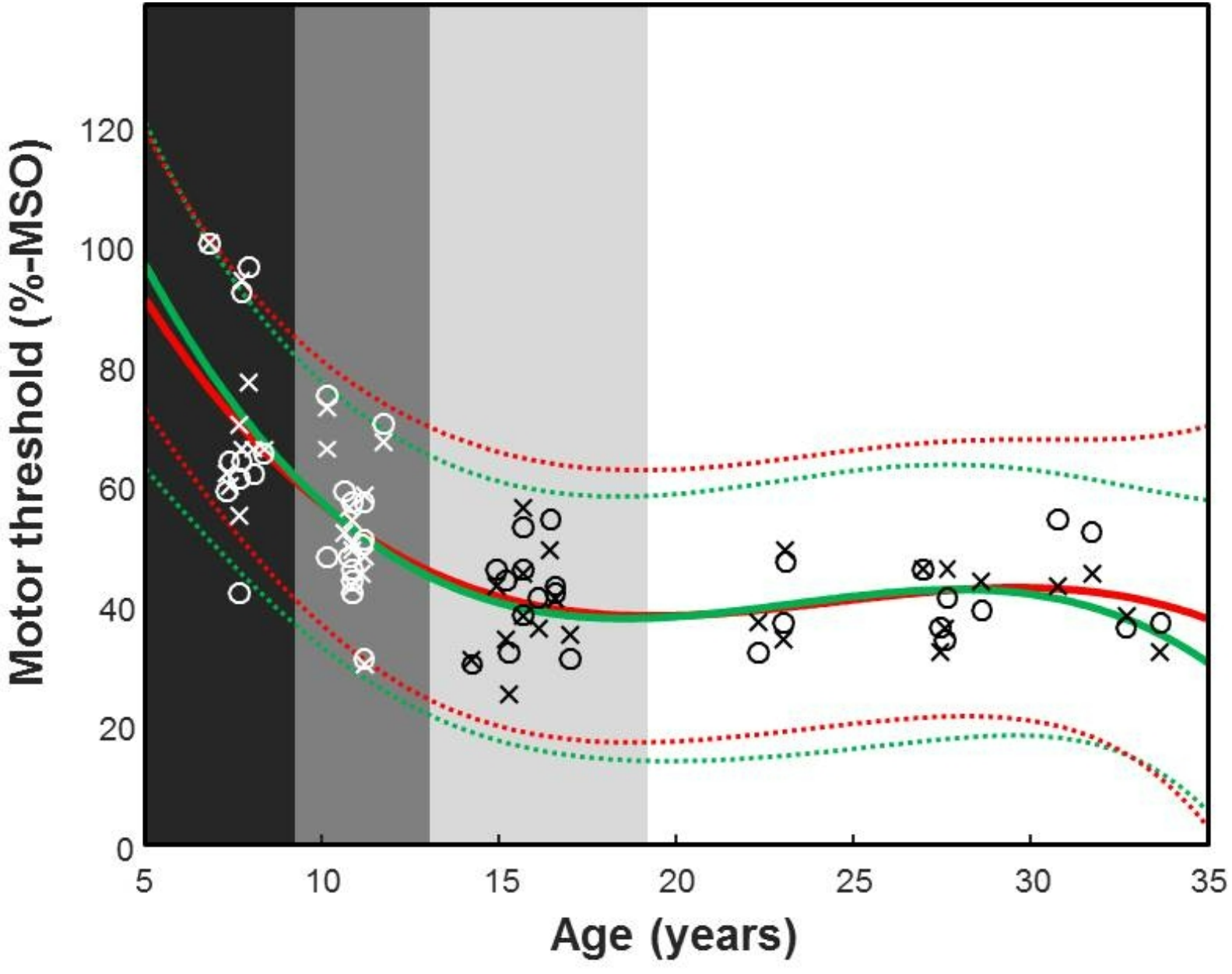
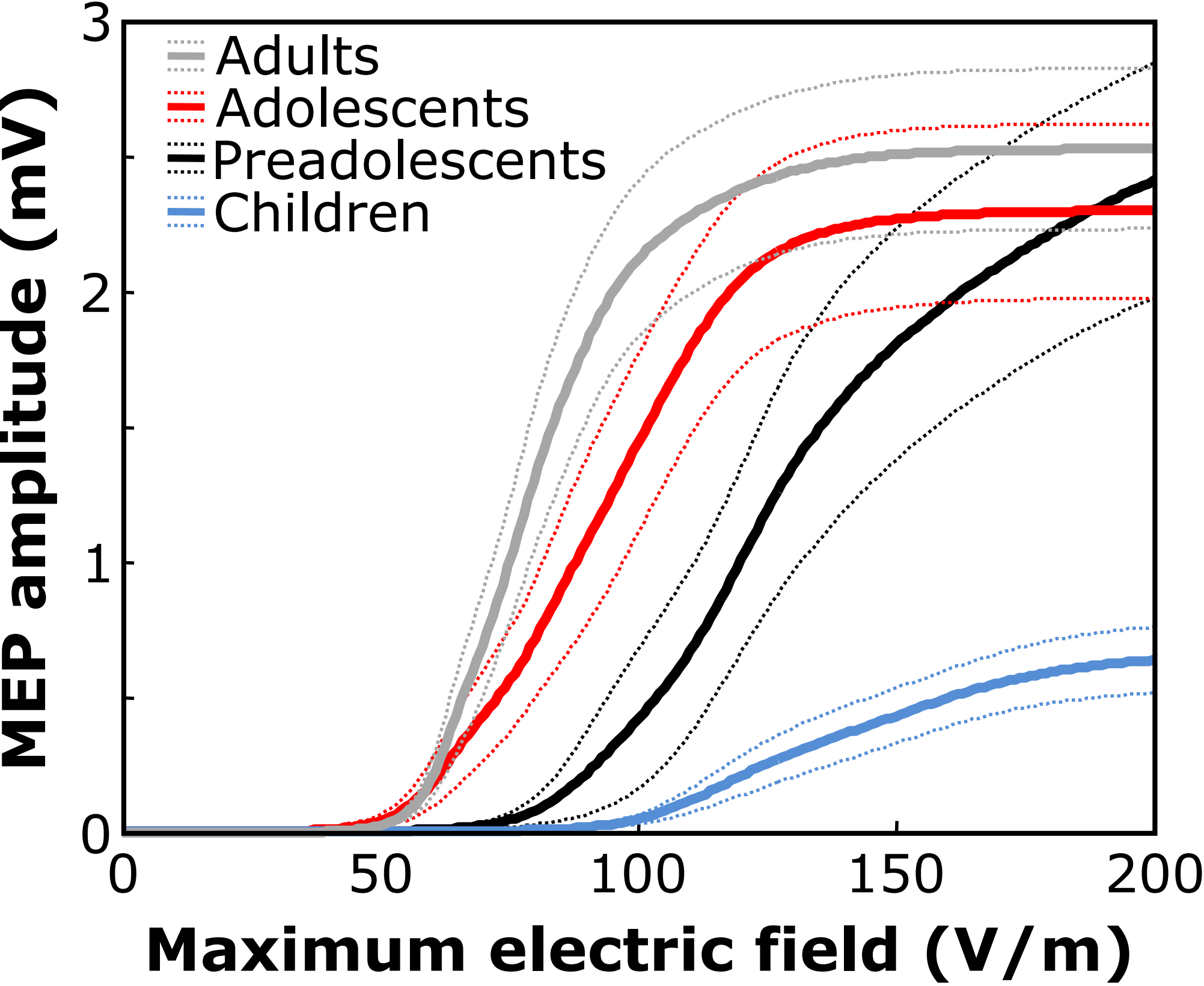
Figure legends

Figure 1. Relationship between the resting motor threshold (rMT) and age. Circles indicate the left and crosses the right hemisphere. A polynomial fit with 95% confidence intervals for both hemispheres is presented (red line for left hemisphere, green line for right hemisphere).

Figure 2. Input–output characteristics of different age groups (mean±SEM). Here, the individual MEP-amplitudes predicted by the optimized Boltzmann-function as a function of TMS-induced electric field were averaged over all subjects and both hemispheres. Individual optimized values were used to compute the Boltzmann function values at each EF intensity based on the conventional input-output curve data with absolute stimulation intensities (%-MSO), and converted to stimulation specific electric fields stored for each stimulus by the navigation software. The functions were drawn from 0 to 200 V/m electric field values, as no higher that 200V/m electric fields were applied on the cortex during this study. Thick lines present the group mean while dashed lines present SEM.

Figure 3. Representative examples of silent period (SP) raw data from one individual in each age group. Five trials (the shortest and the longest removed as used in the analysis) are superimposed. Note the different scaling on the y-axis in the group of adolescents).


Motor threshold (%-MSO)
120
100
80
60
40
20
0
5
10
15
20
25
30
35
Age (years)


Adults
Adolescents
Preadolescents
Children
MEP amplitude (mV)
0
1
2
3
0
50
100
150
200
Maximum electric field (V/m)

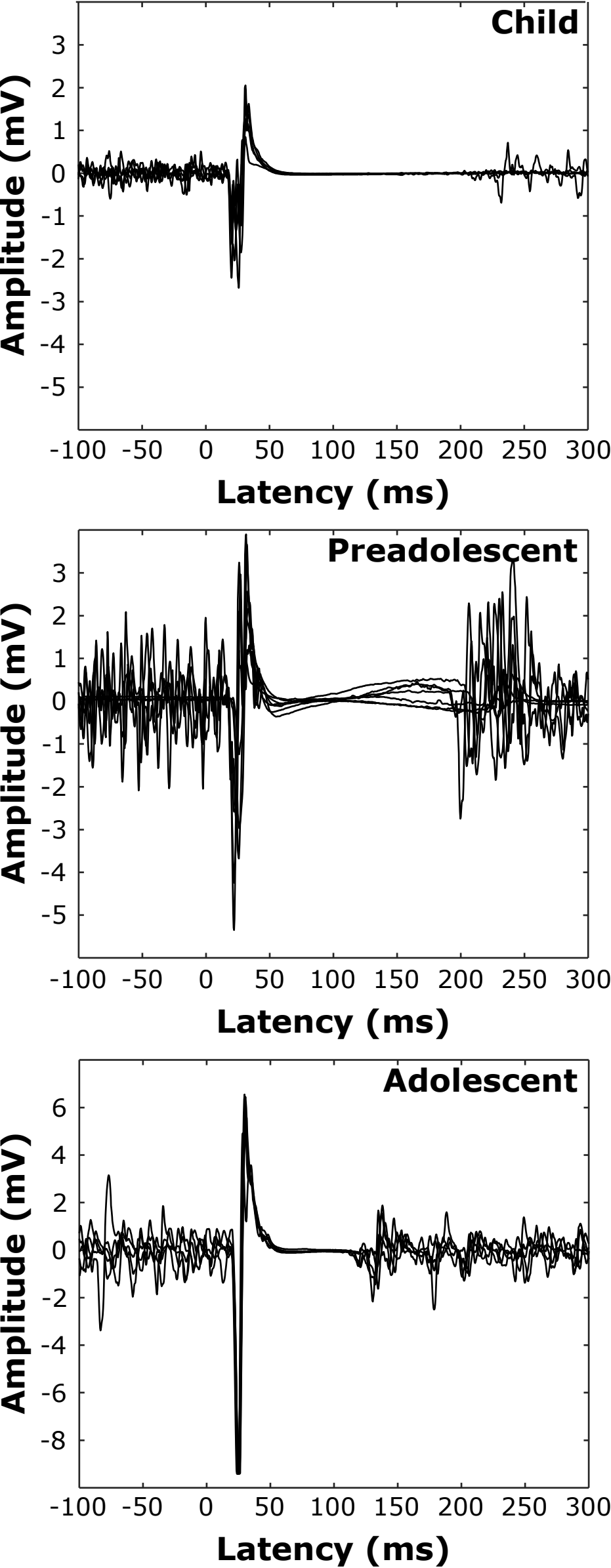

Child
Amplitude (mV)
3
2
1
0
-1
-2
-3
-4
-5
-100 -50 0 50 100 150 200 250 300
Latency (ms)
Preadolescent
Amplitude (mV)
3
2
1
0
-1
-2
-3
-4
-5
-100 -50 0 50 100 150 200 250 300
Latency (ms)
Adolescent
Amplitude (mV)
6
4
2
0
-2
-4
-6
-8
-100 -50 0 50 100 150 200 250 300
Latency (ms)

Table 1. Age, height, scalp-to-cortex distance and manual dexterity expressed group-wise as means (range). The dexterity of the right hand was significantly better than the left hand ($F = 5.11$, $p = 0.029$), the difference being significant in adolescents ($p = 0.042$) marked with asterisk.

	Gender (Female / Male)	**Age** (years; months)	**Height** (cm)	**Scalp-to-cortex distance** (mm)	**Box and Block Test score**	
					Right hand	**Left hand**
Children, n=10	5 / 5	7; 8 (6–9)	130 (126–139)	8.0 (5.2-10.6)	55 (46–65)	51 (43–66)
Preadolescents, n=13	6 / 7	10; 11 (10–12)	147 (135–160)	8.8 (7.3-9.9)	65 (55–75)	65 (56–75)
Adolescents, n=12	6 / 6	15; 9 (14–17)	172 (160–191)	11.7 (8.2-13.6)	77 * (56–94)	73 * (53–87)
Adults, n=12	6 / 6	27;11 (22–34)	171 (160–183)	14.0 (10.0-18.1)	84 (75–98)	83 (71–105)

Table 2. TMS data presented separately for the two hemispheres: rMTs expressed as a percentage of the stimulator output and electric field values on the surface of the cortex, MEP characteristics and SP durations. Mean (SD)(range). Significant effects of the age group in linear mixed model analysis are indicated by superscripts to the indices: [a] children and preadolescents; [b] children and adolescents; [c] children and adults; [d] preadolescents and adolescents; [e] preadolescents and adults; [f] adolescents and adults. The effect of the hemisphere was only observed for the silent period duration in the youngest age group marked with an asterisk. Previously measured mean values for adults aged 20–29 years using the same setup were 91.6 (24.3) ms [78].

	Hemisphere stimulated	rMT (%) [a, b, c, d, e]	rMT (V/m) [b, c, e]	MEP amplitude (μV) [c]	Relaxed MEP latency (ms) [b, c, d, e]	Active MEP latency (ms) [b, c, d, e]	Latency jump (ms)	Silent period (ms) [c]
Children	Left	69.6 (17.5) (42–96)	229 (65) (147–331)	310 (138) (91–470)	20.5 (0.2) (19.8–21.3)	17.0 (1.3) (15.5–19.4)	3.5 (1.2) (0.4–4.8)	152 (38)* (125–224)
	Right	71.6 (14.7) (55–94)	235 (74) (132–349)	358 (181) (130–735)	20.3 (1.0) (19.4–21.3)	16.5 (1.2) (15.7–17.9)	3.7 (0.6) (0.1–4.7)	171 (38)* (131–253)
Preadolescents	Left	54.9 (14.7) (31–91)	186 (103) (107–528)	651 (367) (152–1487)	21.2 (1.4) (19.1–23.8)	18.3 (1.5) (15.9–21.3)	2.8 (1.4) (0.4–5.9)	123 (32) (61–166)
	Right	55.0 (12.7) (30–80)	177 (63) (98–366)	908 (874) (204–3415)	20.9 (1.5) (18.9–24.9)	18.7 (1.7) (15.9–22.6)	2.2 (1.0) (0.2–4.6)	125 (37) (75–195)
Adolescents	Left	41.7 (7.9) (30–54)	121 (30) (73–176)	698 (273) (405–1207)	23.2 (1.1) (21.6–24.7)	20.6 (0.7) (19.6–22.0)	2.6 (1.1) (0.8–4.4)	105 (29) (57–142)
	Right	39.5 (8.3) (25–56)	108 (24) (74–151)	749 (361) (388–1441)	23.1 (1.1) (21.3–24.7)	20.7 (1.1) (19.2–23.3)	2.4 (1.1) (-0.1–3.5)	93 (26) (52–146)
Adults	Left	40.9 (7.2) (32–54)	85 (8) (85–147)	1204 (1199) (245–4693)	22.8 (1.1) (21.3–24.9)	20.6 (1.4) (18.8–23.1)	2.2 (0.4) (1.5–2.8)	*not assessed*
	Right	40.2 (6.0) (32–49)	105 (19) (76–98)	1010 (1141) (95–3539)	22.5 (1.3) (20.5–25.3)	20.4 (1.1) (18.5–21.7)	2.1 (0.7) (1.1–3.7)	*not assessed*

rMT = resting motor threshold, MEP = motor-evoked potential.