Influence of stimulation intensity on paired-pulse suppression of human median nerve somatosensory evoked potentials

Mario A. Gatica Tossi, Ann-Sophie Lillemeier and Hubert R. Dinse

Paired-pulse stimulation, the application of two stimuli in close succession, is a useful tool to investigate cortical excitability. Suppression of the second response after short interstimulus intervals characterizes paired-pulse behavior. Although paired-pulse suppression is often studied as a marker of cortical excitability in humans, little is known about the influence of stimulation intensity on paired-pulse suppression. To systematically explore the effect of stimulus intensity on paired-pulse suppression of median nerve somatosensory evoked potentials (MNSEPs), we recorded single-pulse or paired-pulse MNSEPs in healthy volunteers using stimulation intensities ranging from the sensory threshold to 1.2 times the motor threshold using interstimulus intervals of 10, 30, and 100 ms. Of the various somatosensory evoked potential components, only the N20-P25 component showed an effect of intensity, where higher intensities resulted in stronger paired-pulse suppression. However, when only intermediate intensities were considered, paired-pulse suppression was not or only weakly influenced. Our data suggest that stimulation intensity in contrast to single pulse-evoked MNSEPs has only a weak influence on the paired-pulse suppression of early MNSEPs. Paired-pulse suppression is believed to arise from inhibition generated by intracortical networks. The lack of intensity dependence within the range tested can be considered as a step toward creating invariance against fluctuations of stimulus intensity. Thus, intracortical computations as apparent in paired-pulse behavior might be characterized by different properties compared with feed-forward processing. *NeuroReport* 24:451–456 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins.

NeuroReport 2013, **24**:451–456

Keywords: cortical excitability, median nerve, paired-pulse behavior, paired-pulse suppression, short-term plasticity, somatosensory cortex, somatosensory evoked potentials, stimulation intensity

Institut fur Neuroinformatik, Neural Plasticity Lab, Ruhr University, Bochum, Germany

Correspondence to Hubert R. Dinse, PhD, Institut fur Neuroinformatik, Neural Plasticity Lab, Ruhr University, Building NB 3, D-44780 Bochum, Germany
Tel: +49 234 3225565; fax: +49 234 3214210;
e-mail: hubert.dinse@ruhr-uni-bochum.de

Received 15 February 2013 **accepted** 14 March 2013

Introduction

Paired-pulse somatosensory evoked potentials (SEPs) have provided valuable insights into the phenomenon of short-term plasticity in the somatosensory system. Paired-pulse stimulation is a useful technique to investigate the changes in, and the balance between, cortical excitation and intracortical inhibition. When two stimuli are applied in close temporal succession, the response to the second stimulus is significantly suppressed at short interstimulus intervals (ISIs), but approaches the response magnitude of the first stimulus with increasing ISIs [1]. A recovery curve clearly shows this dependence of paired-pulse suppression (PPS) on the length of ISIs. Paired-pulse behavior has been studied in all sensory modalities, as well as in the motor cortex, and the degree of PPS has been used as a marker of cortical excitability [2–4].

Changes in PPS can be used as indices of plastic changes in the somatosensory cortex, much as paired-pulse transcranial magnetic stimulation protocols have been used to show plastic changes in the motor cortex [5,6]. In the somatosensory cortex, Ragert *et al.* [4] showed that paired-pulse median nerve somatosensory evoked potentials (MNSEPs) recorded with an ISI of 30 ms produced a reliable suppression of the N20-P25 component; this PPS was reduced consistently after repetitive transcranial magnetic stimulation was applied at a frequency of 5 Hz [3] or following intermittent theta burst magnetic stimulation [4]. Similarly, application of tactile coactivation has been shown to reduce PPS in parallel to an improvement of tactile acuity [7].

A huge body of literature describes factors that influence the degree of intracortical inhibition observed in the motor cortex [8,9]. However, this information is lacking for the somatosensory system. For example, although components of early MNSEPs following single-pulse stimulation are affected variously by the rate and intensity of stimulation [1,10,11], little is known about how the intensity of stimulation affects PPS [12,13]. This is in contrast to the wide use of paired-pulse protocols to explore the effects of various forms of interventions on PPS and cortical excitability [2–7]. Conceivably, when testing PPS before and after interventions, the interventions themselves might alter stimulus–response characteristics. As a result, potential changes in PPS could be either because of true alterations of PPS or changes in stimulus–response characteristics, which affect PPS. Furthermore, there is agreement that PPS is generated by inhibition resulting from intracortical processing, but

0959-4965 © 2013 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/WNR.0b013e3283616378

the role of variable stimulation intensity in intracortical processing is not fully understood. Therefore, in the present work, we systematically explored the influence of stimulation intensity on PPS of early MNSEPs components in healthy young adults. Because PPS is intracortical in nature, and therefore less dependent on feed-forward information, we assumed that varying stimulus intensities might have less influence than observed following single-pulse stimulation [1,10,11].

Methods

We tested 27 volunteers (16 women), aged 20–27 years. Participants were right-handed and free of neurological diseases. All participants provided their informed consent. The protocol was approved by the local ethics committee of the Ruhr University Bochum. The experiments were conducted in accordance with the 1964 Declaration of Helsinki.

Median nerve somatosensory evoked potential measurement

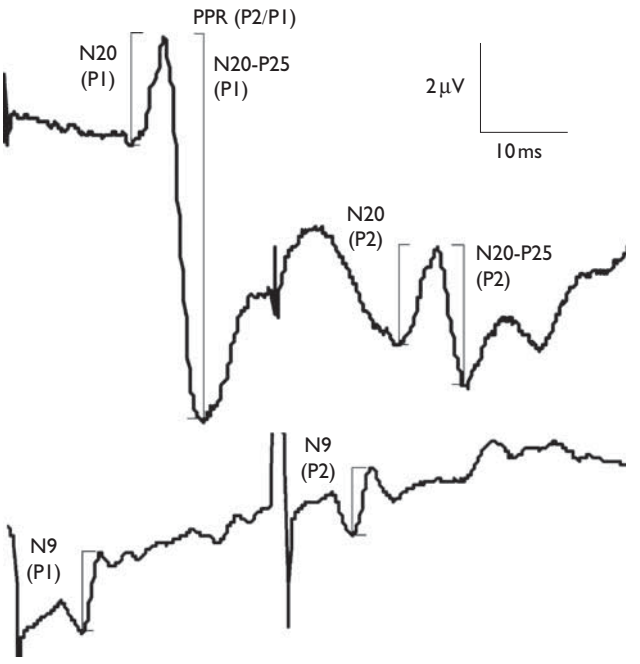
We measured SEPs after electrical stimulation of the right median nerve according to the procedures described previously [3,4,7]. Nerve stimulation was performed with a block electrode placed on the wrist (pulse duration 0.2 ms, repetition rate was 2 Hz). Stimuli were applied either as single pulses or as paired pulses with ISIs of 10 and 30 ms to elicit PPS. A further ISI of 100 ms was used as a control, as at this ISI, early MNSEP components show no significant PPS [1,7]. For each ISI, stimulation was applied at five different intensities: sensory threshold (ST), defined as the intensity at which stimuli were barely perceptible by the participants; motor threshold (MT) that is the intensity at which a visible movement of the thumb was elicited; two equidistant intensities between ST and MT (ST + 1 and ST + 2) and 1.2 times the MT (1.2 × MT). ST and MT were adjusted for each participant. The different ISIs and intensities were presented across participants in a randomized order. During median nerve stimulation and SEP recordings, participants were lying on a bed and were instructed to relax but to stay awake with their eyes closed. SEPs were recorded and stored for offline analysis using conventional Neuropack 8 equipment (Nihon Kohden Europe GmbH, Rosbach, Germany, bandpass filter 2–2000 Hz, sensitivity 2 mV/division). SEP recordings were made on two channels, each using a two-electrode array. On channel 1, an electrode was located 2 cm posterior to the C3 position according to the International 10–20 system (C3′ position); the reference electrode was placed over the midfrontal (Fz) position. On channel 2, nerve-evoked potentials were recorded from the brachial plexus with an electrode located on the right Erb's point and a reference electrode on the ipsilateral acromial process. The electrical potentials were recorded in epochs from 0 to 200 ms after onset of the stimulus. A total of 200 stimulus-related epochs were recorded for each measurement. Although many studies involving paired-pulse SEPs subtract the single-pulse response from the paired-pulse response before assessing the amplitudes, we did not use this approach as we believe that it is inconsistent with the literature that addresses the more general question of adaptation in sensory response using trains of stimuli at high frequencies, of which paired-pulse stimulation is a particular case, and where linear subtraction is not used as a rule [14,15].

The amplitude of the N20 component was assessed from its onset to its peak, occurring at a latency of around 17–21 ms after the time of median nerve stimulation. The amplitude of the N20-P25 complex was determined as the difference between the N20 peak and the peak of the subsequent positivity. Finally, the amplitude N9 component of the MNSEP recorded from Erb's point was measured from its onset to the negative peak occurring at 9 ms. PPS for each of the three components was assessed by calculating a paired-pulse ratio (PPR) of the amplitudes of the second (P2) to the first (P1) response peaks for each condition (Fig. 1).

Data analysis

Statistical analyses were carried out using the Graphpad Prism statistical software v 5.0 (GraphPad Software Inc., La Jolla, California, USA). The effect of stimulation intensity

Fig. 1



Single-participant MNSEP recording of a paired-pulse response using an ISI of 30 ms. For further analysis, the paired-pulse ratio (PPR) was calculated dividing the second response (P2) by the first (P1) separately for the central components N20 and N20-P25 (upper trace) as well as for the peripheral N9 component (lower trace). MNSEP, median nerve somatosensory evoked potential.

on single-pulse MNSEPs was tested by linear regression analysis, separately for each component, using the raw amplitudes as well as the normalized amplitudes and stimulation intensities. Deviation from linearity was assessed using a Runns test. To compare different components, the amplitudes and intensities were normalized and expressed as percentages of the amplitude/intensity obtained at the highest stimulation intensity ($1.2 \times$ MT). The slopes of the linear regressions among the three components using the normalized data were compared with an analysis of covariance. In the paired-pulse conditions, comparisons of the PPR between the 10- and 30-ms ISI conditions were made, separately for each component, with two-way analyses of variance (ANOVA) using the factors 'ISI' and 'Intensity'. The 100-ms ISI control condition was analyzed separately with one-way ANOVA for each component with the factor 'Intensity'.

Results

In all participants, it was possible to determine ST and MT. None of the participants reported any side effects or discomfort during the procedure. The average stimulation intensities for each step were ST = 1.63 ± 0.06; ST + 1 = 2.44 ± 0.08; ST + 2 = 3.23 ± 0.12; MT = 4.04 ± 0.17; and $1.2 \times$ MT = 4.89 ± 0.2.

Single-pulse median nerve somatosensory evoked potentials

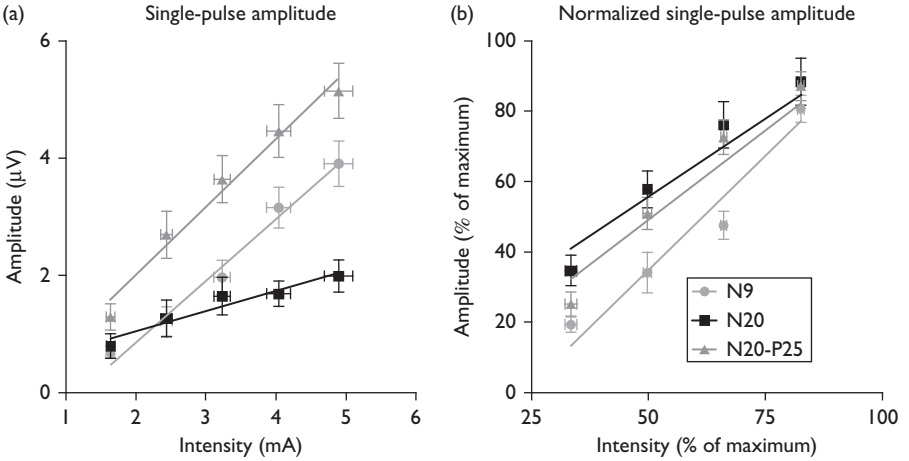
The amplitude of the different components (N9, N20, and N20-P25) increased in proportion to increasing stimulation intensity. A linear regression analysis showed significant positive relationships between stimulation intensity and amplitude for the peripheral N9 component (slope = 0.83; $F_{1,115} = 56.58$; $P < 0.0001$) as well as for the N20 (slope = 0.28; $F_{1,105} = 8.7$; $P = 0.004$) and N20-P25 (slope = 0.8; $F_{1,130} = 31.53$; $P < 0.0001$) components (Fig. 2a).

To compare the three MNSEP components independent of differences in the amplitude range and interparticipant differences in stimulation intensity, the correlation analysis was repeated using the normalized amplitude values (Fig. 2b). This analysis confirmed positive linear relationships between normalized amplitudes and stimulation intensities for all three components: N9 (slope = 1.3; $F_{1,88} = 93.4$; $P < 0.0001$), N20 (slope = 1; $F_{1,83} = 38.6$; $P < 0.0001$), and N20-P25 (slope = 1.2; $F_{1,103} = 90$; $P < 0.0001$). Furthermore, a comparison of the slopes showed no statistically significant difference between components (analysis of covariance, $F_{2,274} = 0.88$, $P = 0.41$).

Analysis of paired-pulse ratios

Two-way ANOVAs with the factors Intensity and ISI were used to compare the conditions in which PPS was expected (10 and 30 ms). For the N9 component, there was no effect of ISI ($F_{1,230} = 0.37$, $P = 0.54$) or Intensity ($F_{4,230} = 0.2$, $P = 0.9$). For the N20 component, although there was a trend toward lower PPR with increasing intensity, neither Intensity ($F_{4,234} = 0.81$, $P = 0.51$) nor ISI ($F_{1,234} = 2.4$, $P = 0.1$) was statistically significant. The PPR for component N20-P25 showed a strong effect of both Intensity ($F_{4,245} = 3.9$, $P = 0.004$) and ISI ($F_{1,245} = 6.9$, $P = 0.009$). The PPR decreased with increasing intensity at the two ISIs tested, with the strongest suppression found at 30-ms ISI. There was no significant interaction between the two factors ($F_{4,245} = 0.28$, $P = 0.88$) (Fig. 3).

Fig. 2



(a) Dependence of amplitude of single-pulse SEPs on increasing stimulation intensity (mA) showing the difference in the range of amplitudes for the three components (N9, N20, and N20-P25). (b) The normalized amplitude of all components (N9, N20, and N20-P25) increases at almost the same rate with intensity (percent of maximal intensity). Linear regression analysis with analysis of covariance showed no significant difference in the slopes between the three components (ANCOVA, $F_{2,274} = 0.88$, $P = 0.41$). ANCOVA, analysis of covariance; SEP, somatosensory evoked potential.

An additional analysis of the influence of intensity on PPR was carried out using one-way ANOVAs, separately for the conditions of ISI 10 and 30 ms; these analyses showed a strong trend that, however, fell short of statistical significance ($F_{4,124} = 2.18$, $P = 0.07$ for ISI 10 ms and $F_{4,121} = 2.15$, $P = 0.08$ for ISI 30 ms).

One-way ANOVA showed that at 100 ms ISI, there was no PPS for any component.

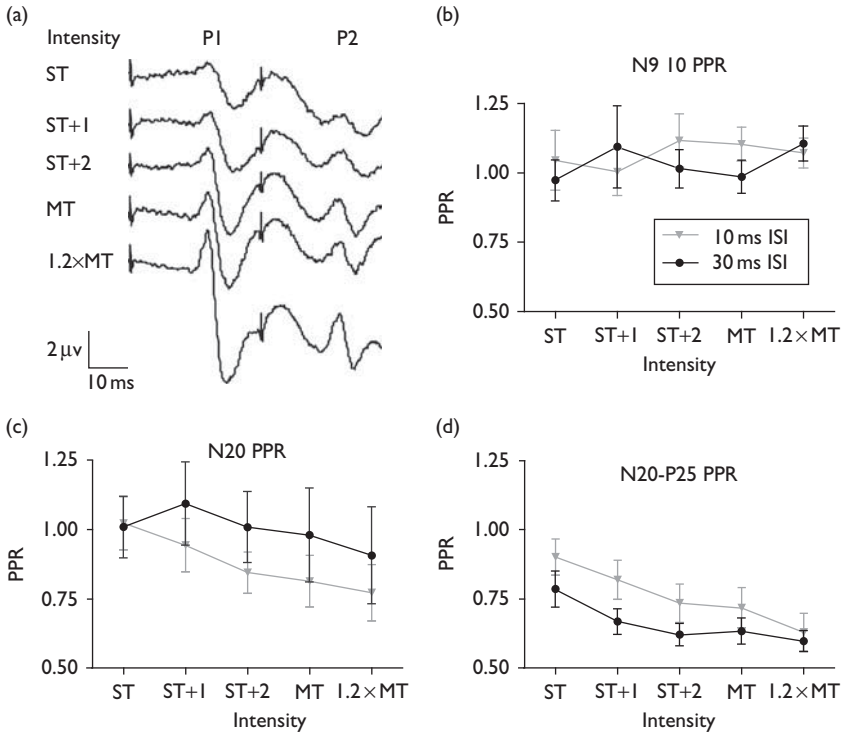
Discussion

Our results showed that the amplitude of single-pulse MNSEPs increased monotonically with stimulation intensity. This observation is in agreement with those of studies reporting a linear increase in amplitude with higher stimulation intensity [10]. Other studies have found an exponential [11] or a sigmoidal relationship [16]. The discrepancy may be attributed, at least in part, to differences in the range of intensities tested. Jousmäki and Forss used intensities between one and three times the ST, approximately the same range used in our experiment, whereas Urasaki and colleagues and Klostermann and colleagues used a much wider range. Thus, although the complete curve of MNSEP change in response to increasing stimulation intensity is nonlinear, it is likely that the range analyzed here corresponded to a segment that behaved linearly. Conceivably, the range we covered reflects a relevant portion of intensities that are often used to study PPS [3,4,7]. Going beyond three times ST, equivalent to 1.2 times MT, would thus result in a ceiling effect, where a further increase of intensity is no longer reflected in a proportional increase in response amplitude.

Intensity dependence of paired-pulse suppression

For N9 and N20, the intensity did not affect PPS significantly. In contrast, for N20-P25, a significant effect of intensity on PSS was observed, with more suppression at higher stimulation intensities, when the data from two ISIs (10 and 30 ms) were pooled (Fig. 3). When each ISI condition was analyzed separately, a trend remained that did not reach statistical significance. This suggests that the effect of intensity on the PPS for N20-P25 was weak.

Fig. 3



Effect of intensity on PPR. (a) Individual participant MNSEP recordings following paired stimulation showing the increase in amplitude of the first (P1) and second (P2) response with increasing intensity for the 30-ms ISI condition. ST, defined as the intensity at which stimuli were barely perceptible; MT, defined as the intensity at which a visible movement of the thumb was elicited; two equidistant intermediate intensities between ST and MT (ST + 1 and ST + 2), and 1.2 times the MT (1.2 × MT). (b) N9 showed no influence of either stimulation intensity or ISI on PPR [two-way ANOVA for ISI ($F_{1,230} = 0.37$, $P = 0.54$) and Intensity ($F_{4,230} = 0.2$, $P = 0.9$)]. (c) N20 showed a nonsignificant tendency toward reduction of PPR with increased intensity [two-way ANOVA for ISI ($F_{1,234} = 2.4$, $P = 0.1$) and intensity ($F_{4,234} = 0.81$, $P = 0.51$)]. (d) N20-P25 showed a significant inverse relationship between intensity and PPR. In addition, suppression was significantly stronger at ISI of 30 ms than at ISI of 10 ms [two-way ANOVA for intensity ($F_{4,245} = 3.9$, $P = 0.004$) and ISI ($F_{1,245} = 6.9$, $P = 0.009$)]. ANOVA, analysis of variance; ISI, interstimulus interval; MNSEP, median nerve somatosensory evoked potential; MT, motor threshold; PPR, paired-pulse ratio; ST, sensory threshold.

Inspection of the PPRs showed that the ratios for N20-P25 were lower than those for N20 at all ISIs and intensities. Indeed, at low stimulation intensities, N20 showed facilitation (PPR > 1) rather than suppression, which was not the case with N20-P25. This agrees with the results of previous studies reporting that N20-P25 is more susceptible to PPS than the earlier N20 component, and less so than the later P30, which is also generated in the primary somatosensory cortex [14,17]. This may be because Brodmann's area (BA) 3b, the origin of N20, is robustly innervated by afferents from the ventral posterolateral nucleus of the thalamus and BA 1 (probably the origin of N20-P25) is weakly innervated by small-diameter fibers from the thalamus as well as corticocortical fibers from BA 3b [18].

Comparison of the recovery curves of N20 and N20-P25 indicates that the two components behave differently. N20 shows the strongest suppression at the shortest ISI (10 ms), whereas for N20-P25 suppression was stronger at 30 ms ISI.

Origin of cortical components and mechanism of paired-pulse suppression

There is general agreement that the N20 component originates in the granular layer (layer IV) of BA 3b, which occupies the posterior bank of the rolandic fissure [1,14,17]. Although not as certain, the origin of the N20-P25 component is probably a radially oriented source that is usually identified as BA1 at the apex of the postcentral gyrus [1,14,17].

It is known that the subcortical P14 (originating in the medial lemniscus) shows little suppression, even at ISIs of 10 ms, and shows full recovery at ISI 30 ms [19]. Assessment of suppression in the somatosensory thalamus using surface electroencephalography has been difficult, but a study in rats found little suppression in the response to repetitive whisker deflections in the ventral posterior medial nucleus of the thalamus [20]. In contrast, under comparable conditions, the neurons in the barrel cortex show considerable attenuation of their response after a few stimuli [21].

Although our experiments were designed to characterize paired-pulse behavior at a systemic level, they cannot provide information about mechanisms. Despite many studies, the cellular mechanism underlying PPS is not fully understood. Animal studies have described synaptic depression of thalamocortical [22] and intracortical [23] synapses, as well as feed-forward inhibition elicited by the activation of GABAergic interneurons by thalamic afferents [24].

The effect of stimulation intensity on PPS has been observed by in-vitro experiments as well. For example, an in-vivo study systematically explored the effect of ISI and stimulus intensity on PPS in the rat hippocampus and reported results similar to those of the present work, that is, shorter ISIs and higher stimulation intensity produced stronger suppression [25]. Those authors proposed that high stimulus intensity would result in the recruitment of more inhibitory interneurons after the first pulse, thus increasing the strength of inhibition and attenuation of the test pulse.

Interestingly, we found a significant effect of stimulation intensity only after pooling the data from two different conditions (ISI of 10 and 30 ms). When each condition was analyzed independently, the trend remained but it was not statistically significant. This suggests that the stimulation intensities commonly used for measuring SEPs are unlikely to affect the outcome significantly, unless very high intensities ($\geq$ 3 times ST) are applied.

This is of considerable relevance as it might minimize confounding when testing PPS before and after interventions, which themselves might alter stimulus–response characteristics. Furthermore, PPS is believed to arise from inhibition generated by intracortical processing. The lack of intensity dependence within the range tested implies a different level of intracortical processing, which can be considered as a step toward creating invariance against fluctuations of stimulus intensity.

Acknowledgements

This study was supported by grants from the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG), SFB grant 874 to H.R.D., and from the International Graduate School of Neuroscience (IGSN) at the Ruhr University Bochum to M.A.G.T.

Conflicts of interest

There are no conflicts of interest.

References

1 McLaughlin DF, Kelly EF. Evoked potentials as indices of adaptation in the somatosensory system in humans: a review and prospectus. *Brain Res Brain Res Rev* 1993; **18**:151–206.
2 Chen R. Interactions between inhibitory and excitatory circuits in the human motor cortex. *Exp Brain Res* 2004; **154**:1–10.
3 Ragert P, Becker M, Tegenthoff M, Pleger B, Dinse HR. Sustained increase of somatosensory cortex excitability by 5 Hz repetitive transcranial magnetic stimulation studied by paired median nerve stimulation in humans. *Neurosci Lett* 2004; **356**:91–94.
4 Ragert P, Franzkowiak S, Schwenkreis P, Tegenthoff M, Dinse HR. Improvement of tactile perception and enhancement of cortical excitability through intermittent theta burst rTMS over human primary somatosensory cortex. *Exp Brain Res* 2008; **184**:1–11.
5 Peinemann A, Lehner C, Mentschel C, Münchau A, Conrad B, Siebner HR. Subthreshold 5-Hz repetitive transcranial magnetic stimulation of the human primary motor cortex reduces intracortical paired-pulse inhibition. *Neurosci Lett* 2000; **296**:21–24.
6 Ziemann U, Hallett M, Cohen LG. Mechanisms of deafferentation-induced plasticity in human motor cortex. *J Neurosci* 1998; **18**:7000–7007.
7 Höffken O, Veit M, Knossalla F, Lissek S, Bliem B, Ragert P, *et al.* Sustained increase of somatosensory cortex excitability by tactile coactivation studied by paired median nerve stimulation in humans correlates with perceptual gain. *J Physiol* 2007; **584**:463–471.
8 Berardelli A, Abbruzzese G, Chen R, Orth M, Ridding MC, Stinear C, *et al.* Consensus paper on short-interval intracortical inhibition and other

transcranial magnetic stimulation intracortical paradigms in movement disorders. *Brain Stimul* 2008; **1**:183–191.
9 Ziemann U. Pharmacology of TMS. *Suppl Clin Neurophysiol* 2003; **56**:226–231.
10 Jousmäki V, Forss N. Effects of stimulus intensity on signals from human somatosensory cortices. *Neuroreport* 1998; **9**:3427–3431.
11 Urasaki E, Wada S, Yasukouchi H, Yokota A. Effect of transcutaneous electrical nerve stimulation (TENS) on central nervous system amplification of somatosensory input. *J Neurol* 1998; **245**:143–148.
12 Allison T. Recovery functions of somatosensory evoked responses in man. Electroencephalogr. *Clin Neurophysiol* 1962; **14**:331–343.
13 Schwartz M, Shagass C. Recovery functions of human somatosensory and visual evoked potentials. *Ann N Y Acad Sci* 1964; **112**:510–525.
14 Arezzo J, Legatt AD, Vaughan HG Jr. Topography and intracranial sources of somatosensory evoked potentials in the monkey. I. Early components. *Electroencephalogr Clin Neurophysiol* 1979; **46**:155–172.
15 Huttunen J. In search of augmentation at human SI: somatosensory cortical responses to stimulus trains and their modulation by motor activity. *Brain Res* 2010; **1331**:74–79.
16 Klostermann F, Nolte G, Losch F, Curio G. Differential recruitment of high frequency wavelets (600 Hz) and primary cortical response (N20) in human median nerve somatosensory evoked potentials. *Neurosci Lett* 1998; **256**:101–104.
17 Allison T, McCarthy G, Wood CC, Darcey TM, Spencer DD, Williamson PD. Human cortical potentials evoked by stimulation of the median nerve. I. Cytoarchitectonic areas generating short-latency activity. *J Neurophysiol* 1989; **62**:694–710.
18 Jones EG. Connectivity of the primate sensory-motor cortex. In: Jones EG, Peters A, editors. *Cerebral cortex, Vol. V. Sensory-motor areas and aspects of cortical connectivity.* New York: Plenum; 1986. pp. 113–183.
19 Emori T, Yamada T, Seki Y, Yasuhara A, Ando K, Honda Y, *et al.* Recovery functions of fast frequency potentials in the initial negative wave of median SEP. *Electroencephalogr Clin Neurophysiol* 1991; **78**:116–123.
20 Hartings JA, Simons DJ. Thalamic relay of afferent responses to 1- to 12-Hz whisker stimulation in the rat. *J Neurophysiol* 1998; **80**:1016–1019.
21 Melzer P, Sachdev RN, Jenkinson N, Ebner FF. Stimulus frequency processing in awake rat barrel cortex. *J Neurosci* 2006; **26**:12198–12205.
22 Chung S, Li X, Nelson SB. Short-term depression at thalamocortical synapses contributes to rapid adaptation of cortical sensory responses *in vivo*. *Neuron* 2002; **34**:437–446.
23 Petersen CC. Short-term dynamics of synaptic transmission within the excitatory neuronal network of rat layer 4 barrel cortex. *J Neurophysiol* 2002; **87**:2904–2914.
24 Cruikshank SJ, Lewis TJ, Connors BW. Synaptic basis for intense thalamocortical activation of feedforward inhibitory cells in neocortex. *Nat Neurosci* 2007; **10**:462–468.
25 Waldbaum S, Dudek FE. Single and repetitive paired-pulse suppression: a parametric analysis and assessment of usefulness in epilepsy research. *Epilepsia* 2009; **50**:904–916.