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Associative plasticity in intracortical inhibitory circuits in human motor cortex

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Abstract

Objective—Paired-associative stimulation (PAS) is a transcranial magnetic stimulation technique inducing Hebbian-like synaptic plasticity in the human motor cortex (M1). PAS is produced by repetitive pairing of a peripheral nerve shock and a transcranial magnetic stimulus (TMS). Its effect is assessed by a change in size of a motor evoked response (MEP). MEP size results from excitatory and inhibitory influences exerted on cortical pyramidal cells, but no robust effects on inhibitory networks have been demonstrated so far.

Method—In 38 healthy volunteers, we assessed whether a PAS intervention influences three intracortical inhibitory circuits: short (SICI) and long (LICI) intracortical inhibitions reflecting activity of $GABA_A$ and $GABA_B$ interneurons respectively, and long afferent inhibition (LAI) reflecting activity of somatosensory inputs.

Results—After PAS, MEP sizes, LICI and LAI levels were significantly changed while changes of SICI were inconsistent. The changes in LICI and LAI lasted 45 minutes after PAS. Their direction depended on the delay between the arrival time of the afferent volley at the cortex and the TMS-induced cortical activation during the PAS.

Conclusions—PAS influences inhibitory circuits in M1.

Significance—PAS paradigms can demonstrate Hebbian-like plasticity at selected inhibitory networks as well as excitatory networks.

Keywords

plasticity; transcranial magnetic stimulation; GABAergic inhibitory interneurones; motor cortex; sensorimotor coupling

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Introduction

From animal studies it is known that intracortical inhibitory circuits are involved in cortical plasticity in two different ways. (i) In vitro studies have demonstrated that decrease of local inhibitory activity accompanies and promotes the development of long-term potentiation (LTP) (Stelzer and Shi, 1994; Castro-Alamancos et al., 1995) synaptic remodeling and cortical receptive field expansion (Chowdhury and Rasmusson, 2002). (ii) Enduring changes in synaptic efficacy have been observed not only at excitatory synapses, but also at inhibitory ones (Woodin et al., 2003). In humans there is indirect evidence that a decrease of local $GABA_A$ergic inhibition in the motor cortex enhances dramatically the excitability in the intracortical circuitry during motor practice (Ziemann et al., 2001) while blockade of $GABA_B$ inhibition prevents the development of a cortical plasticity artificially induced by TMS (McDonnell et al., 2007). These results fit with the former aspect of involvement of cortical inhibition in plasticity. In this paper we do not address the role of a decrease of local inhibition in development of plasticity, we focus on the development of plasticity at the level of inhibitory synapses during artificial induction of plasticity. Various transcranial magnetic stimulation (TMS) techniques can be used to induce non-invasively "artificial" cortical plastic changes. Here we used the paired associative stimulation (PAS) technique, which may represent associative LTP - or LTD-like plasticity at a cell population level (Stefan et al., 2000; Wolters et al., 2003). PAS has not been shown so far to be accompanied by lasting changes of short-interval intracortical inhibition (SICI) involving $GABA_A$ receptors or of afferent inhibition (Stefan et al., 2002; Quartarone et al., 2003; Rosenkranz and Rothwell, 2006). Yet according to the prolongation of the silent period (SP, thought to involve $GABA_B$ inhibition) after PAS (Stefan et al., 2000; Quartarone et al., 2003), implication of $GABA_B$ inhibition in PAS-induced after effects has been suggested. This has to be confirmed as SP is a complex parameter involving spinal as well as cortical mechanisms (Fuhr et al., 1991) and evidence for a contribution of $GABA_B$ receptor activation to the SP is weak and controversial (Paulus et al., 2008).

We investigated the aftereffects of a PAS intervention on the excitability of several intracortical inhibitory circuits: those involving $GABA_A$ (SICI) and $GABA_B$ (LICI) synapses and also those fed by peripheral sensory inputs. Sensory stimulation can change motor cortex excitability. Inhibition of the MEP by peripheral stimulation has been called "long afferent inhibition" (LAI) when the delay between peripheral and TMS stimulation is from 100 to 1000 ms (Chen et al., 1999b; Abbruzzese et al., 2001; Paulus et al., 2008). Transmitters and pathways involved in LAI are unknown.

Methods

Subjects

Experiments were performed on 38 healthy volunteers (19 men, 19 women) aged 19 – 67 years (mean ± SEM, 35.5 ± 6.1 years) with no history of either neurological or psychiatric disease and a normal neurological examination. Results of 3 subjects were discarded from analysis because their MEPs were highly variable due to sleepiness. The study included 3 different experiments, and each experiment included several measures. The number of subjects used in each experiment and the number used for calculating the mean value of each measure are indicated in Fig. 1 and Tables 1 – 2, respectively, as all measures were not obtained in all subjects. The experimental protocol was approved by the NINDS Institutional Review Board, and all subjects gave written informed consent. All subjects were right-handed according to the Oldfield handedness inventory (Oldfield, 1971).

EMG recording

Surface EMG activity (band-pass 10 Hz-2 kHz) was recorded from the right flexor pollicis brevis (FPB) – the target muscle- and the abductor digiti minimi (ADM) muscle, in bipolar belly-tendon arrangements, using a Nicolet Viking electromyograph (Skovlunde, Denmark). Signals were fed into an IBM compatible personal computer (486 DX) with a data acquisition system built with the Labview graphical programming language (sampling rate 5 kHz) (Kaelin-Lang and Cohen, 2000) for further off-line analysis. During the experiments, EMG activity was continuously monitored with visual and auditory feedback to ensure complete relaxation.

Transcranial magnetic stimulation

Subjects were seated in a comfortable reclining chair. A figure-of-eight shaped coil (7 cm inner diameter for each half) connected to a Bistim-module and two Magstim 200 magnetic stimulators (The Magstim Company, Dyfed, UK) was positioned on the scalp over the left M1. The hot spot for the right FPB muscle was defined as the lowest threshold site evoking a MEP response in FPB accompanied by a clear thumb flexion movement. The coil was positioned with the handle pointing backwards at an angle of 45° to the midline (Brasil-Neto et al., 1992). The hot spot was marked with a pen on the cap worn by the subject; this served as visual reference against which the coil was positioned and maintained by the experimenter.

Resting motor threshold (rMT)

The resting motor threshold (rMT) was defined as the minimum TMS intensity (measured by altering the stimulator output intensity in 1% decrements) required to elicit at least five FPB MEPs > 50 μV in 10 consecutive trials (Rossini et al., 1994; Rothwell et al., 1999). TMS stimulus intensities were then expressed as percentage of the right FPB rMT.

Input-output (I-O) curves

With the coil at the hot spot, 7 responses were recorded and averaged at each of a range of intensities. Intensity of stimulation started at the rMT and was increased by steps of 10% × rMT until the MEP size reached a plateau value. Each I-O curve was characterized by 3 parameters: (i) “slope”: the slope of a regression line fitted to the steepest part of I-O curve; (ii) “calculated” resting motor threshold (cMT), the intercept of the regression line with the × axis, and (iii) the plateau value (MEP max).

Long interval intracortical inhibition (LICI)

To evoke LICI a suprathreshold conditioning TMS stimulation (CS90) was delivered 90 ms before a test TMS stimulation (TS) (Valls-Sole et al., 1992; Nakamura et al., 1997; Chen et al., 1999a). We first created an intensity curve for LICI using CS90 intensities of 0.9, 1, 1.1, 1.2 and 1.3 × rMT while keeping the TS at 1.2 × rMT. During experiments TS intensity was adjusted to 1.2 × rMT. CS90 intensity was adjusted in each subject according to the individual intensity curve at the lowest intensity evoking an inhibition. Such a low intensity was chosen to avoid any ceiling effect.

Short interval intracortical inhibition (SICI)

To evoke SICI, a subthreshold conditioning TMS stimulation (CS2.5) was delivered 2.5 ms before a test TMS stimulation (TS) (Fisher et al., 2002). We first created an intensity curve for SICI by using CS2.5 intensities 0.5, 0.6 and 0.7 and 0.8 × rMT (Orth et al., 2003; Stinear and Byblow, 2004), while keeping the TS at 1.2 × rMT. During experiments, the intensity of the TS was adjusted to 1.2 × rMT and intensity of the CS2.5 was adjusted in each subject according to the intensity curve at the lowest intensity evoking an inhibition.

Long afferent inhibition (LAI)

Time course of LAI—The effect of a conditioning stimulation to the right median nerve on FPB and ADM MEPs was tested using 5 different interstimulus intervals (ISIs): 50, 100, 150, 200 and 240 ms. The median nerve was stimulated at wrist through bipolar surface electrodes (cathode proximal, bipolar stimulation, rectangular pulses of 0.2 ms duration) using a Grass S88 stimulator (Grass Instruments Co., Quincy, Mass., USA). Intensity was adjusted to 2.5 × the perceptual threshold (PT) (mean: 4.2 ± 1.12 mA) always below the thumb twitch threshold. TMS intensity was adjusted to 1.2 ×rMT. LAI was calculated as the ratio of the conditioned (with preceding median nerve stimulation) to the test (TS alone) MEPs and expressed as a percentage (± SEM) of the test MEP.

During the main experiments LAI was tested at only two ISIs 150 ms (LAI_{150}) and 240 ms (LAI_{240}), because inhibition peaked at these ISIs in the preliminary time-course experiment Median nerve stimulation and TS were adjusted as described above.

PAS intervention

Paired associative stimulation (PAS) was achieved by pairing an electrical stimulus to the right median nerve stimulation at wrist (2.5 × PT: 5.1 ± 1.67 mA, always below thumb twitch threshold), and a single TMS pulse targeting the right FPB muscle. We adjusted the TMS intensity to evoke a FPB MEP with a peak-to-peak amplitude around 0.5 mV. The original PAS technique (Stefan et al., 2000; Wolters et al., 2003) induces spike-time dependent plasticity (STD) - like effects, as direction of change in amplitude of test MEPs depends on the ISI between peripheral and TMS stimulations. Here the ISI between median nerve and TMS stimulation was set either at 25 ms or at 10 ms, because these ISIs have been shown to be optimal for inducing sustained increase/decrease in motor cortex excitability (Stefan et al., 2000; Wolters et al., 2003). Two hundred and forty pairs of stimuli were delivered at 0.2 Hz over 20 min (Muller et al., 2007). These interventions are referred in the following as PAS_{25} or PAS_{10}. We asked the subjects to count the stimulations in order to maintain attention.

Effect of PAS on overall corticospinal excitability

A screening tool for the efficacy of PAS previously described in the literature is the increased amplitude of a 1 mV test MEP after PAS (Wolters et al., 2003; Stefan et al., 2004). To compare our results with literature data, we compared the mean size of 10 MEPs before and after PAS using a test MEP before PAS adjusted to 0.8 – 1 mV.

Study designs (Fig. 1)

The study designs of the 3 different experiments performed and the number of subjects are illustrated in Fig 1. Experiment 1 was designed to study PAS_{25}-induced modulation of LAI and LICI, experiment 2 to study PAS_{25}-induced modulation of SICI and experiment 3 to verify whether PAS-induced changes of LICI, SICI and LAI followed the rules of STDP-like plasticity. Measurements were done at baseline (T0), shortly after the PAS (T1, 5 to 10 minutes after the end of PAS) and forty to fifty minutes after the end of the PAS (T2). To measure LICI, SICI and LAI_{150} or LAI_{240}, 10 test (TS) and 10 test + conditioning (CS) stimulations were randomly alternated. Intensity of TS was adjusted at T0 at 1.2 × rMT. Since the amount of LAI, SICI and LICI depends on the size of the test MEP (Sanger et al., 2001), the intensity of TS at T1 and T2 was adjusted in order that the test MEP had the same size as at T0 (0.8–1 mV).

Statistical analysis

The intensities used (CS90, CS2.5) and the different measures (LICI, SICI, LAI) done at T0 were compared between the 2 groups (the group tested for PAS_{25} or PAS_{10} respectively) using unpaired t tests.

I-O curves: values of rMT, cMT, slope and the plateau value were compared between T0 and T1 using paired t-tests. RMT and cMT were expressed in percentage of the stimulator output and the plateau values in mV. To calculate the slopes of the I-O curves measures of MEPs were normalized to the plateau value at T0 and intensities were expressed as a percentage of the rMT at T0.

Time course of LAI: conditioned and unconditioned MEP values were compared using paired t- tests at each of the tested ISIs (50, 100, 150, 200 and 240 ms).

The effects of PAS on LAI_{150}, LAI_{240}, LICI and SICI were evaluated by separate repeated-ANOVA (Tables 1 and 2) with the values of the measures at T0, T1 and T2 forming the repeats. Conditional on a significant F-value, post hoc paired samples t-tests (Fisher's PLSD) were performed to explore the strength of main effects.

To compare the effects of the 2 interventions: PAS_{25} and PAS_{10} over time in the target muscle FPB versus the non-target muscle ADM, inhibitions after PAS were expressed as a percentage of the inhibitions at T0 (inhT1/inhT0 and inhT2/inhT0). Subsequently, we performed a repeated ANOVA with "TIME" (inhT1/inhT0 and inhT2/inhT0) as within-subject factor and "INTERVENTION" (PAS_{25} or PAS_{10}) and "MUSCLE "(FPB or ADM) as between-subject factors. Conditional on a significant F-value, post hoc unpaired t-tests were performed to explore each muscle and each time.

To look for a relationship between PAS-induced changes of MEP size and LICI or LAI changes we used linear regression analysis. PAS-induced changes of MEPs or inhibitions were expressed as a percentage of their values at T0 (see above).

A p-value of < 0.05 was considered significant. All data are given as means ± SEM. Statistical analysis was performed with Statview 5.1 software.

Results

For clarity we do not present results of the 3 experiments separately but we present together results of "excitatory" (PAS_{25}) and "inhibitory" (PAS_{10}) PAS for each tested measure: rMT, I-O curves, LICI, SICI, LAI.

Subjects did not report any adverse side effects during the study.

Motor Threshold (rMT)

Neither PAS_{25} nor PAS_{10} modified the *mean* value of the FPB rMT (PAS_{25}: 46.1 ± 1.4 % of stimulator output at T0 versus 45.4 ± 1.4 % at T1; p ns; n = 19) (PAS10: 44.8 ± 5.6 % at T0, 44.5 ± 6 % at T1 and 44.1 ± 5.7 % at T2; p ns; n = 11).

Input-Output curves

cMT was very close to rMT and was not modified after PAS_{25} (46.9 ± 2.4 % at T0 and 45.2 ± 2.2 % at T1; p ns; n = 15). The slope of the I-O curve was significantly enhanced after PAS_{25} (1.7 ± 0.2 at T0 versus 2.2 ± 0.3 at T1, $p< 0.03$; n = 15) while there was a trend for the plateau value to be increased (2.4 ± 0.4 mV at T0 versus 3.2 ± 0.4 mV at T1, p = 0.08; n = 15).

Effect of PAS on LICI

The averaged intensity curve for LICI, obtained in 15 subjects, is presented on Fig. 2B. The lowest intensity evoking an inhibition was 1.1 × rMT in all but 2 subjects. The mean CS90 was 1.12 ± 0.06 × rMT and 1.10 ± 0.03 × rMT for the PAS_{25} and the PAS_{10} group, respectively (p ns). Despite these similar intensities of CS90 in the 2 groups, baseline LICI was quite different between the 2 groups (FPB: 42.3 ± 4 % and 65.1 ± 4 % of the test MEP in PAS_{25} and PAS_{10} group respectively; $p < 0.005$; ADM: 63.5 ± 8 % and 87.7 ± 9 % of test MEP in PAS_{25} and PAS_{10} group, respectively; p ns).

LICI was reduced in the target muscle immediately and 45 minutes after PAS_{25} while it was only slightly modified in the non-target ADM (Table 1). Contrasting with the decrease of LICI after $PAS_{25,}$ PAS_{10} led to a significant increase of the LICI in the target FPB without significant change in the non-target muscle ADM (Table 2).

Effect of PAS on SICI

The averaged intensity curve for SICI, obtained in 12 subjects, is presented on Fig. 2A. The lowest intensity evoking an inhibition was 0.6 × rMT in all but 2 subjects. The mean CS2.5 was 0.65 ± 0.07 × rMT and 0.60 ± 0.07 × rMT in the PAS_{25} and the PAS_{10} group, respectively. Despite these similar intensities of CS2.5 in the 2 groups, baseline SICI was quite different between the 2 groups (FPB: 47.1 ± 3 % and 67.8 ± 4 % of the test MEP in PAS_{25} and PAS_{10} group, respectively; $p < 0.001$; ADM: 49.3 ± 6 % and 77.4 ± 6 % of the test MEP in PAS_{25} and PAS_{10} group, respectively; $p < 0.007$).

Effects of PAS on SICI among subjects were more variable than those on LICI or LAI. After PAS_{25} mean SICI was not modified (Table 1). After PAS_{10} mean SICI was significantly decreased in FPB (Table 2).

We noticed that for subjects with a small baseline SICI, the net effect of PAS_{25} was most of the time a SICI increase while when working on the whole group, who had a stronger baseline SICI, PAS_{25} did not induce any significant effect. An explanation for such a discrepancy is illustrated in Fig. 3: the amount of baseline SICI influenced the PAS - induced effect. All subjects with small baseline SICIs (more than 50%) exhibited a SICI increase after PAS_{25} (Fig. 3A). The amount of baseline SICI also influenced the PAS10-induced effect (Fig. 3B). Neither amount of baseline LICI, nor amount of baseline LAI influenced PAS-induced effects.

Effect of PAS on LAI

Time course of LAI—Eight subjects participated in this preliminary experiment. FPB MEP was inhibited at all but the 50 ms and the 100 ms ISI (50 ms: p ns, 100 ms p = ns, 150 ms: p< 0.02, 200 ms p< 0.05, 240 ms p<0.004) (Fig. 4, black diamonds). Inhibition reached similar maxima for ISI 150 ms (59.5 ± 15 % of the test MEP) and 240 ms (68.3 ± 15 %). Median nerve stimulation induced a small inhibition of ADM MEP at 150 ms (89.2±14 %) and at 240 ms (82.8 ± 14 %) (p ns at all ISIs) (Fig. 4, white triangles).

LAI_{150}—At T0, LAI_{150} was evoked in FPB in all 24 tested subjects but one (16 subjects for PAS_{25} and 8 for PAS_{10}), and in 18 out of the 24 tested in ADM. There was a clear topographic specificity as median nerve stimulation induced a stronger inhibition in the target FPB (LAI_{150} at T0 = 49.7 ± 4 % and 66.3 ± 8 % of the test MEP in PAS_{25} and PAS_{10} group, respectively; $p < 0.04$) than in the non-target ADM (LAI_{150} at T0 = 72 ± 10 % and 70.6 ± 9 % in PAS_{25} and PAS_{10} group respectively, p ns).

After PAS_{25} LAI_{150} was significantly decreased in the target muscle FPB and to a lesser (non significant) extent in the ADM muscle (Table 1). After PAS_{10}, LAI_{150} showed a trend to be increased but it did not reach statistical significance (Table 2).

LAI_{240}—At T0, LAI_{240} was evoked in FPB in 21 out of the 24 tested subjects (14 subjects for PAS_{25} and 10 for PAS_{10}), and in 15 out of the 21 tested in ADM. There was a clear topographic specificity as median nerve stimulation induced a stronger inhibition in the target FPB (LAI_{240} at T0 = 65 ± 6 % and 60.5 ± 9 % of test MEP in PAS_{25} and PAS_{10} group, respectively; p ns) than in the non-target ADM (LAI_{240} at T0 = 87 ± 24 % and 77.5 ± 9 % of test MEP in PAS_{25} and PAS_{10} group, respectively, p ns).

Contrasting with the decrease of LAI_{150} after PAS_{25}, LAI_{240} was significantly increased after PAS_{25} at T1 and T2 (Table 1) while it was significantly decreased after PAS_{10} (Table 2). PAS-induced changes in the non-target ADM were of similar direction than those observed in FPB; these changes were not significant after PAS_{25}, but significant after PAS_{10}.

Comparison of the 2 types of PAS interventions

Baseline inhibitions were different between the groups tested for PAS_{25} and $PAS_{10,}$ so to compare the effects of the 2 interventions over time and across muscles, inhibitions after PAS were normalized to their baseline values. Figure 5 illustrates the normalized inhibitions in FBP and ADM after PAS_{25} (gray lines) and PAS_{10} (black lines). Changes of LICI (Fig. 5A–B), LAI_{150} (Fig. 5E–F) and LAI_{240} (Fig. 5.G–H) had the same direction in both muscles, and were not different at T1 and T2, but had reverse directions after PAS_{25} and PAS_{10}, respectively. This was confirmed by the repeated ANOVA (see statistical analysis) showing a significant effect of INTERVENTION (LICI: $F = 9.9$, $p < 0.003$; LAI_{150}: $F = 13.1$, $p < 0.0008$; LAI_{240}: $F = 10.4$, $p < 0.003$), no effect of MUSCLE or TIME and no significant interaction. For SICI (Fig. 5C–D) the only consistent change was a decrease of SICI after PAS_{10} at T1 in FPB while changes after PAS_{25} were not substantial. This was confirmed by the repeated ANOVA with no significant effect of INTERVENTION ($p = 0.06$), but a significant interaction TIME*MUSCLE*INTERVENTION ($F = 5.1$, $p < 0.03$).

Effect of PAS on overall corticospinal excitability (Fig. 6)

The mean overall corticospinal excitability to FPB after PAS_{25} was significantly enhanced at T1 (not tested at T2) (T0: 1.02 ± 0.07 mV; T1: 1.76 ± 0.17 mV, $p < 0.002$) and not modified to ADM (T0: 1.02 ± 0.17 mV; T1: 1.05 ± 0.18 mV, p ns). After $PAS_{10,}$ the mean overall corticospinal excitability to FPB was significantly decreased to a same extent at T1 and T2 (T0: 1.16 ± 0.04 mV, $p < 0.04$; T1: 0.86 ± 0.12 mV; T2: 0.89 ± 0.15 mV, p ns) while corticospinal excitability to ADM was not modified (T0: 0.73 ± 0.11 mV; T1: 0.64 ± 0.12 mV, p ns; T2: 0.64 ± 0.11 mV, p ns).

The question then arose to know whether the subjects who exhibited the largest MEP increase or decrease after PAS are those in whom the PAS-induced decrease/increase of LICI or LAI_{150} were the largest. Neither after PAS_{25} nor PAS_{10} did we found a correlation between the percentage of change of the test MEP (MEP size at T1/MEP size at T0) and the LICI change (LICI at T1/LICI at T0) or the LAI_{150} change (LAI at T1/LAI at T0). We also did not find any correlation between percentage of change of the test MEP and SICI changes.

Discussion

This work shows that a PAS intervention inducing lasting changes of the overall corticospinal excitability, induces concomitant changes in amount of LICI and LAI. Changes in LICI and LAI have opposite directions according to the interval (10 or 25 ms) used between median

nerve and cortical TMS stimulations during PAS. Changes in SICI are more difficult to interpret as direction of the changes depends on the baseline SICI.

Are PAS-induced changes of inhibitions related to a bi-directional associative synaptic plasticity of inhibitory synapses?

A first possibility to explain the bi-directional PAS-induced decrease/increase of LICI and LAI would be a change in the "gain" of the corticospinal system. Such a change was demonstrated by the increase of the slope of the I-O curve after PAS_{25} (also shown by (Ridding and Taylor, 2001). We did not study the I-O curves after PAS_{10} but a decrease of the slope of the I-O curve after PAS10 has already been described (Rosenkranz et al., 2007). As illustrated in Fig. 7, even in absence of any change of synaptic efficacy in intracortical inhibitory pathways, an increase in the slope of the input-output relationship after PAS may induce an apparent increase in inhibitions. Conversely a decrease of the slope may induce an apparent decrease of inhibitions. In our results LICI and LAI_{150} were *decreased* after PAS_{25} while the slope of the I-O curve *was increased* and they were *increased* after PAS_{10} while the slope was presumably *decreased*, so these changes cannot be ascribed to the changes of the slope.

A confounding factor to interpret PAS-induced changes of inhibitions would be a PAS-induced change of the corticospinal volley evoked by the TMS test stimulation. If PAS favours the development of lasting changes of excitability in cortical circuits responsible for the early recruited I waves it may be that the descending volleys evoking the 1 mV test MEPS be different before and after PAS. An increase of the relative weight of early components versus the latter ones after PAS might decrease by itself the amount of LICI as LICI acts by decreasing the later components of the corticospinal volley (I3 waves) and has no effect on early components (D wave and I1 waves) (Chen et al., 1999a; Di Lazzaro et al., 2002). Epidural recordings of the descending volleys have recently brought a direct demonstration that PAS enhances the amplitude of later descending I waves of the test volley whereas the I1 wave was not modified (Di Lazzaro et al., 2009). Is such a conclusion applicable to our data? Di Lazzaro et al (Di Lazzaro et al., 2009) used a standard PAS intervention with 1 mV MEPs during both the PAS and the testing of excitability of the corticospinal volley. We used lower intensities of TMS during the PAS intervention (0.5 mV) than before and after the intervention (1 mV test MEPs). Using also 1 mV test MEPs and very weak (subthreshold) stimulation during PAS, Kujirai et al (Kujirai et al., 2006) observed an increased effectiveness of PAS when they used anterior-posterior directed currents (known to increase the recruitment of late I waves) instead of posterior-anterior directed ones. It suggests that, even with the use of low TMS intensities, PAS after-effects are due to a change in the excitability of neurons responsible for recruiting the I3 waves in the corticospinal tract and not the early waves.

The third and most plausible explanation is that PAS-induced changes of LICI and LAI are related to enduring changes in excitability of the corresponding pathways. Arguments for attributing bidirectional changes of a test MEP after a PAS_{25} or a PAS_{10} intervention to long-term facilitation/long-term depression-like phenomena governed by temporal Hebbian rules have been developed in several papers (Stefan et al., 2000; Stefan et al., 2002; Wolters et al., 2003; Weise et al., 2006; Rosenkranz et al., 2007). The same arguments can be used here for PAS-induced changes of LICI and LAI_{150}: topographic specificity (changes are prominent in the targeted muscle FPB and absent or less in the control ADM), long duration (>30 minutes), reversibility, direction of the change depending whether the peripheral volley arrives at the cortical level before or after the triggering of the cortical stimulation. However, we did not demonstrate here that changes in inhibitions are NMDA dependant, a characteristic of LTP/LTD.

Such reasoning cannot apply for SICI since the only consistent effect of PAS on SICI was the decrease of SICI after PAS_{10}. Such a change may be caused, at least partly, by the decrease of

the slope of the I-O curve after PAS_{10} (see above). The direction of the PAS_{25}-induced changes in SICI depended on the baseline SICI. Baseline SICI has already been described to influence the effect of an ongoing 1Hz rTMS intervention (Bagnato et al., 2005; Daskalakis et al., 2006). In the light of recent findings (Paulus et al., 2008; Peurala et al., 2008) subjects with large baseline SICI might be not tested in the descending part of the intensity curve where SICI is less contaminated by SICF (and especially at the 2.5 ms interval that we used (Ni et al., 2007)) but later when SICI and SICF are superimposed (see Peurala et al 2008, their Fig. 2 (Peurala et al., 2008)). Contrastingly, subjects with low baseline SICI might be tested in the descending part of the SICI curve, and, in those subjects, PAS-induced SICI changes might reflect more faithfully changes in GABAergic interneurones. If we look at Fig 3, it appears that, for moderate baseline SICI, PAS_{25} induced mainly an increase in inhibition while PAS_{10} induced a decrease. This suggests a possible bi-directional effect according whether the peripheral volley arrives before or after the cortical one at the target cells. Such an interpretation fits with the bi-directional changes of SICI recently demonstrated after theta-burst stimulation of M1: if theta-burst stimulation is applied in an intermittent (excitatory) mode SICI is increased, whereas if applied in a continuous (inhibitory) mode SICI is decreased (Huang et al., 2005; Murakami et al., 2008). Interpretation of SICI changes is weakened by the difference in baseline SICIs between the groups.

LAI_{240} versus LAI_{150}

It was unexpected to find opposite changes of LAI when using 2 different ISIs: 150 ms and 240 ms. As discussed above, as they have an opposite direction to the changes of the slope of the I-O curve, PAS-induced change of LAI_{150} can be reasonably attributed to the development of a LTP/LTD - like bidirectional plasticity. Oppositely, as they follow the direction of the changes of slope of the I-O curve (increase after PAS25, decrease after PAS10) changes of LAI_{240} may be due to the changes of the I-O relationship or at least be masked by it. In such a case PAS-induced changes of LAI_{150} and LAI_{240} would be of same direction but of different amount. The large changes of LAI_{150} would overcome the changes due to the change of the slope while the small changes of LAI_{240} would be masked by it. Another possible explanation comes from the strong inhibitory interaction described between LICI and LAI (Sailer et al., 2002). Then a PAS-induced decreased/increased excitability of interneurones shared by LICI and LAI pathways might explain the increase/decrease of AI_{240}, while a 150-ms delay would be too short for the inhibitory interaction to develop.

Link with previous data in the literature

SP and LICI—Conventional PAS (suprathreshold stimulations, 0.05 to 0.25 Hz) fail to modulate SICI (Stefan et al., 2000; Stefan et al., 2002; Quartarone et al., 2003; Rosenkranz and Rothwell, 2006), yet induced an *increase* in duration of the silent period (SP) (Morgante et al. 2006; Stefan et al. 2000; Stefan et al. 2004). As LICI and SP (at least its second part) have been considered so far as related phenomena (Wassermann et al., 1996), reflecting activation of $GABA_B$ receptors (Roick et al. 1993; Siebner et al. 1998; Werhahn et al. 1999), the increase of duration of SP after PAS was thought to reflect increased $GABA_B$ activity. The results obtained here question the relationship between SP and LICI; indeed what we found, after excitatory PAS, is a *decrease* of LICI and not the increase that should go with a prolongation of the SP (see above). Several papers have recently showed a dissociation between SP and LICI modulation: during voluntary contraction (Hammond and Vallence, 2007) during fatiguing hand exercise (Benwell et al., 2007) during treatment by a $GABA_B$ receptor agonist (McDonnell et al., 2006).

Different PAS protocols—We used a PAS protocol similar to that used by Mueller et al. (Muller et al., 2007) but different from the “conventional” one. It is possible that by changing the relative “weight” of cortical stimulation with respect to the peripheral one (during PAS,

TMS was adjusted to evoke a MEP of 0.5 mV instead of the 1mV used in the original protocol) and by delivering a larger number of pairs (240 instead of 90) we favor the development of plasticity in the inhibitory circuits. Indeed when using rTMS, for a given frequency and a given intensity, the longer the duration of stimulation the more substantial are the induced effects on interneurone excitability (Jung et al., 2008) (see also Fitzgerald et al 2006 (Fitzgerald et al., 2006)). Also while 1 Hz subthreshold rTMS of M1 has no effect on the final motor output (no change in MEP size) it induces effects on interneuronal excitability suggesting that the "lowest" threshold system activated by TMS in the hand area of human motor cortex is inhibitory (Bagnato et al., 2005).

Conclusion

The present data show that PAS paradigms can demonstrate Hebbian-like plasticity in the inhibitory networks responsible for LICI (a $GABA_B$ mediated inhibition) and LAI as well as excitatory networks. Despite some clues indicating that enduring changes of LICI might occur at a presynaptic level on $GABA_A$ interneurones and induce changes of SICI in the opposite direction, further experiments are needed to elucidate the complex relationship between PAS-induced changes of $GABA_A$ and $GABA_B$ mediated inhibitions.

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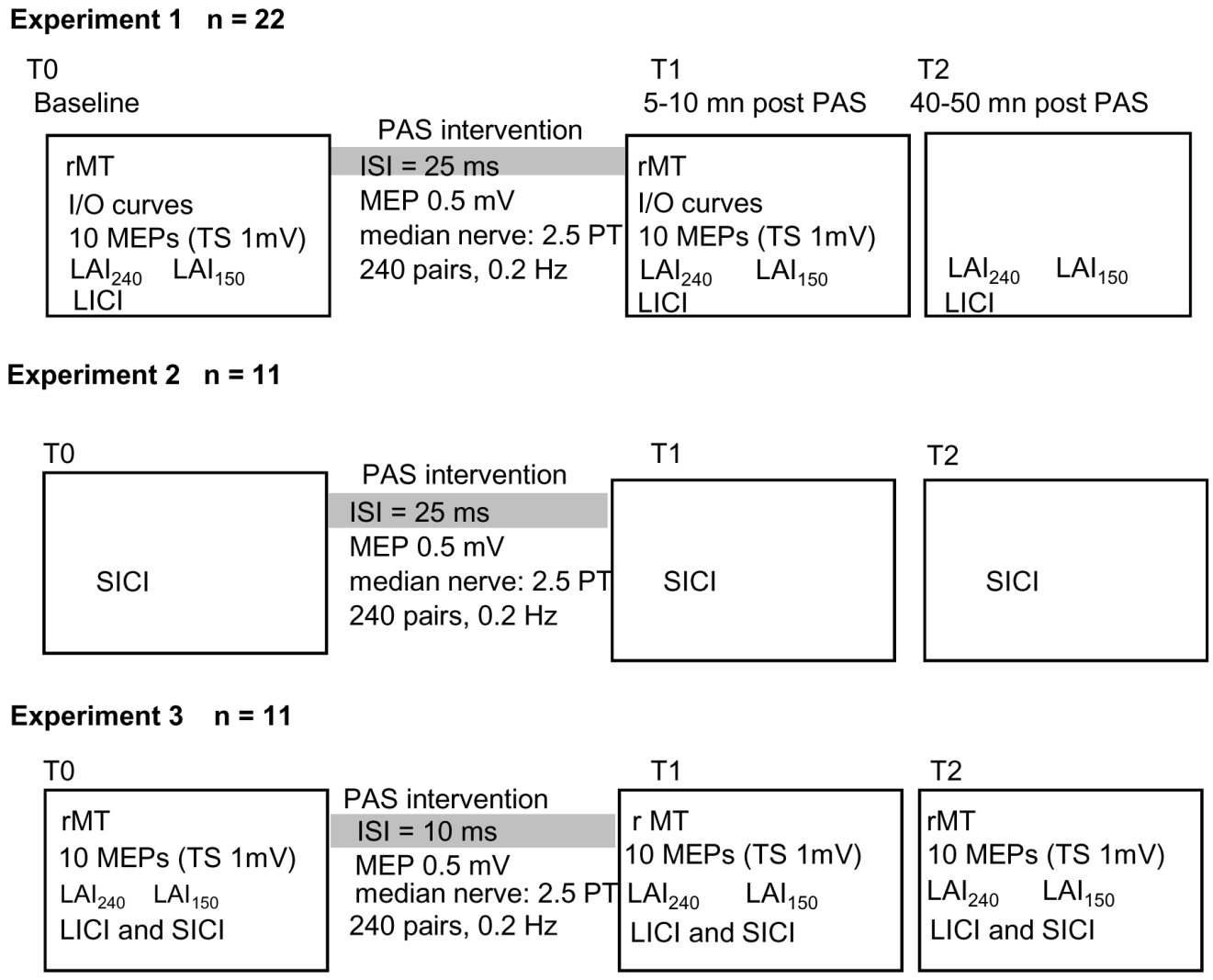

Figure 1.
Experimental designs of the 3 experiments performed.

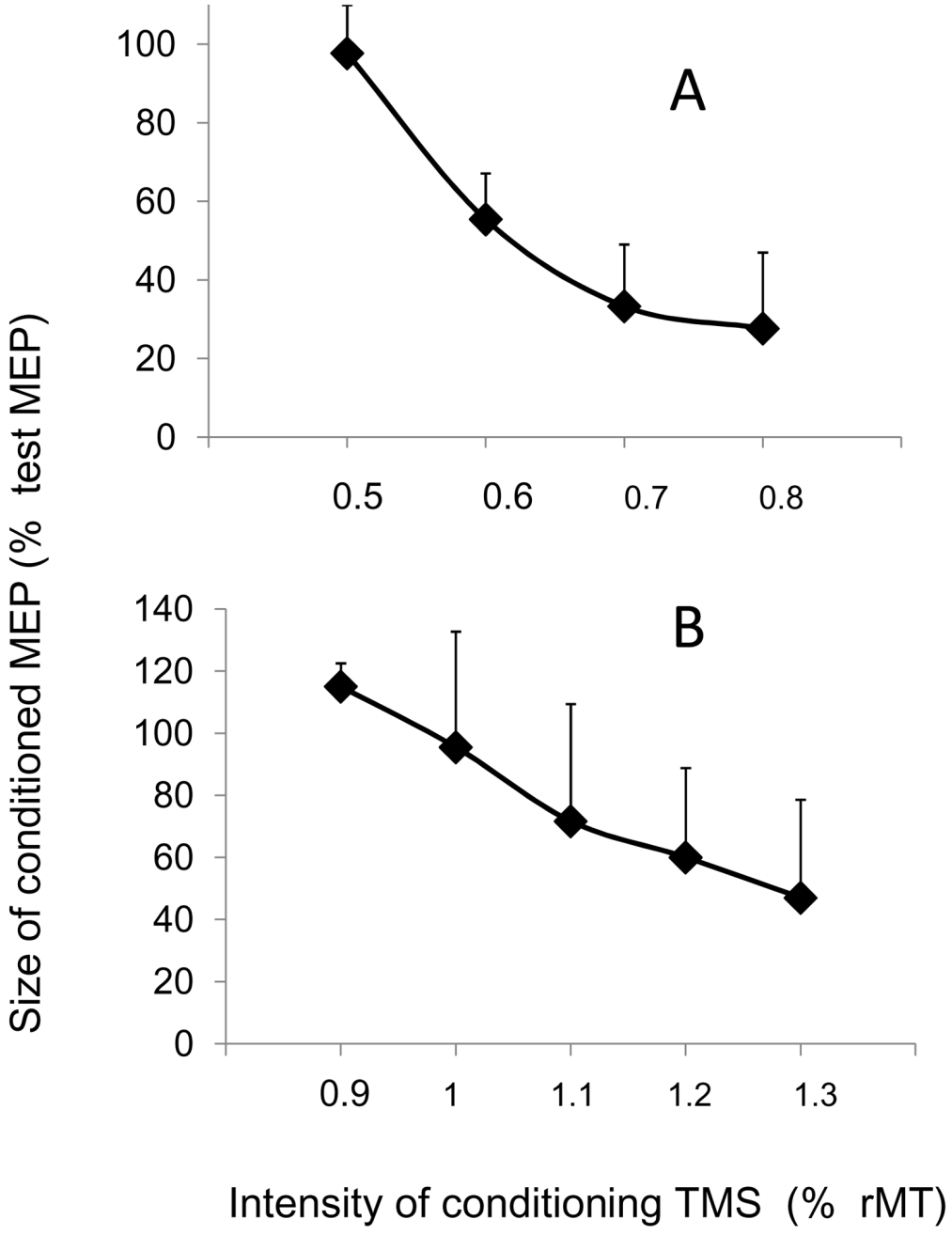

Figure 2. Intensity curves of SICI (A) and LICI (B)

The effect of a conditioning TMS pulse (CS) is tested on a 1 mV test MEP from FPB. In A: the ISI between CS and test stimulation was 2.5 ms; each dot represents the mean of 120 measurements (12 subjects). B: the ISI between CS and test stimulation was 90 ms; each dot represents the mean of 150 measurements (15 subjects).

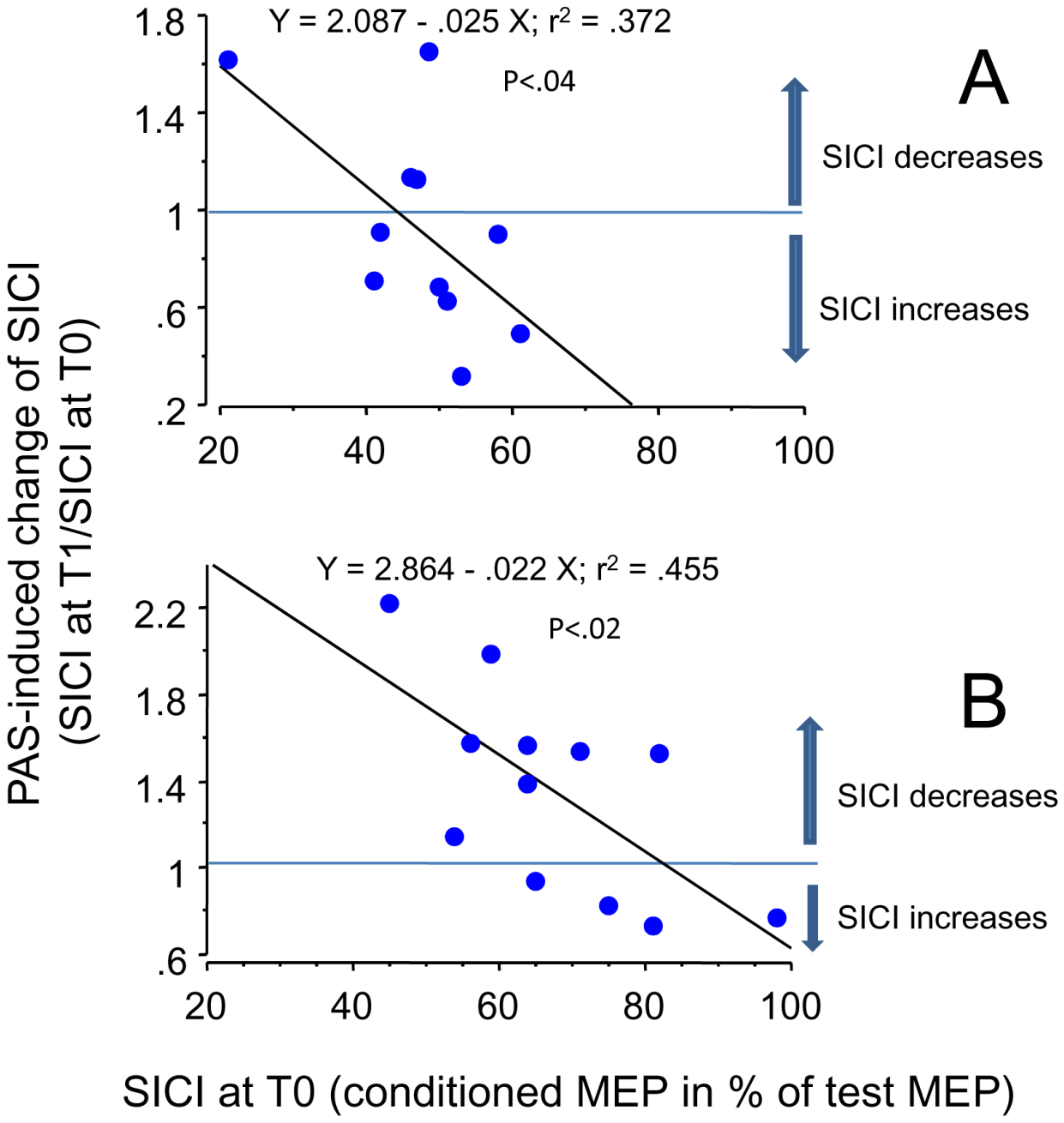

Figure 3. Relationship between the magnitude of baseline SICI in FPB and the PAS-induced change at T2

Each point represents one subject. The *x*-axis represents the magnitude of the baseline inhibition expressed as the size of the conditioned MEP in percentage of the size of the test MEP. The *y*-axis represents the PAS-induced changes in inhibition calculated as "(inhibition at T2/inhibition at T0)". Thus ratios greater than 1 represent a decrease in inhibition and ratios smaller than 1: an increase in inhibition.

The changes in SICI were correlated with the magnitude of baseline SICI after PAS_{25} (A) and also after PAS10 (B).

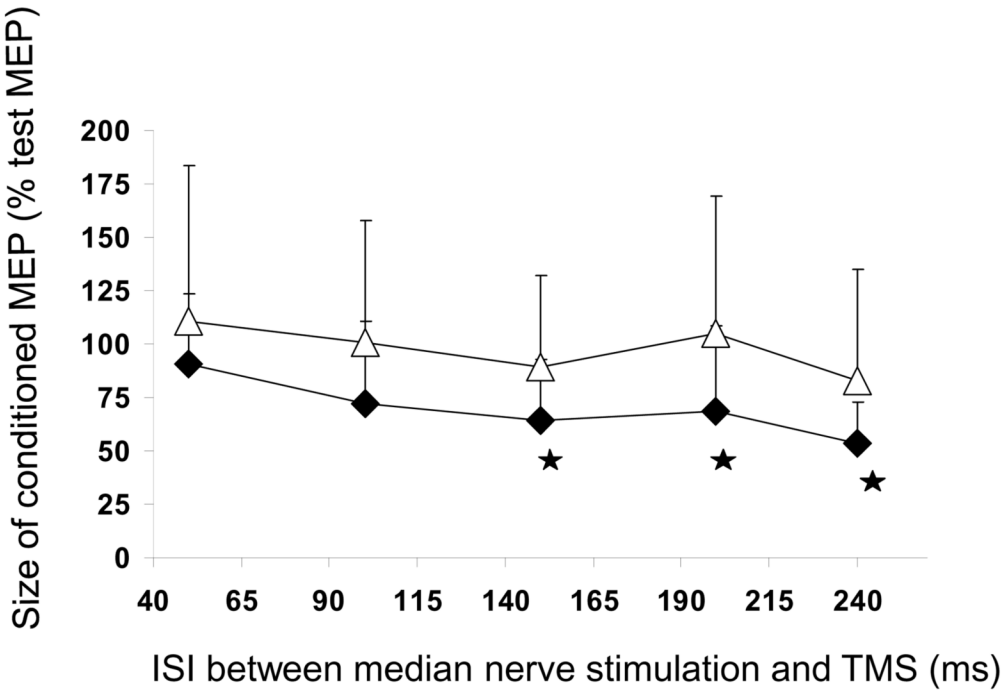

Figure 4. Time course of long afferent inhibition (LAI)

The effect of median nerve stimulation (2.5 × PT) is tested on a 1mV test MEP from FPB (black diamonds) and ADM (white triangles). Each square or diamond represents the mean of 80 measures (8 subjects). Median nerve stimulation inhibits the FPB MEPs at all but the 50 ms and the 100 ms ISI. Stars indicate the ISIs at which the mean conditioned MEP was statistically different from the mean test MEP (paired t-tests).

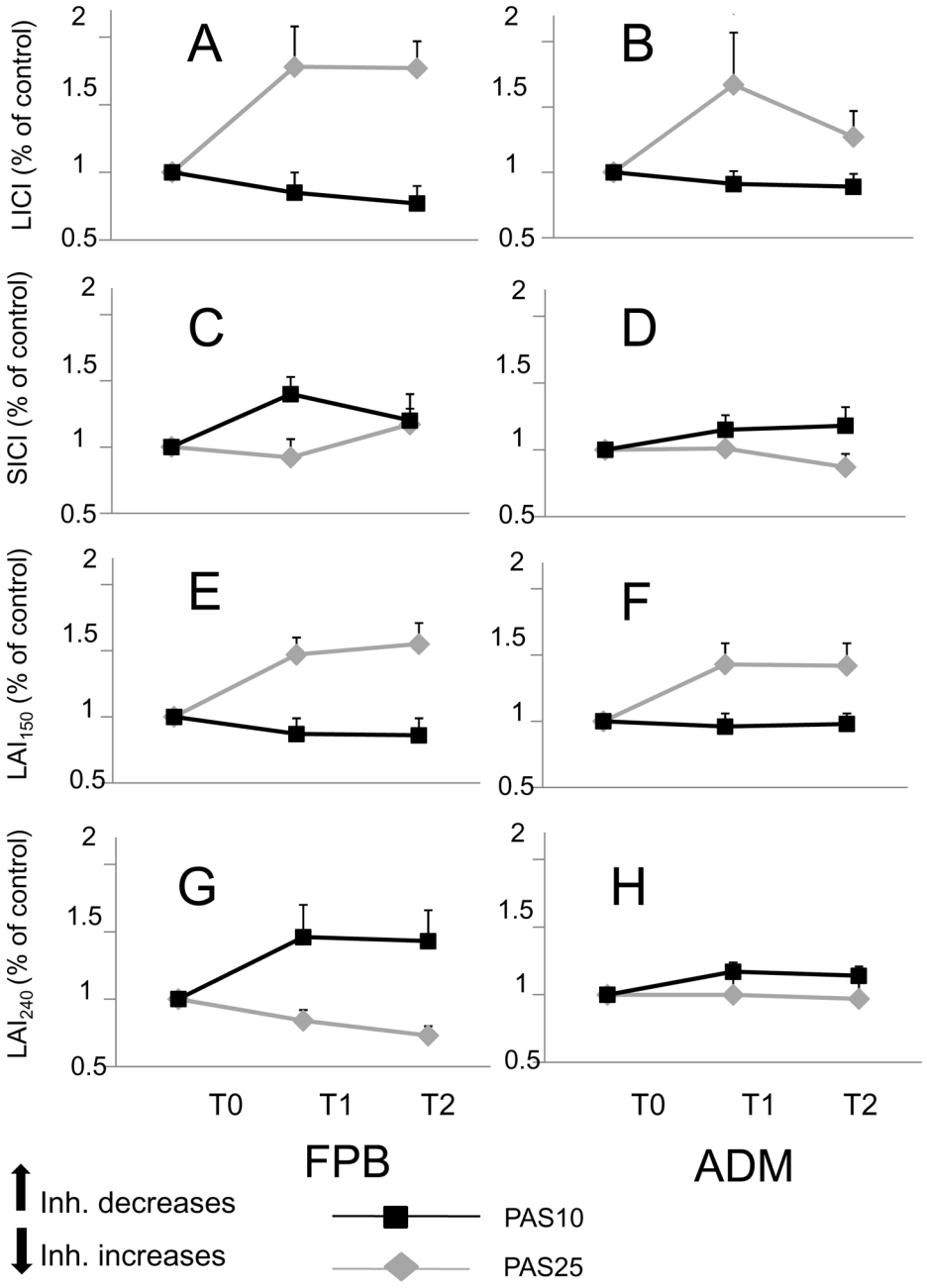

Figure 5. Differential effects of PAS_{25} and PAS_{10} on inhibitions

Graph of interactions between "TIME" (PAS induced change of inhibition at T1 and T2), "MUSCLE" (target muscle: FPB [left column] versus non-target muscle: ADM [right column]) and "INTERVENTION" (PAS_{25} [gray diamonds and lines] versus PAS_{10} [black squares and lines]). The *y*-axis represents the PAS-induced changes of inhibition (inhibition at T1/inhibition at T0 and inhibition at T2/inhibition at T0) with, in A-B: LICI, in C-D: SICI, in E-F: LAI_{150} and in G-H: LAI_{240}. There was a significant effect of "INTERVENTION" for LICI, LAI_{240} and LAI_{150} as the effects of PAS_{25} and those of PAS_{10} go in opposite directions. There was a significant interaction "TIME"*"INTERVENTION"*"MUSCLE" for SICI. The stars indicate a statistically significant result for the post-hoc analysis (unpaired t-tests).

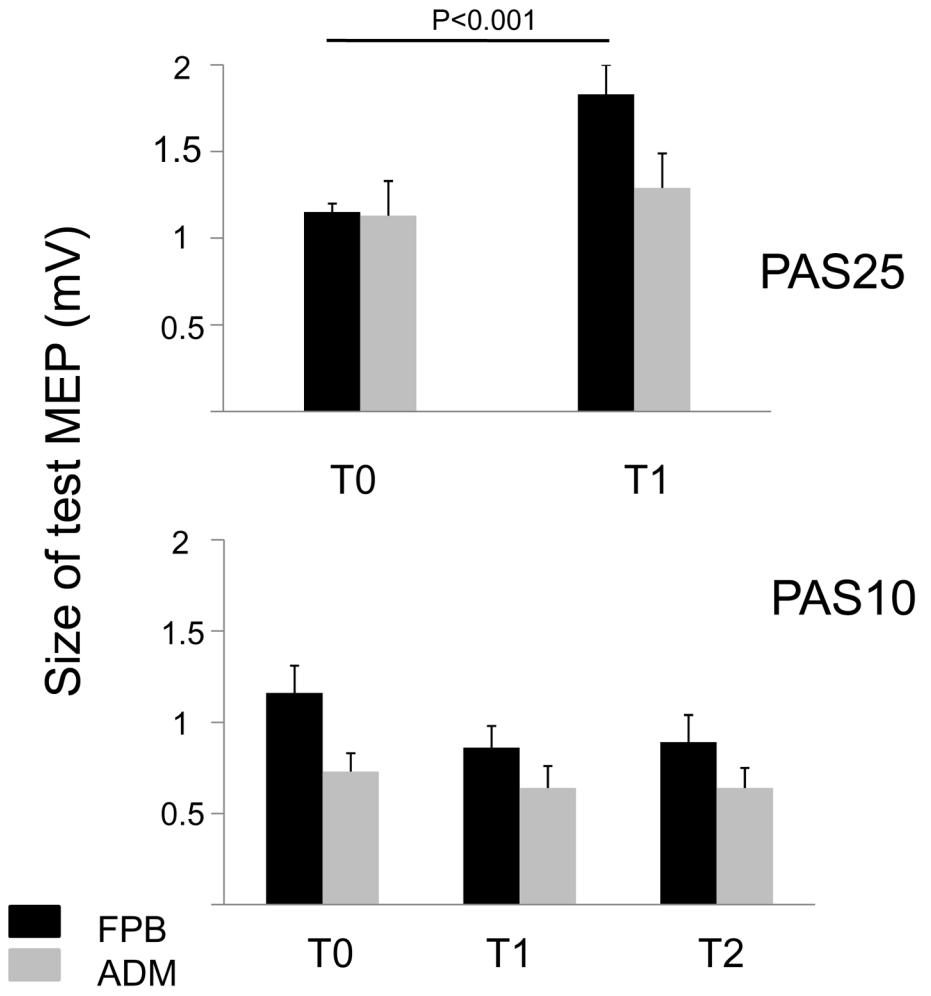

Figure 6. Effects of PAS_{25} and PAS_{10} on overall corticospinal excitability

Upper graph: each column represents the mean of 160 measurements (16 subjects), bottom graph: each column represents the mean of 110 measurements (11 subjects). The size of a test MEP is expressed in mV for the FPB (black columns) and the ADM (gray columns). Intensity was adjusted in order to obtain a 1 mV MEP at baseline (T0). Target muscle was the FPB. Immediately after PAS_{25} (T1) size of the test MEP is increased for FPB, but not for ADM (upper graph). Immediately after PAS_{10} (T1), but also 40 to 50 minutes later (T2), the size of the test MEP is decreased for FPB but not for ADM.

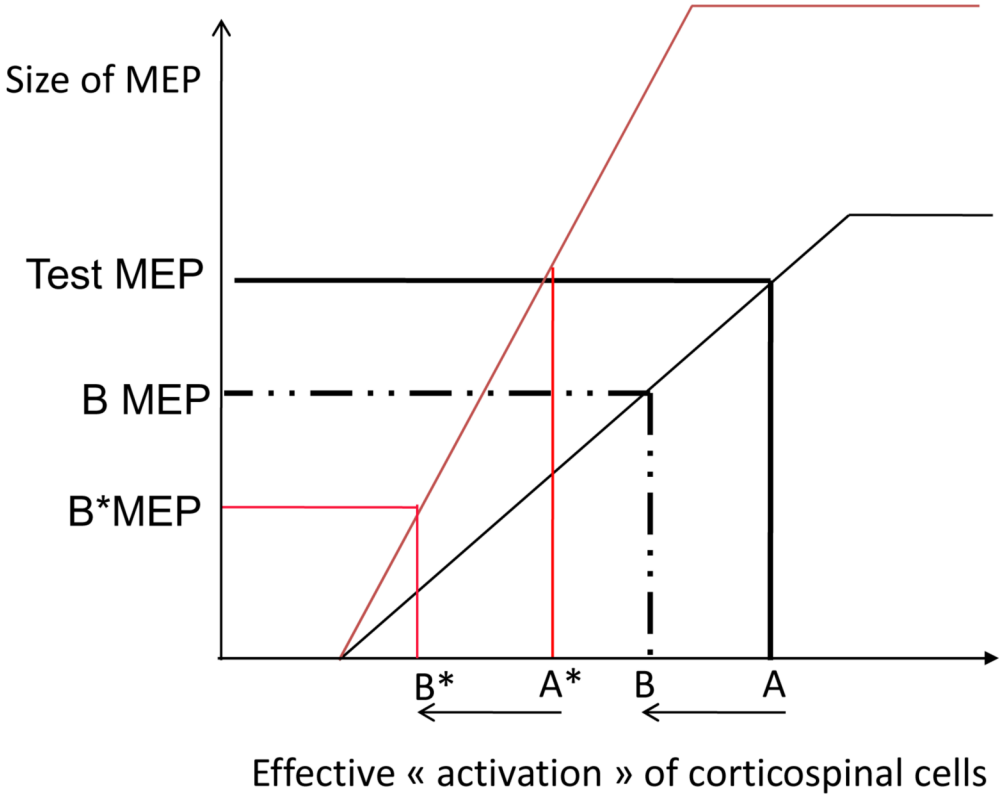

Figure 7. Interpretation of PAS-induced changes of intracortical inhibitions in presence of a change of the input-output curve (I-O curve)

Hypothetical recruitment curve for FPB corticospinal cells is represented before PAS (black thin line) and after PAS (red thin line) under the assumption that PAS has induced a change of the recruitment gain (increase of the slope).

In abscissa: the "final" net input arriving at the corticospinal cells (which is the sum of excitatory and inhibitory inputs); in ordinate: the size of the corresponding MEP.

Before PAS an input "A" results in a test MEP, if the input decreases from A to B due, for example, to activation of inhibitory GABAergic neurons, then the corresponding MEP becomes "B MEP" which is smaller than the test MEP. What would happen if PAS causes an increase of the I-O curve is illustrated. After PAS in order to get a test MEP of the same size as before PAS, a smaller input "A*" is necessary. The same activation of inhibitory interneurones as before PAS occurs then and the final input to corticospinal cells becomes "B*" with A-B = A*-B*. The corresponding MEP is "B*MEP" and it appears clearly than "B*MEP" is smaller than "B MEP". Such an apparent post-PAS "increase" of the amount of the inhibition reflects only the change of the slope of the I-O curve and not a change of the inhibitory drive to corticospinal cells.

Table 1

Mean group data (± SEM) of LICI, SICI and LAI before and after excitatory PAS (PAS25)

EFFECT OF PAS ON LICI											
FPB muscle							**ADM muscle**				
n = 16				LICI	post hoc		n = 16			LICI	post hoc
T0	rANOVA	F = 4.8	p<0.01	42.3 ± 4	p<0.02	p<0.01	T0	rANOVA	p ns	63.5 ± 8	
T1				64.4 ± 7			T1			77.2 ± 9	
T2				68 ± 8			T2			65.6 ± 18	
EFFECT OF PAS ON SICI											
FPB muscle							ADM muscle				
n = 11				SICI	post hoc		n = 8			SICI	post hoc
T0	rANOVA	p NS		47.1 ± 4			T0	rANOVA	p ns	49.3 ± 6	
T1				41.1 ± 5			T1			51.1 ± 11	
T2				52.8 ± 9			T2			41.3 ± 5	
EFFECT OF PAS ON LAI150											
FPB muscle							ADM muscle				
n = 16				LAI150	post hoc		n = 16			LAI150	post hoc
T0	rANOVA	F = 9.4	p<0.0007	49.7 ± 4	p< 0.001	p<0.0005	T0	rANOVA	p ns	72 ± 10	
T1				73.1 ± 8			T1			89.7 ± 10	
T2				74.8 ± 7			T2			82.5 ± 9	
EFFECT OF PAS ON LAI240											
FPB muscle							ADM muscle				
n = 14				LAI240	post hoc		n = 11			LAI240	post hoc
T0	rANOVA	F = 4.9	p<0.02	65 ± 6	p<0.05	p< 0.005	T0	rANOVA	p ns	87 ± 24	
T1				53 ± 6			T1			75.5 ± 11	
T2				47.7 ± 6			T2			73.8 ± 12	

Table 2

Mean group data (± SEM) of LICI, SICI and LAI before and after inhibitory PAS (PAS10)

EFFECT OF PAS ON LICI											
FPB muscle						**ADM muscle**					
n = 10				LICI	post hoc	n = 10				LICI	post hoc
T0	rANOVA	F = 4	p<0.04	65.1 ± 4	p<0.01	T0	rANOVA	p ns		87.7 ± 9	
T1				51 ± 10		T1				81.7 ± 12	
T2				46 ± 9		T2				72 ± 12	
EFFECT OF PAS ON SICI											
FPB muscle						ADM muscle					
n = 12				SICI	post hoc	n = 12				SICI	post hoc
T0	rANOVA	F = 3.6	p<0.05	67.8 ± 4	p<0.01	T0	rANOVA	p ns		77.4 ± 6	
T1				87.2 ± 7		T1				85.2 ± 10	
T2				76.7 ± 6		T2				84 ± 7	
EFFECT OF PAS ON LAI150											
FPB muscle						ADM muscle					
n = 8				LAI150	post hoc	n = 8				LAI150	post hoc
T0		rANOVA	p ns	66.3 ± 8		T0	rANOVA	p ns		70.6 ± 7	
T1				58.4 ± 9		T1				78.9 ± 12	
T2				53.1 ± 7		T2				72.1 ± 8	
EFFECT OF PAS ON LAI240											
FPB muscle						ADM muscle					
n = 10				LAI240	post hoc	n = 10				LAI240	post hoc
T0	rANOVA	F = 3.6	p<0.05	60.5 ± 9	p<0.02	T0	rANOVA	F = 3.9	p<0.04	77.5 ± 9	
T1				86.1 ± 13		T1				92.6 ± 10	
T2				77.1 ± 12		T2				86.3 ± 10	