The two sides of associative plasticity in writer’s cramp

David Weise,1 Axel Schramm,1 Katja Stefan,1,3 Alexander Wolters,2 Karlheinz Reiners,1 Markus Naumann1,4 and Joseph Classen1

1Human Cortical Physiology and Motor Control Laboratory, Department of Neurology, University of Wuerzburg, Wuerzburg and 2Department of Neurology, University of Rostock, Rostock, Germany
Present addresses: 3Department of Neurology, University of Rostock, Rostock and 4Neurologische Klinik und klinische Neurophysiologie, Augsburg, Germany

Correspondence to: Prof. Dr. Joseph Classen, Human Cortical Physiology and Motor Control Laboratory, Department of Neurology, University of Wuerzburg, Josef-Schneider Strauss 11, 97080 Wuerzburg, Germany
E-mail: classen_j@klinik.uni-wuerzburg.de

Neuronal plasticity is to be kept within operational limits to serve its purpose as a safe memory system that shapes and focuses sensory and motor representations. Temporal and spatial properties of motor cortical plasticity were assessed in patients with writer’s cramp using a model of long-term potentiation (LTP) and long-term depression (LTD) of synaptic efficacy. Paired associative stimulation (PAS) combined repetitive electric stimulation of the median or ulnar nerve (MN or UN) with subsequent transcranial magnetic stimulation of the contralateral dominant motor cortex at 21.5 ms (MN-PAS21.5; UN-PAS21.5) or 10 ms (MN-PAS10). Motor-evoked potentials were recorded from abductor pollicis brevis (APB) muscle and abductor digiti minimi (ADM) muscles in 10 patients with writer’s cramp and 10 matched healthy control subjects. Following MN-PAS21.5 or UN-PAS21.5 in non-dystonic subjects, motor responses increased if the afferent PAS-component came from a homologous peripheral region and remained stable with a non-homologous input. In contrast, following either MN-PAS21.5 or UN-PAS21.5 in non-dystonic subjects, motor responses increased if the afferent PAS-component came from a homologous peripheral region and remained stable with a non-homologous input. In contrast, following either MN-PAS21.5 or UN-PAS21.5, both APB- and ADM-amplitudes increased in patients. Compared with controls, this increase started earlier, its magnitude was larger and its duration longer. Following MN-PAS10 in controls, APB-amplitudes decreased, while ADM-amplitudes increased. In writer’s cramp, the decrease of APB-amplitudes started earlier and lasted longer. Of note, ADM-amplitudes were decreased, too. LTP-like as well as LTD-like plasticity is abnormal with respect to both gain and spatial organization. These findings may help to develop a pathophysiological model explaining core features of focal dystonia.

Keywords: dystonia; long-term depression; long-term potentiation; paired associative stimulation; plasticity; transcranial magnetic stimulation

Abbreviations: ADM = abductor digiti minimi; ANOVAR = repeated measures analyses of variance; APB = abductor pollicis brevis; LTD = long-term depression; LTP = long-term potentiation; MEPs = motor-evoked potentials; PAS = paired associative stimulation; TMS = transcranial magnetic stimulation


Introduction

Dystonia is a motor disorder that is characterized by sustained involuntary muscle contractions resulting from co-contraction of antagonistic muscles and overflow into extraneous muscles (Hallett, 1998). It has been proposed that the motor features of dystonia may reflect deficiency in centre-surround suppression (Hallett, 1998, 2004). Surround suppression is a well-known organizational characteristic of sensory systems and refers to the phenomenon of changing the response properties of neurons by stimulation outside the neuron’s classical receptive field. Perhaps best understood in the visual system (Webb et al., 2005; Smith, 2006; Smith et al., 2006), surround suppression is supposed to optimize information transmission (Vinje and Gallant, 2000). In the motor system, an analogue to surround suppression may facilitate voluntary movements and inhibit competing movements (Mink, 2003). Surround suppression in the motor system has been proposed to map anatomically to the direct and indirect basal ganglia pathways (Mink, 1996; Hallett, 1998; Mink, 2003). In theory, failure of surround suppression could entirely be due to a
deficiency of neuronal GABA-mediated inhibition (Hallett, 2004). This hypothesis is supported by substantial evidence for deficient GABAergic inhibition in dystonia and by the anatomical organization of the basal ganglia output (Mink, 2003). The compelling evidence for deficient inhibition in dystonia was derived from neurophysiological studies (reviewed in Berardelli et al., 1998), magnetic resonance spectroscopy of the motor cortex (Levy and Hallett, 2002), histopathological evidence of a deficit in striatal inhibitory interneurons in a heredo-degenerative form of dystonia (Goto et al., 2005) and in an animal model of dystonia (Gernert et al., 2000). However, several of the most pronounced abnormalities of inhibition are present also in non-affected limbs (Ridding et al., 1995) and in asymptomatic carriers of a gene mutation (DYT1) known to be associated with dystonia (Edwards et al., 2003). These observations raise the possibility that deficient inhibition alone may not be sufficient to give rise to a dystonic phenotype.

Experience-dependent changes of synaptic efficacy such as long-term potentiation (LTP) and long-term depression (LTD) are believed to shape sensory receptive fields and motor representations in the brain (Buonomano and Merzenich, 1998; Martin et al., 2000). The importance of LTP in cortical map plasticity is widely recognized. Although LTD is less well studied than its counterpart LTP, it may be of similar physiological importance as it drives the activity-dependent reduction of responses to deprived or behaviourally irrelevant stimuli (Bear et al., 1987; Glazewski and Fox, 1996; Nelson and Turrigiano, 1998). In particular, it may be a significant contributor to sculpting centre-surround suppression (Carandini et al., 2002). Therefore, both synaptic phenomena LTP and LTD may well be involved in the pathogenesis of dystonia. Demonstration of abnormal synaptic plasticity would have the advantage of providing an explanation for the fact that dystonic syndromes frequently emerge in the setting of extensive practice and may progress to involve adjacent body regions.

Previously, we have developed and characterized a protocol, termed paired associative stimulation (PAS), which may probe synaptic changes non-invasively in the human motor cortex. PAS was shaped after animal models of Hebbian associative LTP and LTD (Stefan et al., 2000; Wolters et al., 2003), and consists of pairing repetitively low-frequency median nerve stimulation with transcranial magnetic stimulation (TMS) of the homotopic representation in the primary motor cortex. Following PAS, motor cortical excitability, as probed by the magnitude of TMS-evoked motor potentials, was changed (Stefan et al., 2000; Stefan et al., 2002; Wolters et al., 2003). This change was long-lasting, reversible and topographically specific (Stefan et al., 2000; Wolters et al., 2003). The direction of PAS-induced cortical excitability change depended on the exact sequence of events induced in the primary motor cortex by each of the stimulation modalities (Wolters et al., 2003), similar to the spike-timing dependent plasticity of synaptic efficacy observed in brain preparations of animals (Dan and Poo, 2004). Excitability increased when the events induced by the TMS pulse in the motor cortex followed those generated by the afferent stimulation, whereas excitability was suppressed when the sequence of events was reversed by minimally changing the interstimulus interval. Pharmacological properties of the PAS-induced excitability changes (Stefan et al., 2002; Wolters et al., 2003) were consistent with properties of LTP and LTD. Further support for the synaptic nature of PAS-induced changes in the central nervous system was provided by observations (Ziemann et al., 2004; Stefan et al., 2006) that PAS-induced LTP-like plasticity was temporarily occluded by prior motor training.

Using this model, Quartarone and co-workers (Quartarone et al., 2003) have previously observed that PAS-induced LTP-like enhancement of motor cortical plasticity was increased, and its spatial specificity was compromised in writer’s cramp patients. However, it is not known, whether abnormalities of associative plasticity extend to PAS-induced depression of excitability. Furthermore, it remains an open question, whether temporal evolution and decay of PAS-induced plasticity, properties with implications for cortical map plasticity potentially independent from its magnitude, are affected.

In the present study, we first provide a novel methodological framework to address these questions. Measurements of corticospinal excitability were extended to capture the dynamics of PAS-induced changes of excitability more comprehensively. To enhance temporal resolution of PAS-induced after-effects, the interstimulus interval between the two interacting stimulus modalities was shortened to 21.5 ms sacrificing some of the protocol’s efficacy (Stefan et al., 2000). We further establish that PAS-induced LTD-like plasticity exhibits a highly specific spatial organization in healthy controls. We, then, tested bidirectional (LTP- and LTD-like) plasticity in writer’s cramp patients using the previously characterized versions of the PAS-protocols. This comprehensive approach allowed us to identify abnormalities of LTD-like plasticity, and to disentangle the effects of altered gain of plasticity from disruption of its spatial organization. In addition, the present study helps to resolve some ambiguities in the interpretation of abnormal LTP-like plasticity as established previously by Quartarone and co-workers (Quartarone et al., 2003). Some of the results have been published previously in abstract form (Weise et al., 2004).

Patients and methods

Subjects

The study was approved by the Ethics Committee of the University of Würzburg and all participants gave their written informed consent. Experiments were performed on 10 patients with writer’s cramp (5 men, 5 women), aged 26–51 years (mean ± SD, 38.5 ± 9.6 years) and on 10 healthy subjects, aged 25–53 years (38.3 ± 10.2 years) with normal results on neurological examination who were matched for age, sex and handedness. Writer’s cramp was classified as ‘simple’ if dystonic features were present only with writing and as ‘dystonic’ if muscle cramps also interfered with other
motor tasks (Sheehy and Marsden, 1982). All patients and subjects were right-handed, except one patient and one control subject who were left-handed. Demographic information on the patients and controls is summarized in Table 1.

**Stimulation**

Focal TMS was performed using a figure-of-eight shaped magnetic coil (outer diameter of each wing 7 cm) connected to a Magstim 200 stimulator (Magstim, Whittington, Dyfed, UK). The coil was held tangentially to the skull with the handle pointing backwards and laterally at an angle of 45° to the sagittal plane. Electric mixed (median or ulnar) nerve stimulation was performed at the wrist using a standard stimulation block (cathode proximal) connected to a Digitimer DS7A stimulator (Digitimer model DS7A; Digitimer, UK). Stimulus duration was set at 200 μs and stimulus intensity at 300% of the perceptual threshold. For the digital cutaneous stimulation ring electrodes around the proximal and middle or distal phalanges of dig.I or dig.V were used and connected to a Digitimer DS7A stimulator (Digitimer model DS7A; Digitimer, UK). Stimulus duration was set at 200 μs and stimulus intensity at 300% of the perceptual threshold. For the digital cutaneous stimulation, at least 5 stimuli detected out of 10 presented stimuli were used and connected to a Digitimer DS7A stimulator (Digitimer model DS7A; Digitimer, UK).

**Electromyographic recordings**

Surface EMG activity was recorded from the thenar abductor pollicis brevis (APB) and hypothenar abductor digiti minimi (ADM) muscles of the dominant hand using disposable surface electrodes (Ag-AgCl; Dantec Medical, Skovlund, Denmark) with the active electrode mounted on the muscle bellies and the inactive electrode placed over the base of the metacarpophalangeal joint of dig.I and dig.V. Raw signals were amplified using a differential amplifier (CED 1902, Cambridge Electronic Design, Cambridge, UK) and bandpass-filtered between 1 and 2000 Hz. EMG signals were sampled at 5000 Hz, digitized using an analogue–digital converter (CED 1401 plus, Cambridge Electronic Design, Cambridge, UK) and stored in a laboratory computer for display and later off-line analysis.

**Experimental procedures**

Subjects were seated in a comfortable reclining chair. The perceptual thresholds of the mixed nerve and the cutaneous nerve stimulation (at least 5 stimuli detected out of 10 presented stimuli) were determined. The optimal position of the magnetic coil for eliciting motor-evoked potentials (MEPs) in the target muscle of the dominant hand was assessed over the contralateral motor cortex at a moderately suprathreshold stimulation intensity and marked directly on the scalp with a soft-tip pen. At the optimal site, the resting motor threshold (RMT) was determined as the stimulator intensity needed to produce a response of at least 50 μV in the relaxed target muscle in at least 5 of 10 consecutive trials (Rossini et al., 1994). Thereafter, the stimulator intensity sufficient to evoke a peak-to-peak amplitude of 1 mV in the relaxed target muscle was determined (SI1mV). Subjects were instructed to keep the target muscles at complete rest during the whole experiment. Complete muscle relaxation was continuously monitored by visual and auditory feedback from the surface EMG.

**Paired associative stimulation**

PAS was performed on the dominant hemisphere in all subjects in three different variants in different sessions separated by at least 2 days. The sequence of the protocols was pseudo-randomized between subjects. Electric stimulation of the median or ulnar nerve (MN or UN) of the dominant hand was combined repetitively (0.1 Hz, 180 pulses) with TMS delivered subsequently at 21.5 ms (MN-PAS21.5; UN-PAS21.5) or 10 ms (MN-PAS10) to the contralateral motor cortex. With MN-PAS21.5 and MN-PAS10, the magnetic coil was placed over the optimal cranial position for eliciting an MEP in the contralateral APB. With UN-PAS21.5, the coil was placed over the “hot spot” for the ADM. The general experimental set-up is illustrated in Fig. 1.

Attention was monitored during intervention by testing the ability of the subjects to detect and recall the number of weak electric stimuli randomly applied to target region of PAS (Stefan et al., 2004). Six to eight stimuli (stimulus width 100 μs, 150% of the perceptual threshold) were delivered to dig.I and dig.V in MN-PAS or dig.V in UN-PAS. Care was taken to deliver the electric pulses to the digit asynchronously with the paired associative stimuli. After PAS, subjects were asked to report the count of the stimuli they had identified. Subjects were not provided with any feedback as to the accuracy of the number reported.

For baseline measurements, MEPs of the resting APB and ADM muscles were collected in 60 trials before each intervention. The intensity, duration and somatotopy of the cortical excitability changes were probed by collecting 180 TMS pulses immediately after intervention and 60 pulses after 45 and 75 min, each. The stimulus intensities (SI1mV, as assessed before PAS) and the stimulation rates of 0.1 Hz ± 20% were identical before and after intervention.

**Data analysis**

MEP-amplitudes of the APB and ADM were measured peak-to-peak in each individual trial before and after intervention. For
A similar general pattern emerged after UN-PAS21.5 (Fig. 2B). Several minutes after the termination of UN-PAS21.5, ADM-amplitudes began to increase, reaching their maximum between 20 and 55 min post-intervention. In contrast, APB-amplitudes remained unchanged. ANOVA\(_{\text{AMD}}\) [TIME \((t_0, t_1, \ldots, t_5)\), NERVE (MN, UN), MUSCLE (APB, ADM)] performed on the entire dataset of both interventions revealed a significant main effect of TIME \((F = 5.626; P < 0.000)\), and significant interactions of NERVE \(\times\) MUSCLE \((F = 16.541; P = 0.003)\) and TIME \(\times\) NERVE \(\times\) MUSCLE \((F = 4.329; P = 0.003)\). None of the other main factors or interactions was significant. The TIME \(\times\) NERVE \(\times\) MUSCLE interaction was due to the fact that the PAS21.5-induced increase of corticospinal excitability was restricted to the muscle representation receiving topographically congruent (‘homotopic’) stimulation by both stimulation modalities. When the peripheral stimulation was topographically incongruent (‘heterotopic’), PAS21.5-intervention was ineffective. Post hoc \(t\)-tests performed for each intervention separately revealed a significant increase in MEP size of the homotopically conditioned muscles. Following MN-PAS21.5, APB-amplitudes increased in epoch \(t_2\) (20–30 min; \(P = 0.005\)) compared with baseline (Fig. 2A). After UN-PAS21.5, ADM-amplitudes increased in epochs \(t_2\)–\(t_5\) (10–55 min; \(t_2\); \(P = 0.013\); \(t_3\); \(P = 0.001\); \(t_4\); \(P = 0.002\)) (Fig. 2B). MEP-amplitudes recorded from the heterotopically conditioned muscles were not statistically significantly different from baseline in any of the post-intervention epochs following either MN-PAS21.5 or UN-PAS21.5. To simplify the analysis of PAS21.5-induced effects and to increase the statistical power of the analysis, factors NERVE and MUSCLE were reduced to a single factor TOPO with two levels, ‘homo’ and ‘hetero’. For ‘homo’, results obtained with homotopic conditioning (APB-amplitudes with MN-PAS21.5 and ADM-amplitudes with UN-PAS21.5) were considered. Correspondingly, ‘hetero’ comprised the results of heterotopic stimulation (ADM-amplitudes with MN-PAS21.5 and APB-amplitudes with UN-PAS21.5). Consistent with this interpretation, ANOVA\(_{\text{AMD}}\) [TIME \((t_0, t_1, \ldots, t_5)\); TOPO (homo, hetero)] revealed a significant effect of TIME \((F = 6.004; P < 0.001)\), TOPO \((F = 14.999; P = 0.001)\) and TIME \(\times\) TOPO \((F = 3.564; P = 0.005)\) (Fig. 2C). MEP-amplitudes recorded from homotopically conditioned muscles increased first statistically significantly at \(t_2\) (10–20 min; \(P = 0.001\)). A significant increase was also noted at \(t_5\) (20–30 min; \(P < 0.001\)) and \(t_4\) (45–55 min; \(P = 0.006\)), but at \(t_3\) (75–85 min) the increase was no longer statistically significant \((P = 0.066)\).

**Results**

**PAS21.5-induced increase of cortical excitability in healthy control subjects: dependency on time and topography**

Baseline physiological and behavioural data are shown in Table 2. Changes in baseline-normalized MEP-amplitudes of APB and ADM induced by MN-PAS21.5 or UN-PAS21.5 are illustrated in Fig. 2. MN-PAS21.5 led to an increase of the APB-amplitudes (Fig. 2A). The increase appeared to start several minutes after the termination of the PAS21.5-intervention, reaching a maximum 20–30 min post-intervention. ADM-amplitudes remained essentially unchanged.

**PAS21.5-induced effects in patients with writer’s cramp and comparison to healthy controls**

Writer’s cramp patients did not differ significantly from healthy controls in any of the baseline physiological parameters (Table 2). Similarly, attention, as assessed...
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by the number of errors in detecting a weak electrical stimulus delivered to the target finger, was comparable between patients and controls (Table 2). Following either MN-PAS21.5 or UN-PAS21.5, MEP-amplitudes increased in both APB and ADM (Fig. 3A and B). ANOVA_RRM [TIME (t₀, t₁, . . ., t₅), NERVE (MN, UN), MUSCLE (APB, ADM)] performed on the entire dataset of both interventions revealed a significant main effect of TIME (F = 8.23; P < 0.001), while neither of the other main effects nor any of the interaction terms was significant. In particular, NERVE × MUSCLE (F = 0.014; P = 0.909) and TIME × NERVE × MUSCLE (F = 0.276; P = 0.924) were not significant. To facilitate comparison with observations in non-dystonic controls, results from MN-PAS21.5 or UN-PAS21.5 were combined in patients. ANOVA_RRM [TIME (t₀, t₁, . . ., t₅), TOPO (homo, hetero)] revealed a significant effect of TIME (F = 10.295; P < 0.001), while TOPO (F = 0.012; P = 0.913) and TIME × TOPO (F = 0.196; P = 0.963) were not significant. The lack of TIME × TOPO interaction suggested that, unlike in the control subjects, the time-dependent effect of the PAS21.5 intervention was not different between muscle representations receiving topographically congruent (homotopic) or incongruent (heterotopic) stimulation. MEP-amplitudes (considering APB as well as ADM muscle) were larger than baseline in all epochs following PAS21.5 (t₁, P < 0.001; t₂, P < 0.001; t₃, P < 0.001; t₄, P = 0.006; and t₅, P < 0.001; Fig. 3C). Therefore, this observation suggested that the increase of excitability was earlier and the increase persisted longer than in controls. There were no obvious differences between patients treated with botulinum toxin and those untreated (data not shown).

To explore the difference between control subjects and patients directly, a three-way factorial ANOVA with factors TIME (t₀, t₁, . . ., t₅), TOPO (homo, hetero) and HEALTH (control, writer’s cramp) was performed. Building on the results obtained in healthy controls we used an unsaturated model which tested solely the effect of HEALTH on PAS21.5-induced effects. A significant effect was found for TIME × HEALTH (F = 9.313, P < 0.001), but not for TIME × TOPO × HEALTH (F = 0.939; P = 0.507). Thus, the analysis suggested that the time-dependent modulation of MEP-amplitudes differed between patients and healthy controls. Post hoc analysis revealed that MEP-amplitudes in patients exceeded those in controls for all post-interventional time epochs except t₅ (45–55 min) (t₁, P < 0.001; t₂, P = 0.001; t₃, P = 0.003; t₄, P = 0.053; and t₅, P < 0.001). However, due to the lack of significance of the TIME × TOPO × HEALTH interaction, this analysis did not provide statistical evidence that the topographical gradient between homotopically and heterotopically conditioned muscles differed time-dependently between patients and controls.

We considered the possibility that the lack of topographically different modulation of excitability was confounded by the PAS-induced increase of excitability. The timing characteristic of the topographical gradient of PAS-induced
plasticity was examined further in the combined PAS21.5-experiments (MN-PAS21.5 and UN-PAS21.5). An asymmetry ratio of MEP-amplitudes from both recorded muscles was defined as the ratio of homotopically conditioned muscles over heterotopically conditioned muscles. An ANOVA was performed with factors TIME ($t_0$, $t_1$, ..., $t_5$) and HEALTH (writer's cramp, controls). This revealed a significant main effect of TIME ($F = 2.581; P = 0.028$) and a strong trend of TIME × HEALTH ($F = 2.172; P = 0.059$), suggesting that the time-dependent topographical gradient of modulation of MEP-amplitudes differed between patients and control subjects. Post hoc analysis suggested that the difference arose in the later epochs representing the time between 45 and 85 min ($t_4: P = 0.044$; and $t_5: P = 0.018$; Fig. 4).

MEP-amplitudes increased earlier and remained elevated longer in patients. The relative timing of the PAS-induced change of MEP-amplitudes was compared between patients and controls. Onset and decay of MEP-amplitude changes were considered relative to the maximum of the PAS21.5-induced effect. In patients, MEP-amplitudes reached one-third of their maximum increase on average 18.8 ± 4.6 min after the termination of the PAS-intervention, and MEP-amplitudes fell below one-third of the maximal amplitude after 89.5 ± 7.2 min. Similar values were obtained in control subjects (16.4 ± 2.5 and 91.9 ± 7.4 min, respectively). Likewise, when the threshold for MEP-amplitude increase was set to 25 or 50% of the maximum increase, no difference between patients and controls emerged (data not shown). Because the timing of relative MEP-amplitude changes was similar in patients and controls, a disturbance of the gain of plasticity may be sufficient to explain the change in its build-up and decay.

Fig. 2 Effect of PAS21.5 on MEP-amplitudes recorded from APB and ADM raw data from a representative subject. EMG traces represent the average of 60 trials. Pre: before PAS; Post: 20–30 min post-PAS. Group data from 10 control subjects. (A) MN-PAS21.5. (B) UN-PAS21.5. (C) Combined data from MN-PAS21.5 and UN-PAS21.5. Following PAS, MEP-amplitudes increased in the homotopically stimulated muscle representation only, while MEP-amplitudes recorded from a heterotopically stimulated muscle remained essentially constant. Asterisks indicate significant difference from baseline (two-tailed, one-sample t-test), after false discovery rate correction.
PAS10-induced effects in healthy controls and in patients with writer’s cramp

Following MN-PAS10 in healthy controls, MEP-amplitudes recorded from APB muscle decreased, while those recorded from ADM increased (Fig. 5A). ANOVA with factors TIME (t₀, t₁, ..., t₅) and TOPO (homo, hetero) revealed significant effects of TOPO (F = 10.593; P = 0.010) and TIME × TOPO (F = 2.652; P = 0.035) while TIME (F = 1.900; P = 0.113) was not significant. After false discovery rate correction, APB-amplitudes were significantly reduced compared with baseline at 45–55 min post-MN-PAS10 (P = 0.001; Fig. 5A). There was a strong trend for ADM-amplitudes to increase after PAS, but the increase just failed to be significant in any particular time epoch after false discovery rate correction (t₁: P = 0.159; t₂: P = 0.026; t₅: P = 0.031; and t₅: P = 0.016; t₅: P = 0.051).

Again, in writer’s cramp patients a different pattern emerged (Fig. 5B). On average, APB-amplitudes were decreased in all epochs following MN-PAS10. MEP-amplitudes recorded from ADM, a heterotopically conditioned muscle, were also decreased by MN-PAS10. ANOVA with factors TIME (t₀, t₁, ..., t₅) and TOPO (homo, hetero) revealed significant effects of TIME (F = 4.587; P = 0.002), while TOPO (F = 0.042; P = 0.8412) and TIME × TOPO (F = 1.015; P = 0.420) were not significant. The lack of the TIME × TOPO interaction suggested that the spatial specificity of the MN-PAS10-induced decrease of MEP-amplitudes was lost. In all epochs following PAS10, MEP-amplitudes (considering responses from APB and ADM) were reduced statistically significantly compared with baseline (t₁, P = 0.009; t₂, P = 0.009; t₅, P = 0.016; t₅, P = 0.001; and t₅, P = 0.010)
To explore the MN-PAS10 related difference between control subjects and patients, a three-way factorial ANOVA with factors TIME ($t_0$, $t_1$, ..., $t_5$), TOPO (homo, hetero) and HEALTH (control, writer’s cramp) was performed. In analogy to the analysis done with PAS21.5, we used an unsaturated model which tested only the effect of HEALTH on PAS10-induced effects. This analysis revealed significant interactions of TIME $\times$ HEALTH ($F = 3.880$, $P < 0.001$) and TIME $\times$ TOPO $\times$ HEALTH ($F = 3.137$, $P < 0.001$). The latter interaction provided statistical evidence that the topographical gradient between homotopically and heterotopically conditioned muscles differed time-dependently between patients and controls. The interaction TIME $\times$ TOPO $\times$ HEALTH was due to the fact that ADM-amplitudes were substantially smaller in patients than in healthy control subjects. Post hoc analysis revealed statistically significant smaller ADM-amplitudes in writer’s cramp patients compared with controls for all time epochs ($t_1$, $P = 0.041$; $t_2$, $P = 0.008$; $t_3$, $P = 0.018$; $t_4$, $P < 0.001$; and $t_5$, $P = 0.004$). In contrast, APB-amplitudes did not differ between patients and controls at any single time epoch after MN-PAS10.

**Discussion**

Dystonic patients exhibited profoundly abnormal dynamic responses of corticospinal excitability toward two Hebbian associative stimulation protocols which were previously...
shown to induce neuronal plasticity of different polarity. Plasticity was abnormal with respect to its magnitude and temporal properties, as well as with respect to its spatial organization.

**Novel properties of bidirectional PAS-induced plasticity in healthy controls**

The present study employed a facilitating PAS-protocol which differed from the previously published PAS-protocol which utilized an interstimulus interval of 25 ms (Stefan et al., 2000) by the shorter interstimulus interval of 21.5 ms between the different PAS-components and by the larger number of interventional pairs. This change in the protocol was associated with a reduction of the maximum magnitude of PAS-induced increase of excitability. Of interest for the present study, the maximum enhancement was only reached after a delay of ~30 min after the termination of the PAS-intervention suggesting a slower build-up of excitability changes compared with our previous observations (Stefan et al., 2000). This delayed increase is consistent with recent observations by Morgante et al. (2006) who used the same interstimulus interval. Conceivably, the reduced efficacy of the facilitating PAS-protocol has unmasked a temporal evolution of excitability change which was impossible to detect previously. Similar temporal dynamics of plasticity have been observed in an animal study of associative LTP in hippocampal slice preparations (Magee and Johnston, 1997). Therefore, the temporal properties of PAS-induced enhancement of plasticity are consistent with LTP as the underlying mechanism. However, it should be pointed out that inferences about mapping of PAS-induced cortical excitability changes onto cellular phenomena such as LTP/LTD are limited, due to the non-invasive nature of the technique, and due to the lack of appropriate comparative studies in animals. In particular, changes of intrinsic neuronal excitability (Daoudal and Debanne, 2003; Zhang and Linden, 2003; Li et al., 2004) may be pre- or postsynaptic mechanisms involved synergistically.

The present study revealed hitherto unknown spatial properties of PAS10-induced plasticity in healthy subjects: PAS10-induced decrease in excitability was confined to the homotopically stimulated muscle. In previous studies, PAS-induced enhancement of excitability was either less pronounced or absent in heterotopically conditioned muscle representations (Stefan et al., 2000; Ridding and Taylor, 2001; Quartarone et al., 2003). However, with PAS applied at ISI = 10 ms, MEPs recorded from the heterotopically stimulated muscles did not remain unchanged but were even increased (Fig. 5). This observation suggests that PAS10-induced plasticity may have stronger and qualitatively different spatial properties than facilitating PAS-protocols. Interestingly, stimulation-induced LTD-like changes of pain perception were surrounded by heterosynaptic LTP-like facilitation in adjacent skin regions (Klein et al., 2004).

Stimulation-induced depression of synaptic efficacy can be associated with heterosynaptic strengthening of nearby synapses (Royer and Pare, 2003). Our findings, therefore, would be compatible with the hypothesis that induced LTD-like plasticity displays a similar spatial organization in multiple systems, with important behavioural correlates.

**Enhanced gain of LTP/LTD-like plasticity in writer’s cramp**

The present study confirmed and extended observations by Quartarone and co-workers (Quartarone et al., 2003). Using a similar PAS-protocol (interstimulus interval of 25 ms and a stimulation frequency of 0.05 Hz), these authors demonstrated that facilitatory effects on TMS-evoked MEPs recorded from the target muscle were enhanced in writer’s cramp patients (Quartarone et al., 2003). Recent results have shown that the magnitude of the modulation of the cortical excitability induced by plasticity-inducing protocols is strongly influenced by the activation history of the targeted neuronal circuit (Siebner et al., 2004; Ziemann et al., 2004; Stefan et al., 2006). Therefore, it appears worthwhile to consider the possibility that an enhanced facilitatory effect of PAS-induced plasticity in patients may be due to a similar phenomenon, possibly reflecting a reduced spontaneous use of the affected dystonic hand. In this case, enhancement of facilitatory PAS-induced plasticity would not be abnormal itself, but could sufficiently be explained by a simple activity-dependent lateral shift of the synaptic modification threshold (Bienenstock et al., 1982; Bear et al., 1987; Abraham and Tate, 1997) between enhancing and suppressing induction conditions. However, in this case the efficacy of PAS10 should be diminished, because PAS10 has been shown to lead to LTD-like plasticity in healthy controls (Wolters et al., 2003) and the formation of LTD is decreased by prior synaptic inactivity (Kirkwood et al., 1996). Importantly, PAS10-induced depression of cortical excitability in patients exceeded that obtained in healthy controls (Fig. 5). Even when only the homotopically conditioned muscle representation was considered, PAS10-induced depression was no smaller in the patients. Therefore, our findings render it unlikely that the enhanced facilitatory effect of PAS21.5 (or PAS at ISI = 25 ms) was merely a consequence of an unrecorded health-status-dependent difference in the activation history of the cortex. That PAS10-induced plasticity was not reduced in patients suggests, instead, complex changes in the control of LTP- and LTD-like plasticity (see below). Because attention is a powerful modulator of PAS-induced plasticity (Stefan et al., 2004), it is conceivable that a larger facilitating PAS-effect in writer’s cramp was due to greater attention to the target hand. This possibility is unlikely as attention was similar in the patients and control subjects. Together, these arguments support the view that PAS-induced LTP-like plasticity was genuinely altered in writer’s cramp.
That PAS10-induced suppression of cortical excitability was more pronounced in writer’s cramp is in keeping with the majority of previous studies examining inhibitory plasticity in dystonia. Repetitive TMS of 1 Hz applied to the premotor cortex was previously shown to suppress metabolic activity at the site of stimulation as well as at the primary motor cortex and supplementary motor area. In patients, the reduction of metabolic activity was more pronounced than in healthy subjects (Siebner et al., 2003). Similarly, theta-burst stimulation, a recently introduced novel repetitive TMS protocol (Huang et al., 2005), depressed cortical excitability more in dystonic than in healthy subjects (Edwards et al., 2004). Moreover, 1 Hz repetitive TMS applied to the primary motor cortex of patients increased (previously defective) intracortical inhibition while leaving intracortical inhibition in healthy controls unchanged (Siebner et al., 1999). Because, as outlined above, physiological properties of PAS10-induced plasticity resemble those of associative LTD (Wolters et al., 2003), the present study possibly provides the strongest evidence so far that enhanced responses to depressing protocols may be due to enhancement of LTD-formation in writer’s cramp. A single study in writer’s cramp patients reported the absence of a suppressing effect of cathodal transcranial direct current stimulation (cTDCS) on corticospinal excitability (Quartarone et al., 2005). cTDCS reliably induces depression of MEP-amplitudes in healthy controls (Nitsche and Paulus, 2000). However, because cTDCS most likely operates through non-synaptic effects (Ardolino et al., 2005), the absence of its efficacy in dystonia does not argue against an enhancement of LTD-like synaptic plasticity.

As outlined above, the present protocol allowed us to detect abnormalities in the temporal properties of PAS-induced plasticity in addition to the amplitude of modulation. In patients the PAS21.5 effect on corticospinal excitability started earlier and lasted longer than in healthy controls (Fig. 3). Thus, the present results suggest a disturbance of the temporal properties of plasticity formation in writer’s cramp. Because the speed of build-up and decay did not differ from the controls when considered relative to the maximum PAS-induced effect, both deviations, enhanced magnitude and altered dynamics of synaptic plasticity, may belong to the same abnormality. In summary, the findings reviewed thus far point to an increased gain of synaptic motor cortical plasticity in writer’s cramp relating to the formation of both LTP- and LTD-like phenomena.

Properties of dystonia may be matched to abnormalities of associative plasticity
Many parallels suggest that properties of dystonia may be tightly linked to aberrant PAS-induced plasticity. We found that neuronal plasticity may lead to de-differentiation of motor representations. Overflow to extraneous muscles, a cardinal property of focal dystonia, may, therefore, be related to abnormalities of spatial properties of associative plasticity. Additionally, loss of spatial differentiation may be relevant for progression of dystonia toward adjacent body segments. In the sensory system, LTD is fundamental in reducing cortical responsiveness to behaviourally irrelevant or unused sensory stimuli (Allen et al., 2003). Because loss of spatial focus was most striking in PAS10-induced plasticity, we speculate that failure of spatial focus of LTD-like plasticity may have more devastating effects on motor organization than a disturbance of its LTP-like counterpart. Previous work (Ziemann et al., 2004; Stefan et al., 2006) has demonstrated that the type of plasticity that is probed by PAS may be closely related to neuronal mechanisms involved in motor learning by repeated practice. Failure of
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Is aberrant neuronal plasticity related to previously identified abnormalities of neuronal inhibition?

Exactly what caused neuronal plasticity to transgress normal boundaries in writer’s cramp remains unknown. Because our protocol comprised activation of an afferent somatosensory pathway as well as external activation of the motor cortex, the spatial abnormalities of PAS-induced plasticity could be due to a disturbance in any neuronal processing stage before or within the motor cortex. Indeed, previous findings have provided evidence both for spatial de-differentiation of somatosensory representation (Bara-Jimenez et al., 1998; Elbert et al., 1998; Tinazzi et al., 2000) and for a disturbance of sensorimotor integration (Abbruzzese et al., 2001; Tamburin et al., 2002; Bertolasi et al., 2003) in dystonia. However, while somatosensory de-differentiation may contribute to spatial de-differentiation in motor cortex, it would not easily explain the increased gain of motor cortical plasticity. Deficiencies in homeostatic dynamic plasticity mechanisms [such as synaptic scaling (Turrigiano and Nelson, 2004)] that normally help to maintain average neuronal activity levels may compromise regulation of either magnitude or spatial organization of induced plasticity. Yet another possibility might be that subtle disturbances in dopaminergic transmission, which are implicated in some forms of dystonia, may have contributed to aberrant plasticity. Indeed, suppression of PAS-induced plasticity, reversible upon dopaminergic medication, has been noted in recent studies of plasticity in parkinsonian patients (Morgante et al., 2006; Ueki et al., 2006). While we cannot exclude contribution of any of these mechanisms, we are tempted to speculate that both abnormalities, spatial de-differentiation and increased gain, are intimately related to abnormalities of neuronal inhibition that have been identified previously both in the motor and somatosensory system. According to this hypothesis, defective inhibition may not itself lead to a dystonic phenotype but only by virtue of its effects on synaptic plasticity. Local GABA-mediated inhibition is of crucial importance in spatially focusing LTD-dependent plasticity (Foeller et al., 2005). Therefore, spatial derangement of the expression or formation of LTD-like plasticity inhibition in dystonia may well be explained by failure of neuronal inhibition. Furthermore, GABAergic inhibition is a gate to formation of LTP in somatosensory (Dykes, 1997) and motor cortex (Hess et al., 1996). In an animal model of dystonia, deficient striatal GABAergic inhibition (Gernert et al., 2000) was associated with enhanced LTP-formation (Kohling et al., 2004) as tested in striato-cortical slice preparations. Therefore, it is conceivable that enhancement of PAS-induced LTP-like plasticity in dystonia was promoted by neuronal disinhibition. These considerations would explain why intense occupational hand usage which likely is associated with cortical disinhibition (Floyer-Lea et al., 2006) and intense repetitive afferent conjoint stimulation is a risk factor for developing dystonia. Conversely, our findings might suggest that a rational approach to treating dystonia may be to pharmacologically augment inhibition while attempting to normalize motor representations through re-training or therapeutic interventional stimulation. Indeed, interaction with neuroplasticity mechanisms may underlie the beneficial effect induced by tiagabine, a GABA-reuptake inhibitor, on dystonia in an animal model (Kreil and Richter, 2005).

Future studies may reveal that aberrant plasticity does not have an early and primary pathogenic role as suggested here, but evolves only secondarily—a consequence rather than a cause of dystonia. However, it is important to note that, even in this case, plasticity may be instrumental in stabilizing pathological movement representations. Maladaptive neuronal plasticity has been proposed to be related to abnormal perceptual experiences, such as phantom limb sensation, tinnitus, central pain (Elbert and Rockstroh, 2004; Jastreboff and Hazell, 2004; Zhuo, 2005) and emotional and affective disorders (Rainnie et al., 2004). Our findings support extension of this concept to the motor domain and underline that neuronal plasticity is advantageous only if kept within useful limits (Quartarone et al., 2006).

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References


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