Changes in spinal excitability after PAS (paired associative stimulation).

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Summary

Repetitive pairing of a peripheral stimulation with a magnetic transcortical stimulation (PAS) is widely used to induce plastic changes in the human motor cortex non-invasively. Based on the contrast between PAS-induced increase of corticospinal excitability and absence of PAS-induced increase of the spinal F wave size it has been generally accepted that PAS-induced plasticity is cortical in origin. Here, instead of F waves, we used H reflex recruitment curves to assess spinal excitability, and we demonstrate that PAS induces parallel changes in cortical and spinal excitability.
Since its description in 2000 (Stefan et al., 2000) the PAS (paired associative stimulation) technique has been extensively used in clinical research to induce plastic changes in the human motor cortex non-invasively. The use of PAS has opened the way to test the possible role of an aberrant cortical plasticity in pathophysiology of various motor disorders, e.g., for dystonia (Quartarone et al., 2003) and for Parkinson’s disease (Morgante et al., 2006). Reproducing stimulation protocols developed to induce associative LTP/LTD in animal cortical slices, PAS consists of pairing repetitively a peripheral (most often electrical median nerve stimulation at the wrist) and a cortical stimulation targeting a median nerve innervated muscle. Cortical stimulation is achieved using single shock transcranial magnetic stimulation (TMS). Changes in corticospinal excitability evoked by the PAS intervention are monitored at different delays after the intervention by measuring the electromyographic amplitude of a test MEP (motor evoked potential). MEP is a complex response as it reflects activity of: (i) many groups of cortical cells (cortico-cortical cells, corticospinal cells), (ii) and also spinal cells, not only motoneurons, but also interneurones through different pathways (mono or polysynaptic) (see the review of Petersen et al. 2003). So when we observe PAS-induced change in size of MEPs the question arises whether the excitability changes have occurred at cortical and/or subcortical or spinal cord levels. This issue was answered by comparing the PAS-induced changes of MEPs with those of F waves and occasionally with those of motor responses evoked by brainstem stimulation (Stefan et al., 2000; Wolters et al., 2003). F waves are due to the backfiring of few antidromically activated spinal motoneurones (Eccles, 1955), so a change in their size might be ascribed to a change in spinal motoneurone excitability. The limitation of such a control is that F wave sensitivity to short-term changes in motoneuronal excitability appears to be quite low (Lin & Floeter, 2004), and that F waves and MEP are probably not generated by the same population of spinal motoneurones (Eccles, 1955). We have reinvestigated whether the PAS intervention induces any change in spinal excitability by using the technique of the H reflex. H reflexes are more sensitive than F waves to detect changes in excitability of the spinal motoneurones (Eccles, 1955). In addition, while F waves recruit preferentially the larger faster conducting motoneurones (Kimura et al., 1984), stimulation of both Ia
afferents and the corticospinal tract in animal experiments recruit the motoneurons in an orderly manner according to the size principle (Henneman et al, 1965; Somjen et al, 1965). Nevertheless it has been pointed out that, in humans, MEPs and H reflexes might not always reflect activation of the same population of motoneurones (see discussion).

The recruitment curves of the wrist flexors (FCR) M waves and H reflexes were recorded before and 20-40 minutes after a PAS intervention in 9 right handed healthy volunteers (mean age 36.44 years ± 8.6, range: 24-51). To evoke an H reflex the right median nerve was electrically (1 ms rectangular pulses) stimulated through bipolar surface electrodes in the medial aspect of the arm (two cm above the elbow). EMG signals from FCR were filtered (10 Hz-2 kHz) and digitized (sampling frequency 5 kHz). To record ascending phase and plateau of the H reflex recruitment curve, we used intensities ranging from 0.6 MT (motor threshold) to 1.5 MT; 10 reflexes were averaged at each intensity. It was verified that the M wave size after the PAS intervention was similar to that before the PAS. If it was not the case the corresponding file was discarded from analysis. In order to compare changes in spinal excitability with those of cortical excitability, the mean size of 10 FCR MEPs was also calculated before, and 10-20 minutes after the PAS intervention at each of 3 or 4 different TMS intensities: from 1.1 to 1.4 x rMT. Resting motor threshold (rMT) was defined as the minimum intensity needed to evoke, prior to the PAS, a MEP of 50 µV in 5 out of 10 consecutive trials in the relaxed FCR muscle. Sizes of M waves, H reflexes and MEPs were expressed in percentage of the FCR maximal M wave (Mmax). The FCR Mmax was calculated before and after the PAS intervention.

PAS was induced by pairing every 5 second a median nerve (1.1 MT) and a TMS (1.2 rMT) stimulation for 20 minutes (median nerve stimulation to TMS interstimulus interval: 20ms). During PAS and when measuring MEPs sizes, TMS was adjusted spatially to target the FCR muscle. Using a Magstim 200 stimulator (Magstim, Whitland, Dyfed, UK) and a figure-of-eight coil (outer diameter 8 cm) the hot spot for FCR muscle was defined as the lowest threshold site evoking a MEP response in FCR accompanied by a clear wrist flexion movement. The coil was positioned with the handle pointing backwards at an angle of 45° to the midline; the direction of the induced current in the brain was from posterior to anterior. The hot spot for right FCR stimulation 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.

The group results are presented on Figure 1 A-B. H reflex sizes were measured at \( I_{50} \) (the stimulus intensity required to obtain a response 50% of the maximum). For the whole group, mean size of the M wave ± SD was 3.69 ± 2.6 % of Mmax and that of the H reflex was 7.98 ± 6 % of Mmax. After the PAS intervention the size of the M wave was the same (3.49 ± 2.5) while H reflex size was significantly increased to 13.10 ± 12% (Wilcoxon ranked test \( p < .011 \)). In order to get a valid comparison of PAS-induced modulation of H reflex and MEP, we used in the comparison the MEP, the size of which was the closest to the H reflex size. Mean MEP size was 5.4 % of Mmax ± 3.9 before PAS (mean TMS intensity: 1.21 x rMT ± .08), and this increased significantly after PAS to 9.5 ± 10 (Wilcoxon ranked test \( p < .021 \)). When individual results of the 9 subjects were examined (Figure 1 C) it appears that PAS-induced modulation (size of the response post PAS minus its size pre PAS divided by its size pre PAS) of the H reflex paralleled that of the MEP in 7 out of the 9 subjects. In six out of these 7 subjects both the H reflex and the MEP were increased by PAS (subjects 1 to 6 on Fig.1 C) while for subject 7 PAS was ineffective in modifying the MEP as well as the H reflex. In 2 subjects (subjects 8 and 9) there was a clear dissociation between the PAS-induced effect on the MEP and the H reflex: PAS induced a clear facilitation of the MEP but no (subject 8) or a very small modification (subject 9) of the H reflex.

To get more insight into possible mechanisms of the increase in spinal excitability we examined the whole recruitment curves. Results of a representative subject (subject 3) are presented in Fig.2 (middle column). We calculated for each subject: the H reflex threshold (intensity evoking a H reflex of 2 % of Mmax), the Hmax value and the slope of the ascending limb of the recruitment curve. This slope was obtained by fitting to a linear regression function the steepest part of the ascending limb of the recruitment curve. These 3 parameters were averaged across subjects and compared before and after PAS. They were used to draw the hypothetical recruitment curves of the whole group presented on Fig. 2 left part. Mean threshold for evoking the H reflex was not modified by PAS (.848 MT ± .14 before PAS versus .846 ± .15 after), Hmax value was increased but this increase did not reach statistical significance (21 % of Mmax ± 14 before versus 25 ± 23
after). The most striking difference was an increase of the slope of the ascending phase of the recruitment curve: \(0.75 \pm 0.7\) before versus \(1.12 \pm 1\) after (Wilcoxon ranked test \(p < 0.02\)).

In most of the PAS experiments reported in the literature, the target muscle was a hand muscle: FDI (first dorsal interosseus) (Pitcher et al., 2003), APB (abductor pollicis brevis) or ADM (abductor digiti minimi) (Stefan et al., 2000; Wolters et al., 2003). It is almost impossible to obtain an H reflex in these muscles, and it explains why the F wave technique has been usually chosen to explore PAS-related change in spinal excitability. It could be argued that the PAS-induced changes in spinal excitability that we describe here are more or less specific to forearm muscles and would not exist for hand muscles. As a control we performed for the APB muscle the same experiment as previously done for the FCR muscle with TMS targeting the APB muscle (PAS: ISI = 25 ms, intensity of stimulation of median nerve at wrist: 3 x PT). Among a subgroup of 10 subjects chosen for their especially big FCR H reflexes we found 3 subjects in whom it was possible to evoke a suitable H reflex in the APB muscle from a stimulation of the median nerve at the wrist level. In 2 of them PAS induced an increase in size of the H reflex and of the MEP (see the results of one of these 2 subjects on the right part of Fig. 2), in the third one, neither the MEP nor the H reflex was modified after PAS.

APB F waves are reported in the literature not to be changed by PAS. We wondered whether this apparent lack of susceptibility to changes in spinal cord excitability could be related to the experimental conditions: (i) small number of averaged F waves whereas a sample size of at least 50-75 F waves is necessary to approximate the F wave size with an accuracy of \(\pm 25\%\) (Lin & Floeter, 2004); (ii) discrepancy between the F waves and the MEPs sizes, 100-300 \(\mu\)V and 1 mV respectively (Stefan et al., 2000; Wolters et al., 2003). Even if F wave and MEP would recruit the motoneurons in the same order (which is unlikely), the sensitivity of each to the facilitatory PAS intervention would depend on their respective size (Capaday, 1997). We performed complementary experiments to look at the effect of PAS on APB F waves. Seven subjects participated in these experiments. F waves were evoked by a supramaximal stimulation of the median nerve at wrist. Fifty to 100 F waves were recorded and averaged before and 20-40 minutes after the PAS. The PAS-induced modulation of F waves was compared with that of the APB MEP. TMS
intensity was adjusted in order that MEP and F sizes (in % of Mmax) before PAS were similar. On average the MEP was facilitated by PAS (2.46 % of Mmax ± 1.1 before PAS versus 4.47 ± 1.9 after; Wilcoxon ranked test, p <.02) (Fig.3 B) while F wave size did not change (2.56 ± 1.6 versus 3.11 ± 2.4; Wilcoxon, p = 0.7) (Fig.3 A). Inspection of individual results (fig.3 C) show that PAS-induced modulation of F waves was not uniform as a PAS-induced facilitation of the MEPs was accompanied by an increase in size of F waves in subjects 4 and 6, no change in subject 1 and a decrease of F waves in subjects 2 and 3.

In this pilot study we have shown that a PAS intervention, targeting the motor representation of wrist flexor or hand muscles, does induce a change in the recruitment curve of the H reflex 20-40 minutes after the end of the PAS intervention. As the M waves were exactly matched before and after PAS, modification in the size of the H reflex can not be ascribed to a change in the excitability of the peripheral afferents in the median nerve or a change in the neuro-muscular transmission and does reflect an enduring modification of the spinal excitability. From our data it is not possible to make any conclusion regarding the exact spinal site(s) where PAS-induced changes develop or the mechanism supporting such a short-term plasticity. The possible sites are the synapses interposed in the pathways controlling presynaptic inhibition to Ia afferents, the synapse between Ia afferents and motoneurons, or the spinal motoneurons by themselves. Changes in the slope of the H reflex input-output relationship can be due to an increase in efficacy of the afferent volley through a decrease of presynaptic inhibition or homosynaptic depression, to a change in the recruitment gain of the reflex (Kernell & Hultborn, 1990) or a change in the intrinsic properties of the motoneurons (Carp et al., 2006). The hypothesis that PAS might induces changes of presynaptic inhibition fits with the recent finding that activation of the target muscle accelerates PAS changes (Kujirai et al, 2006), and presynaptic inhibition is known to be strongly decreased during voluntary contraction (Meunier & Pierrot-Deseilligny, 1989). Changes in the tonic level of presynaptic inhibition to Ia afferents might rely on changes in the strength of the synapses interposed in presynaptic inhibitory pathways. Presynaptic interneurones receive both projections from peripheral afferents and descending pathways and development of an associative plasticity at their level is conceivable, although never been demonstrated.
PAS-induced facilitation of the H reflex may also rely on a change of the descending corticospinal tonic control to presynaptic interneurons (Meunier, 1999). The fact that an increase of the H reflex is always accompanied by a MEP increase while sometimes MEP facilitation exists without concomitant H reflex facilitation fits with such a “cortical” origin of spinal changes in excitability. As presynaptic inhibition of Ia terminals is stronger on terminals supplying slow motor units than on terminals on fast motor units (Zengel et al., 1983), a decrease of presynaptic inhibition can cause an increase of the slope of the recruitment curve. Homosynaptic depression is another presynaptic mechanism playing a role in short term regulation of synaptic transmission and may be due to changes in the probability of transmitter release at the synapse (Kuno, 1964). PAS-induced change in the recruitment gain is unlikely. Such a change has been described when an afferent volley (for example a cutaneous one) has a differential effect on early recruited versus late recruited motoneurons (Nielsen & Kagamihara, 1993); here the two volleys involved in the PAS intervention (the afferent and the corticospinal ones) are known to initially recruit the small motoneurons (Bawa & Lemon, 1993). Changes in motoneuron electrophysiological properties cannot be ruled out; nevertheless, they probably underlie more long-term spinal plasticity than short-term one as that observed here. Shift in motoneuron firing threshold and change in the level of the after-hyperpolarisation have been documented after operant conditioning of the primate H reflex (see the review of Wolpaw & Carp 2006) or after increased chronic activity in rats (Beaumont & Gardiner, 2002).

Although slightly smaller than that of the MEP (63.9% of its control value) the amount of PAS induced modulation of H reflex (48.5%) is impressive. Does it mean that the largest part of the MEP facilitation is due to the development of a spinal plasticity with only a small part due to a genuine cortical plasticity? Such a conclusion is certainly excessive. Indeed, development of spinal plasticity seems to depend on that of cortical plasticity as we observed sometimes an increase of the MEP without a parallel increase of the H, but never the opposite. Furthermore, even if H reflexes and MEPs have similar sizes, it does not mean that they reflect activation of the same population of motoneurons (Morita et al., 1999). It may be due to the complex nature of the descending volley regarding the single synchronized Ia volley or also to the fact that presynaptic inhibition
can cause changes in the gain with which the Ia stimulation recruits the motoneurons while TMS can not (as presynaptic inhibition is not distributed to corticospinal fibres (Nielsen & Petersen, 1994); different distributions in the motoneuronal pool of corticospinal fibers and Ia afferents seem to be unlikely. Also changes in excitability in spinal interneuronal pathways may influence H reflex size as H reflex is not exclusively monosynaptic (Burke et al., 1984).

Respective reliability of F waves and H reflexes in assessing spinal cord excitability is difficult to assess. F waves are easily recordable from hand muscles but not from forearm muscles, whereas H reflexes are recordable from forearm muscles but rarely from hand muscles. Nevertheless, we have shown that the PAS-induced modulation of hand muscles F waves is highly variable leading to a lack of effect on averaged data while PAS-induced modulation of MEPs is very consistent. In summary, we conclude that the absence of a change in F-waves measures, even in hand muscles, does not allow us to eliminate any associated change in spinal excitability.

We have still to elucidate why, contrary to TMS evoked responses, muscle responses evoked by transcranial or brainstem electrical stimulations (TES) (Stefan et al., 2000; Ridding & Uy, 2003; Wolters et al., 2003) are not modified by PAS. The small number of subjects tested with electrical stimulation (1 to 3) is probably not the unique cause of such a discrepancy between the large PAS-induced change of H reflexes and the lack of effect on TES induced MEPs. As these responses are generated down stream to the cortex, any change in spinal excitability should be reflected on them.
References


Modulation by PAS of H reflexes and MEPs (% of control size)

A

B

C

Modulation by PAS of H reflexes and MEPs (% of control size)

- MEP
- H reflex
Modulation by PAS of F wave and MEP (% of their control values)