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TOPICAL REVIEW

Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects

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The ability to induce cortical plasticity with non-invasive brain stimulation (NBS) techniques has provided novel and exciting opportunities for examining the role of the human cortex during a variety of behaviours. Additionally, and importantly, the induction of lasting changes in cortical excitability can, under some conditions, reversibly modify behaviour and interact with normal learning. Such findings have driven a large number of recent studies examining whether by using such approaches it might be possible to induce functionally significant changes in patients with a large variety of neurological and psychiatric conditions including stroke, Parkinson's disease and depression. However, even in neurologically normal subjects the variability in the neurophysiological and behavioural response to such brain stimulation techniques is high. This variability at present limits the therapeutic usefulness of these techniques. The cause of this variability is multifactorial and to some degree still unknown. However, a number of factors that can influence the induction of plasticity have been identified. This review will summarise what is known about the causes of variability in healthy subjects and propose additional factors that are likely to be important determinants. A greater understanding of these determinants is critical for optimising the therapeutic applications of non-invasive brain stimulation techniques.

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Abbreviations BDNF, brain derived neurotrophic factor; cTBS, continuous theta burst stimulation; EEG, electroenchephalography; GABA, gamma-amino butyric acid; ISI, interstimulus interval; iTBS, intermittent theta burst stimulation; LTD, long term depression; LTP, long term potentiation; M1, primary motor cortex; MEP, motor evoked potential; NBS, non-invasive brain stimulation; NMDAR, *N*-methyl-D-aspartate receptor; PAS, paired associative stimulation; rTMS, repetitive transcranial magnetic stimulation; tACS, transcranial alternating current stimulation; TBS, theta burst stimulation; tDCS, transcranial direct current stimulation.

Plasticity can be defined as 'any experience dependent enduring change in neuronal or network properties either morphological or functional' (Donoghue *et al.* 1996). The ability to induce cortical plasticity with non-invasive brain stimulation (NBS) techniques has provided novel and exciting opportunities for examining the role of the human cortex during a variety of behaviours. Additionally, and importantly, the induction of lasting changes in cortical excitability can, under some conditions, reversibly modify behaviour and interact with normal learning. Such findings have been the driving force behind a large number of recent studies examining whether by using such approaches it might be possible to induce functionally significant changes in patients with a large variety of neurological and psychiatric conditions including stroke, Parkinson's disease and depression. However, even in neurologically normal subjects the variability in the neurophysiological and behavioural response to such brain stimulation techniques is high. This variability at present limits the therapeutic usefulness of these techniques. The cause of this variability is multifactorial and to some degree still unknown. However, a number of factors that can influence the induction of plasticity have been identified (Table 1). This review will summarise what is known about the causes of variability in healthy subjects and propose additional factors that are likely to be important determinants. A greater understanding of these determinants is critical for optimising the

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Table 1. Factors that may influence NBS-induced plasticity

Factor	Study	NBS	Protocol characteristics	Effect
Priming	Iyer *et al.* (2003)	rTMS	1 Hz rTMS primed with 6 Hz rTMS	↑ in LTD-like response
	Lang *et al.* (2004)	rTMS	5 Hz rTMS (SI: 100% AMT) primed with anodal and cathodal tDCS	↑ in M1 excitability when primed with cathodal tDCS and ↓ in M1 excitability when primed with anodal tDCS
	Siebner *et al.* (2004)	rTMS	1 Hz rTMS (SI: 90% RMT) primed with anodal and cathodal tDCS	↑ in M1 excitability when primed with cathodal tDCS and ↓ in M1 excitability when primed with anodal tDCS
	Nitsche *et al.* (2007)	$PAS_{25\,ms}$	21, 45 and 90 pulse pairs at 0.05 Hz; TMS SI: SI_{1mV}. Primed with anodal and cathodal tDCS	↑ in M1 excitability when primed with anodal tDCS and ↓ in M1 excitability when primed with cathodal tDCS
	Todd *et al.* (2009)	cTBS	cTBS (600 pulses) primed with either 2 or 6 Hz rTMS or iTBS (600 pulses)	↑ in M1 suppression when primed with iTBS
	Hamada *et al.* (2009)	QPS	QPS of M1 (ISI: 1.5, 5, 10, 30, 50 and 100 ms) primed with QPS of SMA (ISI: 5)	↓ in QPS-5 ms induced M1 facilitation and reversal of QPS-30 ms induced M1 inhibition to facilitation
	Hamada *et al.* (2009)	QPS	QPS of M1 (ISI: 1.5, 5, 10, 30, 50 and 100 ms) primed with QPS of SMA (ISI: 50 ms)	Reversal of QPS-5 and 10 ms induced M1 facilitation to inhibition, ↑ in QPS-30 ms induced M1 inhibition, ↓ in QPS-50 and 100 ms induced M1 inhibition
	Müller *et al.* (2007)	PAS_{N20+2}, PAS_{N20-5}, PAS_{random}	2 PAS protocols repeated. First PAS protocol was either PAS_{N20+2}, PAS_{N20-5}, PAS_{random}. Second PAS protocol was always PAS_{N20+2}.	Response to facilitatory PAS protocol ↓ when preceded by similar PAS protocol. Conversely, response to facilitatory PAS protocol ↑ when preceded by inhibitory PAS protocol.
	Ragert *et al.* (2009)	iTBS	iTBS (600 pulses) of left M1 primed with 1 Hz rTMS of right M1	↓ in LTP-like plasticity in left M1 when primed with 1 Hz rTMS over right M1
Prior voluntary motor activity	Stefan *et al.* (2006)	$PAS_{10\,ms} + PAS_{25\,ms}$	90 pulse pairs at 0.1 Hz; TMS SI: 130% RMT	↓ in LTP-like plasticity following motor training (no change in LTD-like plasticity)
	Rosenkranz *et al.* (2007)	$PAS_{10\,ms} + PAS_{25\,ms}$	200 pulse pairs at 0.25 Hz; TMS SI: SI_{1mV}	Reversal of M1 facilitation to inhibition when motor practise preceded stimulation
	Gentner *et al.* (2008)	cTBS	300 pulses at 70% RMT	Reversal of M1 facilitation to inhibition when voluntary isometric contraction preceded stimulation
	Ziemann *et al.* (2004)	$PAS_{N20} + PAS_{N20-5\,ms}$	200 pulse pairs at 0.25 Hz; TMS SI: SI_{1mV}	↑ in LTD-like plasticity and ↓ in LTP-like plasticity following repeated fast thumb abduction task
	Iezzi *et al.* 2008	TBS	cTBS (300 pulses) and iTBS (600 pulses) at 80% AMT	short period of phasic finger movements prior to TBS reversed effects seen following cTBS (to facilitation) and iTBS (to inhibition)

Table 1. continued

Factor	Study	NBS	Protocol characteristics	Effect
Parallel voluntary motor activity	Antal *et al.* (2007)	tDCS	1.0 mA cathodal and anodal stimulation of M1 for 10 min	↓ in M1 plasticity when subjects performed a motor task during stimulation
	Huang *et al.* (2008)	TBS	cTBS (300 pulses) and iTBS (600 pulses) at 80% AMT	↓ in M1 plasticity when subjects performed an isometric contraction during stimulation
Aerobic exercise	Cirillo *et al.* (2009)	$PAS_{25\,ms}$	90 pulse pairs at 0.05 Hz; TMS SI: 130% RMT	↑ in M1 plasticity in physically active individuals
Age	Müller-Dahlhaus *et al.* (2008)	$PAS_{N20+2\,ms}$	225 pulse pairs at 0.25 Hz; TMS SI: SI_{1mV}	↓ in M1 plasticity with age
	Tecchio *et al.* (2008)	$PAS_{25\,ms}$	150 pulse pairs at 0.2 Hz; TMS SI: SI_{1mV}	↓ in M1 plasticity with age in females only
	Fathi *et al.* (2010)	$PAS_{25\,ms}$	240 pulse pairs at 0.2 Hz; TMS SI: SI_{1mV}	↓ in M1 plasticity with age
	Todd *et al.* (2010)	rTMS	Intermittent, sub-threshold, 6 Hz rTMS	↓ in M1 plasticity with age
Attention	Stefan *et al.* (2004)	PAS	132 pulse pairs at 0.2 Hz; TMS SI: 130% RMT	↑ in M1 plasticity when subjects directed attention towards target hand
	Conte *et al.* (2007)	rTMS	10 trains of 10 stimuli at 5 Hz; SI of 120% RMT	↑ in M1 plasticity when subjects directed attention towards target hand
	Antal *et al.* (2007)	tDCS	1 mA cathodal and anodal stimulation of M1 for 10 min	↓ in M1 plasticity when subjects performed a cognitive task
	Conte *et al.* (2008)	rTMS	Trains of 10 stimuli at 5 Hz and 1 Hz; SI of 120% RMT	↑ in LTP (but not LTD) like M1 plasticity when subjects directed attention towards their target hand
Sex	Chaieb *et al.* (2008)	tDCS	1 mA cathodal and anodal stimulation of V1 for 7 or 10 min	↑ in V1 plasticity in females compared to males following anodal tDCS only
	Kuo *et al.* (2006)	tDCS	1 mA cathodal and anodal stimulation of M1	↑ in M1 plasticity in females compared to males following cathodal tDCS only
	Tecchio *et al.* (2008)	$PAS_{25\,ms}$	150 pulse pairs at 0.2 Hz; TMS SI: SI_{1mV}	↓ in M1 plasticity with age in females only
	Inghilleri *et al.* (2004)	rTMS	8 trains of 10 stimuli at 5 Hz; SI of 120% RMT	↓ in M1 plasticity on day 1 of menstrual cycle (low oestrogen)
	Fumagalli *et al.* (2010)	tDCS	2 mA anodal or cathodal over ventral prefrontal cortex or occipital cortex	In females only, tendency for cathodal prefrontal cortex stimulation to ↓ utilitarian response while anodal stimulation ↑ utilitatian responses
Pharmacological influences	Wolters *et al.* (2003)	$PAS_{10\,ms}$	90 pulse pairs at 0.05 Hz; TMS SI: 130% RMT	↓ in LTD-like plasticity in the presence of dextromethorphan
	Stefan *et al.* (2002)	$PAS_{25\,ms}$	90 pulse pairs at 0.05 Hz; TMS SI: SI_{1mV}	↓ in LTP-like plasticity in the presence of dextromethorphan
	Liebetanz *et al.* 2002	tDCS	1 mA cathodal and anodal stimulation of M1	↓ in LTP-like and LTD-like plasticity in the presence of dextromethorphan

Table 1. continued

Factor	Study	NBS	Protocol characteristics	Effect
	Nitsche *et al.* (2003)	tDCS	1 mA cathodal and anodal stimulation of M1	↓ in LTP-like and LTD-like plasticity in the presence of dextromethorphan
	Huang *et al.* (2007)	cTBS + iTBS	cTBS (300 pulses) and iTBS (600 pulses) at 80% AMT	↓ in LTP-like and LTD-like plasticity in the presence of dextromethorphan
	Nitsche *et al.* (2004*b*)	tDCS	1 mA cathodal and anodal stimulation of M1	↑ duration of the effect of anodal tDCS in presence of amphetamine
	Kuo *et al.* (2007)	tDCS + $PAS_{10\,ms}$ + $PAS_{25\,ms}$	1 mA cathodal (9 min) and anodal (13 min) tDCS. PAS: 90 pulse pairs at 0.05 Hz; TMS SI: SI_{1mV}	↑ in PAS-induced M1 plasticity in the presence of cholinesterase inhibitor rivastigmine.
	Kuo *et al.* (2008)	tDCS + $PAS_{25\,ms}$	1 mA cathodal (9 min) and anodal (13 min) tDCS. PAS: 90 pulse pairs at 0.05 Hz; TMS SI:??	↑ in PAS-induced M1 facilitation in the presence of the dopamine precursor levodopa
	Nitsche *et al.* (2009*a*)	tDCS + $PAS_{10\,ms}$ + $PAS_{25\,ms}$	1 mA cathodal (9 min) and anodal (13 min) tDCS. PAS: 90 pulse pairs at 0.05 Hz.	↓ in $PAS_{10\,ms}$ induced M1 plasticity in the presence of the D_2 antagonist sulpiride
	Monte-Silva *et al.* (2009)	tDCS + $PAS_{10\,ms}$ + $PAS_{25\,ms}$	1 mA cathodal (9 min) and anodal (13 min) tDCS. PAS: 90 pulse pairs at 0.05 Hz; TMS SI: SI_{1mV}	↓ in tDCS and $PAS_{25\,ms}$ induced plasticity in the presence of high or low doses of ropinirole (D_2/D_3 dopamine agonist)
	Ziemann *et al.* (1998)	rTMS	0.1 Hz rTMS (SI: 120% RMT) for ∼30 min in the presence of ischemic arm block	↓ in M1 facilitation in the presence of benzodiazepine lorazepam and lamotrigine
	Nitsche *et al.* (2004*b*)	tDCS	1 mA cathodal (9 min) and anodal (13 min) tDCS	↑ in M1 facilitation following anodal tDCS in the presence of D-cycloserine
	Nitsche *et al.* (2006)	tDCS	1 mA cathodal (9 min) and anodal (13 min) tDCS	↓ in M1 plasticity in the presence of sulpiride; ↑ in M1 suppression in the presence of pergolide
	McDonnell *et al.* (2007)	$PAS_{N20+2\,ms}$	225 pulse pairs at 0.25 Hz, TMS SI: SI_{1mV}	↓ in LTP-like M1 plasticity in the presence of $GABA_B$ receptor agonist baclofen
	Lang *et al.* (2008)	rTMS	1 Hz rTMS for 20 min, SI of 90% RMT	↑ in LTD-like M1 plasticity in the presence of D_1/D_2 receptor agonist pergolide
	Swayne *et al.* (2009)	iTBS	600 pulses at 80% AMT	↑ in M1 facilitation in the presence of nicotine
	Nitsche *et al.* (2009*b*)	tDCS	1 mA cathodal (9 min) and anodal (13 min) tDCS	↑ in M1 facilitation following anodal and cathodal tDCS in the presence of serotonin reuptake blocker citalopram
	Ziemann *et al.* 2002	rTMS	0.1 Hz rTMS (SI: 120% RMT) for ∼30 min in the presence of ischemic arm block	d-amphetamine ↑ M1 facilitation with nerve block alone, but ↓M1 LTP-like response to rTMS + nerve block
Genetics	Cheeran *et al.* (2008)	TBS + $PAS_{25\,ms}$	cTBS (300 pulses) and iTBS (600 pulses) at 80% AMT. PAS: 200 pulse pairs at 0.25 Hz; TMS SI: SI_{1mV}	↓ in TBS and PAS-induced M1 plasticity in individuals with the BDNF polymorphism Val66Met

Table 1. continued

Factor	Study	NBS	Protocol characteristics	Effect
Time of day	Sale *et al.* (2007)	$PAS_{25\,ms}$	132 pulse pairs at 0.2 Hz; TMS SI: 130% RMT (short) or 90 pulse pairs at 0.05 Hz; TMS SI: SI_{1mV} (long)	↑ in M1 plasticity in the afternoon compared with morning
	Sale *et al.* (2008)	$PAS_{25\,ms}$	90 pulse pairs at 0.05 Hz; TMS SI: SI_{1mV}	↑ in M1 plasticity in the afternoon when endogenous cortisol levels are low
	Marshall *et al.* (2004)	tDCS	Bilateral frontal anodal tDCS at 0.26 mA cm^{-2} 15 s on/15 s off for 30 min	Stimulation during slow wave sleep increased retention of declarative memory. Stimulation during the wake retention interval did not affect retention.

Abbreviations: SI_{1mV}, stimulus intensity producing MEPs of ∼1 mV; $PAS_{x\,ms}$, interval between peripheral nerve and M1 stimulation equal to x ms; PAS_{N20+2}, interval between peripheral nerve and M1 stimulation equal to somatosensory evoked potential N20 component latency + 2 ms; PAS_{N20-5}, interval between peripheral nerve and M1 stimulation equal to somatosensory evoked potential N20 component latency – 5 ms; PAS_{random}, interval between peripheral nerve and M1 stimulation randomised between PAS_{N20+2} and PAS_{N20-5}; LDLPFC, left dorsolateral prefrontal cortex; V1, primary visual cortex; QPS, quadripulse stimulation; SMA, supplementary motor area.

therapeutic applications of non-invasive brain stimulation techniques.

Commonly employed NBS techniques

Before we describe factors which are known to influence the induction of plasticity by NBS techniques we will firstly briefly outline the commonly applied forms of NBS.

Repetitive transcranial magnetic stimulation (rTMS) techniques involve applying regular trains of transcranial magnetic stimuli over the target cortical region. In general, low frequency (<1 Hz) trains reduce cortical excitability and higher frequency (>5 Hz) trains increase cortical excitability (see Ridding & Rothwell, 2007). However, the response is dictated by a complex interaction between stimulus frequency, intensity and duration. It should be noted here that changes in cortical excitability (as indexed by changes in the amplitude of peripherally recorded motor evoked potentials (MEPs)) are commonly used as a marker of synaptic plasticity in human neurophysiological studies. More recently, new rTMS approaches have been developed which involve the application of high frequency bursts of stimuli at theta frequencies, so called theta burst stimulation (TBS). The temporal pattern in which these bursts are applied determines whether the protocols are facilitatory (intermittent TBS; iTBS) or depressant (continuous TBS; cTBS) (Huang *et al.* 2005).

Paired associative stimulation (PAS) involves applying pairs of peripheral and central stimuli repeatedly (Stefan *et al.* 2000). The peripheral stimulus normally consists of an electrical pulse applied to the median nerve at the wrist and the central stimulus consists of a single TMS pulse applied over the cortical region of interest (usually the motor cortex). Approximately 100 paired stimuli are applied over about 30 min and the outcome is determined by the interval between the peripheral and central stimuli. At an interstimulus interval (ISI) of 25 ms (PAS_{25}) the intervention increases cortical excitability while at an ISI of 10 ms (PAS10) a decrease in excitability is seen (Stefan *et al.* 2000; Wolters *et al.* 2003).

It has been known for a long time that direct current stimulation can produce long lasting excitability changes in the nervous systems of animals. These findings were the driver of more recent development of transcranial direct current stimulation (tDCS) approaches for neurophysiological investigation in human subjects (Priori *et al.* 1998). tDCS involves applying small (approx. 1 mA) direct currents to the scalp with pad electrodes. The outcome is determined by the placement of the anodal and cathodal stimulation pads (Nitsche & Paulus, 2000). For example, placement of the anode over the motor cortex (anodal tDCS) and the cathode over the contralateral orbit produces a lasting increase in motor cortical excitability when the current is applied for approximately 10 min. In contrast, when the electrodes are reversed (cathodal tDCS), a decrease in motor cortical excitability is seen. Both rTMS and tDCS techniques are considered safe if guidelines are adhered to (Nitsche *et al.* 2008; Priori *et al.* 2009). However, rare seizure induction has been reported following rTMS. Given this, and the simplicity and low cost, it may be that tDCS will be more extensively used for clinical applications (Priori *et al.* 2009).

Although these techniques exert their effects through different mechanisms it is likely that the changes induced in the cortex are, in many ways, similar. For example,

the changes induced by all techniques are reversible and last from a few minutes up to about 1 h. In addition, the changes are all dependent upon NMDA receptor function. There is compelling evidence that the effects of these NBS techniques are largely due to long term potentiation (LTP)-like and long term depression (LTD)-like mechanisms (see Cooke & Bliss 2006; Thickbroom, 2007; Wagner *et al.* 2007; Ziemann *et al.* 2008). Most of the evidence regarding the mechanisms responsible for NBS induced plasticity is indirect. However, a very recent study (Fritsch *et al.* 2010) used a mouse motor cortex slice preparation to study the response to anodal tDCS. In this important study it was found that tDCS, when combined with low frequency stimulation, induced lasting NMDA receptor dependent synaptic potentiation. Interestingly, in addition this form of stimulation enhanced, and was dependent upon, BDNF secretion and TrkB activation. The significance of these results for our understanding of the mechanisms responsible for the excitability changes seen following tDCS in relaxed human subjects is not clear but might be more directly relevant for studies combining tDCS with training.

History of synaptic activity

Although no single factor which might influence plasticity induction has been studied extensively perhaps the best studied to date is the influence of the history of synaptic activity within a stimulated cortical region. It is well known from animal experiments that the history of synaptic activity in a targeted brain region can influence the subsequent response to a period of stimulation designed to induce LTP or LTD. For example, the reliable induction of associative LTD within the dentate gyrus of the rat can be significantly improved by theta frequency priming stimulation (Christie & Abraham, 1992). Of note, these authors demonstrated that the priming effects were specific to theta frequency stimulation and it may be that activity at this frequency has a particular role in facilitating changes in synaptic efficacy. Such findings have led to the development of the theory of homeostatic metaplasticity. This theory, for which there is considerable supporting evidence, proposes that the threshold for LTP/LTD induction is flexible and dependent on the recent history of postsynaptic activity: high activity increases LTP threshold and, concomitantly, decreases LTD threshold whereas low activity has the opposite effects (for review, see Abraham, 2008).

These results have promoted the investigation of priming effects in human subjects using non-invasive brain stimulation techniques. Iyer *et al.* (2003) were the first to demonstrate that similar priming effects could be observed in the human motor cortex. That study showed that 10 min of priming stimulation, in the form of subthreshold (below motor threshold) 6 Hz repetitive transcranial magnetic stimulation (rTMS), increased the LTD-like response to a subsequent period of depressant 1 Hz rTMS. The LTD-like response was measured as a long-term (>60 min) depression of motor evoked potential (MEP) amplitude. At variance with the LTD/LTP studies in animal experiments, it remained untested though to which extent the 6 Hz rTMS priming itself altered motor cortical excitability.

More recently, Todd *et al.* (2009) examined the potential of a number of priming protocols for modulating the response to a subsequent period of rTMS. In this study continuous theta burst stimulation (cTBS), which by itself typically induces a LTD-like depression of MEP amplitude (Huang *et al.* 2005), was primed either with 10 min of intermittent 2 Hz or 6 Hz rTMS or with intermittent theta burst stimulation (iTBS). Neither 2 Hz nor 6 Hz priming stimulation influenced the subsequent response to cTBS. However, when cTBS was primed with iTBS there was a significantly stronger and longer lasting LTD-like response compared to when cTBS was applied following sham priming stimulation.

Priming effects have also been examined using repeated paired associative stimulation (PAS) protocols. The LTP-like response to a facilitatory PAS protocol was decreased compared to control when preceded by a period of similar facilitatory PAS (Müller *et al.* 2007). Conversely, when preceded by a PAS protocol that induces a LTD-like MEP decrease the LTP-like response to the subsequent facilitatory PAS protocol was increased. The response to the second PAS protocol correlated in a linear and negative manner with the response to the first, priming, PAS protocol, i.e. a strong LTP-like priming effect was particularly effective in suppressing the LTP-like response of the second PAS protocol. The results from the studies by Todd *et al.* (2009) and Müller *et al.* (2007) are in accordance with the concept of homeostatic metaplasticity.

Several studies have employed tDCS to prime the motor cortex prior to stimulation with rTMS. Siebner *et al.* (2004) demonstrated that the response to a 15 min period of 1 Hz rTMS was modulated bi-directionally by tDCS. Anodal tDCS increased the depressant response to 1 Hz rTMS while when primed with cathodal tDCS an increase in MEP amplitude was seen. In a similar study, Lang *et al.* (2004) examined the effect of anodal *vs.* cathodal priming tDCS on a weak facilitatory 5 Hz rTMS protocol. The priming stimulation consisted of a 10 min period of either anodal or cathodal tDCS to the motor cortex prior to a 20 s train of 5 Hz rTMS. Five-hertz rTMS applied following sham stimulation failed to induce changes in MEP amplitude. However, following cathodal priming stimulation, 5 Hz rTMS induced a significant MEP increase above baseline levels, while following anodal priming stimulation, 5 Hz rTMS resulted in a significant

MEP decrease. Both of these studies are consistent with homeostatic control mechanisms and are examples of metaplasticity.

The effects of priming facilitatory PAS with transcranial direct current stimulation (tDCS) have also been examined (Nitsche *et al.* 2007). Anodal tDCS of motor cortex typically induces an LTP-like increase in MEP amplitude while cathodal tDCS induces an LTD-like decrease (Nitsche & Paulus, 2001; Nitsche *et al.* 2003). The LTP-like response to PAS was facilitated when primed by a period of prior anodal tDCS and reduced when primed by a period of cathodal tDCS. Therefore, these outcomes are not characteristic of a homeostatic response pattern. However, when the PAS and tDCS were administered simultaneously a different pattern of response was seen. In this case, anodal tDCS reduced the LTP-like response to PAS and cathodal tDCS increased it. Therefore, when administered simultaneously the response pattern was consistent with a homeostatic process. The reasons for this difference are currently not clear but the findings of Nitsche and colleagues (2007) point out that the delay between two interacting NBS protocols plays an important role in determining magnitude and direction of the interaction. This warrants more systematic exploration in future experiments.

It is also possible to bias the response to a NBS plasticity protocol by modifying excitability in a distant but connected brain area prior to NBS. For example, a period of priming stimulation applied to the primary motor cortex (M1) of one hemisphere can influence the response to a subsequent rTMS protocol applied to the contralateral M1. Ragert *et al.* (2009) applied 1 Hz rTMS to the right M1. This resulted in a decrease of excitability in the stimulated right M1, and concomitantly, in an increase of excitability in the non-stimulated left M1. This in turn reduced the normal facilitatory effect induced by iTBS applied to the left M1. Also, the application of priming stimulation to the supplementary motor area has been shown to modify the response to a subsequent period of NBS applied to M1. Hamada *et al.* (2009) used a recently developed quadripulse rTMS stimulation protocol for both priming and test stimulation. Various intervals between the stimuli of a quadripulse were investigated to examine both facilitatory and depressant effects. The results were generally consistent with homeostatic metaplasticity rules, with for example priming stimulation over the supplementary area at a facilitatory interval of 5 ms reducing the facilitatory response to test stimuli over M1 at the same facilitatory timing.

It is possible to change the history of synaptic activity within a cortical region by engageing that region behaviourally. For example, performing a voluntary contraction influences synaptic activity within M1. This can be thought of as behavioural priming and several studies have examined the response to NBS techniques following a period of motor activity. Ziemann *et al.* (2004) demonstrated that a period of motor training (ballistic thumb abductions), which resulted in skill acquisition, modified the response to a subsequent PAS protocol. The motor training increased the response to a PAS protocol designed to induce LTD-like plasticity and, in contrast, reduced the response to a PAS protocol designed to induce LTP-like plasticity. Similar results have been described by in a more recent study (Stefan *et al.* 2006) where it was shown that training on a dynamic submaximal isometric motor task blocked the response to a subsequent PAS protocol (PAS25) which under control conditions produces an LTP-like MEP facilitation. In this study the motor training did not influence the response to a PAS protocol (PAS10) which under control conditions reduces excitability. Rosenkranz *et al.* (2007) studied the effect of both short term and longer term training on PAS plasticity. Subjects were trained for five consecutive days on a motor training task consisting of rapid thumb abductions. After the training on day 1 the normal LTP-like response seen with the PAS25 protocol was reversed to inhibition. However, in contrast to this, PAS plasticity was no longer influenced following training on day 5. The authors proposed that by day 5 new synapses might have been formed which allowed PAS susceptibility to be restored.

Gentner *et al.* (2008) demonstrated that a brief tonic voluntary contraction could influence the response to a short period of 300 pulses of continuous theta burst stimulation (cTBS300). When applied without a prior contraction cTBS300 facilitated MEPs. However, when preceded by a contraction the facilitation was replaced by a MEP depression. Of note, even without activation but when the duration of the cTBS was doubled (cTBS600) the facilitation seen with cTBS300 was reversed into a MEP depression. Iezzi and colleagues (2008) demonstrated that phasic movements can also influence the response to TBS. Without prior phasic movements, cTBS elicited inhibitory, and iTBS elicited facilitatory after-effects on MEPs. However, following a short period of phasic finger movements the after-effects of both cTBS and iTBS were reversed. These studies demonstrate that prior behavioural engagement of a cortical region can influence in a homeostatic manner the response to a subsequent period of NBS. In addition, longer trains of stimuli can, in themselves, exert homeostatic influences on the outcome.

The outcome of NBS can also be modified when it is applied to the cortex *during* behavioural engagement of the stimulated region of cortex. Huang *et al.* (2008) reported that both LTP-like and LTD-like plasticity induced by TBS protocols were reduced when stimulation was applied to the motor cortex during voluntary activation of the contralateral hand. Further evidence that behavioural engagement *during* NBS can influence the response comes from a study examining the effect of motor

task performance on plasticity induction with anodal and cathodal tDCS (Antal *et al.* 2007). The motor task resulted in a switch of the normal LTP-like effect induced by anodal tDCS to an LTD-like effect, while the normal LTD-like effect induced by cathodal tDCS was enhanced (Antal *et al.* 2007).

In summary, these studies provide convergent evidence that the magnitude and direction of the response to NBS plasticity protocols depend on the history of activation and the current state of the stimulated cortex. These effects may be utilized purposefully to shape and optimise the response to NBS.

Regular exercise/activity

There is now good evidence that regular aerobic exercise can modify plasticity (for review see Kramer & Erickson, 2007) and improve learning and memory in both animals and humans (see van Praag, 2009). The reasons for this are thought to be multifactorial but likely include increased cerebral blood flow (Xiong *et al.* 2009), angiogenesis (Swain *et al.* 2003), as well as an increase in neurotrophic factors (Klintsova *et al.* 2004). In accord, Cirillo *et al.* (2009) recently demonstrated that the response to an LTP-like PAS protocol was significantly greater in highly active individuals than in sedentary individuals. The mechanisms for this enhancement are not known at present but may include several of the above.

Influence of age

It is well recognised that ageing is associated with impairments in learning and memory. Additionally, ageing is also associated with an altered capacity for processes important for synaptic plasticity such as LTP (for review see Barnes, 2003). There is some evidence that the capacity for NBS induced plasticity declines with age in both healthy and neurologically impaired subjects. A number of studies have employed the technique of PAS to investigate age-dependent effects on plasticity induction. For example, the magnitude of PAS induced plasticity in motor cortex is larger for young than for older subjects (Müller-Dahlhaus *et al.* 2008). Similarly, Fathi *et al.* (2010) used facilitatory PAS to study the effect of age in three groups of healthy subjects. While there was a significant LTP-like response to PAS in both the young and middle aged subjects, elderly subjects (aged 60–79 years) did not respond (Fathi *et al.* 2010). Tecchio *et al.* (2008) also used an LTP-like plasticity inducing PAS protocol to examine age related changes in plasticity. However, in this case there was only evidence for an influence of age on the response in females, with there being reduced plasticity in the older, post-menopausal, females.

Further evidence that ageing might be associated with a reduced capacity for motor cortical plasticity has been provided by several studies using various rTMS protocols. Todd *et al.* (2010) recently reported that the response to an excitability depressant subthreshold 6 Hz rTMS protocol is reduced in healthy aged adults.

Attention

The attentional focus of subjects has been shown to influence the magnitude of experimentally induced plasticity. This may be especially important in protocols involving longer trains of stimuli, such as during PAS. Stefan *et al.* (2004) demonstrated that when subjects directed their attention to the target hand, the amount of plasticity induced by PAS in the contralateral motor cortex was greater than that induced when attention was directed to the non-target hand or the subjects attention was diverted by a complex cognitive task. The effects of a similar attentional protocol have been explored using short trains of facilitatory 5 Hz rTMS (Conte *et al.* 2007, 2008). These authors demonstrated that the MEP facilitation seen during and after the rTMS train was larger when subjects directed their attention to the target hand. Effects of attentional focus have also been demonstrated using tDCS (Antal *et al.* 2007). When subjects engaged in a cognitive task during tDCS of the motor cortex both the LTP-like response to anodal stimulation and the LTD-like response to cathodal stimulation were reduced. The authors proposed that this might be due to cortical areas not involved in the cognitive task being deactivated, which might interfere with neuroplasticity processes.

Sex

There is good evidence from animal experiments that there are significant sex differences in processes important for cortical plasticity (McEwen, 1994; Galea *et al.* 2006). Therefore, sex constitutes a potentially important determinant of NBS induced plasticity although to date there are only a few studies examining this issue. In a retrospective examination of data collected from their previous studies Chaieb *et al.* (2008) examined the influence of sex on the response of the visual cortex to tDCS. A number of cortical excitability measures (visual evoked potentials, phosphene threshold and contrast measurement) were used. There were no sex effects on the response 10 min following cathodal tDCS but following anodal tDCS there was a significantly greater facilitatory effect in the females.

In another retrospective study Kuo *et al.* (2006) examined the response of males and females to tDCS applied over the motor cortex. They reported that, in the female subjects, the LTD-like response to cathodal tDCS

was prolonged compared to the males. In addition, using a short tDCS stimulation protocol that evoked no significant effects the female subjects demonstrated more MEP depression. In contrast, there was no significant difference between male and female subjects with facilitatory, anodal tDCS.

There is some behavioural evidence that there may be sex differences in the response to NBS in non-motor-cortical areas. Fumagalli and colleagues (2010) recently applied tDCS to the ventral prefrontal cortex while subjects performed a task involving utilitarian decision making. Cathodal tDCS tended to decrease utilitarian responses, and anodal tDCS significantly increased utilitarian responses, but only in females.

These findings support the notion that there may be sex differences in the response to NBS with females being somewhat more responsive. However, the nature of the effects may be influenced by the protocol employed and the brain region targeted for stimulation. In addition, the response of female subjects may be influenced by their hormonal status. There is emerging evidence that female sex hormones can exert significant influence on plasticity induction with NBS techniques. For example, short-term plasticity induced by 5 Hz rTMS is less on day 1 of the menstrual cycle (low oestrogen) compared to day 14 of the menstrual cycle (high oestrogen) (Inghilleri *et al.* 2004).

Pharmacological influences

Cortical synaptic plasticity can be significantly influenced by central nervous system active drugs (for reviews see, Gu, 2002; Möhler, 2006). Accordingly, numerous studies examined the influence of a large variety of neuropharmacological agents on the plasticity response to NBS techniques (for review, Ziemann *et al.* 2006). The extent and direction of NBS induced plasticity can be highly significantly modulated by many neuropharmacological agents.

Many forms of long-term cortical synaptic plasticity are dependent on NMDA receptor (NMDAR) activation. Accordingly, the NMDAR antagonist dextromethorphan blocks the induction of LTP-like and LTD-like plasticity induced with the PAS protocol (Stefan *et al.* 2002; Wolters *et al.* 2003) as well as the LTP-like and LTD-like effects seen following both anodal and cathodal tDCS (Liebetanz *et al.* 2002; Nitsche *et al.* 2003). Similarly, the LTP-like and LTD-like effects induced by iTBS and cTBS are both blocked by the administration of the NMDAR antagonist memantine (Huang *et al.* 2007). In contrast, D-cycloserine, a partial NMDAR agonist, prolonged the effect of anodal tDCS but had little effect on cathodal tDCS (Nitsche *et al.* 2004*b*).

The inhibitory neurotransmitter γ-amino butyric acid (GABA) is also of substantial importance for controlling plasticity induction. The benzodiazepine diazepam, a positive allosteric modulator at the $GABA_A$ receptor, and tiagabine, a GABA reuptake inhibitor, blocked LTP-like plasticity induced by a PAS protocol (U. Ziemann, unpublished observations). The $GABA_B$ receptor antagonist baclofen also reduced LTP-like plasticity induced with PAS (McDonnell *et al.* 2007). It was proposed that this effect is driven by an increase in inhibitory postsynaptic activity. It is possible to reduce the level of $GABA_A$ inhibition in the cortex by using a temporary peripheral ischaemic nerve block (Ziemann *et al.* 1998). Under these conditions of a disinhibited motor cortex, a low frequency 0.1 Hz rTMS protocol, which on its own has no overt effect on motor cortical excitability, induced an LTP-like increase in MEP amplitude (Ziemann *et al.* 1998). This effect was blocked by pre-treatment with the benzodiazepine lorazepam, which acts as a positive allosteric modulator of $GABA_A$ receptors.

Neuromodulating neurotransmitters such as dopamine, acetylcholine, noradrenaline and serotonin can modify various forms of NBS induced plasticity (Nitsche *et al.* 2006; Ziemann *et al.* 1998). Blocking the activity of dopamine D2 receptors by the application of sulpiride almost completely abolished the LTP-like and LTD-like responses to both anodal and cathodal tDCS (Nitsche *et al.* 2006). Further, when the dopamine D1/D2 receptor agonist pergolide was co-administered with sulpiride to examine the role of D1 receptors, the sulpiride-induced blockade of tDCS-induced plasticity did not recover. Pergolide alone led to a marked lengthening of the MEP depressant effect of cathodal tDCS. These findings supported the notion that D2 receptor activation has a consolidation-enhancing effect on tDCS-induced changes of excitability in the human cortex, and underscore the importance of the dopaminergic system and of the balance between D1 and D2 receptor activity for human neuroplasticity (cf. also Nitsche *et al.* 2009*a*). Pergolide also increased magnitude and duration of the LTD-like response induced by 1 Hz rTMS (Lang *et al.* 2008).

The influence of dopamine on plasticity induction was examined further using the dopamine precursor, L-dopa, which was administered prior to both tDCS and PAS protocols (Kuo *et al.* 2008). L-Dopa reversed the LTP-like enhancement of MEP amplitude seen with anodal tDCS to a LTD-like effect but prolonged the LTD-like effect induced by cathodal tDCS. Conversely, the effects of facilitatory PAS were enhanced and prolonged when L-dopa had been administered. The authors hypothesized that the differences of dopaminergic modulation between tDCS *vs.* PAS-induced plasticity are explained by dopamine focusing synapse-specific excitability-enhancing neuroplasticity (induced by PAS) rather than non-specific plasticity (induced by tDCS) in human cortical networks (Kuo *et al.* 2008). Also, Monte-Silva *et al.* (2009) examined the influence of dopamine on plasticity induced by anodal

and cathodal tDCS and facilitatory and depressant PAS. The influence of different dosages of the dopamine D2/D3 receptor agonist ropinirole was investigated. They found a dose-dependent effect on the response to anodal and cathodal tDCS and facilitatory PAS. At both lower and higher dosages the response to stimulation was reduced. In contrast, there was no effect on depressant PAS. This study is important because the dose–response relationship was investigated for the first time. The findings suggest that modulation of dopamine D2 receptor activity exerts dose-dependent suppressive or facilitatory effects on neuroplasticity in the human motor cortex consistent with an inverted U-shaped dose–response curve.

The influence of acetylcholine on experimental plasticity induction has been investigated by examining the influence of the acetylcholine esterase inhibitor rivastigmine (Kuo *et al.* 2007). In the presence of rivastigmine, the LTP-like response to anodal tDCS was blocked and there was a tendency for a reduced LTD-like response to cathodal tDCS. In contrast, the LTP-like response to facilitatory PAS was increased and the LTD-like response to depressant PAS was both stronger and longer lasting. The authors proposed that these varying effects are due to the nature of the two protocols. PAS induces changes in a more specific set of synapses than tDCS which has somewhat generalised and non-specific effects. It is proposed further that cholinergic mechanisms are important for optimising the detection of afferent signals during information processing. Facilitation of synapse specific plasticity (such as induced by PAS) would be compatible with such a role. Further investigation of cholinergic modulation of plasticity induction by NBS has been reported by Swayne *et al.* (2009). In this study the influence of nicotine on LTP-like plasticity induction by iTBS was investigated. Pre-treatment with nicotine resulted in a significant enhancement in the facilitatory effect to iTBS, in terms of both magnitude an duration.

The effects of the indirect adrenergic drug *d*-amphetamine on plasticity induction has been studied using a model of ischaemic nerve block-induced temporary peripheral deafferentation in combination with low-frequency 0.1 Hz rTMS (Ziemann *et al.* 2002). While *d*-amphetamine enhanced the short-lasting increase in MEP amplitude induced by the ischaemic nerve block alone, it suppressed the LTP-like response seen when the ischaemic nerve block was combined with low-frequency rTMS. The authors suggested that this depressive effect of *d*-amphetamine is compatible with studies in animal preparations where it favoured LTD over LTP. Conversely, *d*-amphetamine enhanced and prolonged the LTP-like plasticity induced by anodal tDCS (Nitsche *et al.* 2004*a*). Additionally, administration of propanolol, a β-adrenergic antagonist, diminished the duration of the tDCS induced after effects. The authors suggested that this is evidence that adrenergic receptors play a role in the consolidation of the plasticity seen with this induction protocol.

Finally, one study has investigated the effects of serotonergic modulation on NBS-induced plasticity. The selective serotonin reuptake inhibitor citalopram resulted in an enhancement of magnitude and duration of the LTP-like effect induced by anodal tDCS, whereas the LTD-like plasticity induced by cathodal tDCS was reversed to an LTP-like effect, thereby favouring facilitatory plasticity effects in the human brain (Nitsche *et al.* 2009*b*).

It needs to be borne in mind that all of the studies quoted here have manipulated levels of neuromodulators/neurotransmitters in healthy subjects. It is important to consider that different effects may be seen when pharmacological interventions are applied to patient populations.

There are a number of other factors that have been shown, in a small number of studies, to significantly influence the response to non-invasive brain stimulation protocols.

Genetics

Neurotrophins are key molecules in the activity-dependent modulation of local protein synthesis in neuronal dendrites to consolidate long-term changes in synapse structure and functional efficacy (Bramham, 2008). Along this line, it became clear very recently that genetic polymorphisms of neurotrophins significantly influence the induction of plasticity with NBS in human cortex. The most studied genetic influence so far is that of a common single nucleotide polymorphism of the brain derived neurotrophic factor (BDNF) gene. BDNF is released in an activity dependant manner and has a significant role in promoting changes in synaptic efficacy (Lu, 2003; Bramham, 2008). A significant percentage of the population have a single nucleotide polymorphism in the BDNF gene that leads to an amino acid substitution (valine to methionine) at codon 66 (val66met). In a pioneering study, Kleim *et al.* (2006) demonstrated that neurophysiological changes typically associated with a simple motor learning task (increase in MEP amplitude and motor map of the trained representation) are reduced in human subjects with the val66met polymorphism. These findings strongly support the notion that BDNF is involved in mediating experience-dependent plasticity of human motor cortex. More recently, it was shown that the changes induced by several NBS techniques are likewise influenced by this genetic variation (Cheeran *et al.* 2008). Subjects with the val66met polymorphism of the BDNF gene had a reduced or absent response to both facilitatory and depressant TBS. With the technique of PAS there were differences in the specificity of the response. While there was a similar PAS induced LTP-like facilitation when

investigated for the target muscle, there was significantly less spread to non-target muscles. Finally, val66met carriers also had reduced homeostatic metaplasticity tested with the cathodal tDCS/1 Hz rTMS model.

Time of day

The plasticity response to a given stimulation protocol is significantly regulated by circadian rhythms, as has been shown, for instance, in mouse hippocampus (Chaudhury *et al.* 2005). Along this line, Sale *et al.* (2007) demonstrated that the response to PAS in human motor cortex is significantly influenced by the time of day at which it is applied. Subjects were tested with either a short (132 paired stimuli at 0.2 Hz) or a long (90 paired stimuli at 0.05 Hz) facilitatory PAS protocol on three occasions. With both protocols, there was significantly more PAS induced plasticity in the afternoon than in the morning. In a follow-up study this finding was replicated and it was shown that at least some of this effect was due to diurnal variations in cortisol levels (Sale *et al.* 2008).

Slow wave sleep is of key importance for the consolidation of plasticity and memory. Application of anodal tDCS over prefrontal cortex enhanced consolidation of declarative memory specifically when applied during slow wave sleep but not when applied during the wake retention interval (Marshall *et al.* 2004). Procedural memory was not affected. The memory enhancing effect was associated with a tDCS induced increase in slow oscillatory electroencephalographic (EEG) activity considered to facilitate processes of neuronal plasticity.

Endogenous brain oscillations

There is compelling evidence that slow oscillatory EEG activity (<1 Hz) predominantly originating in prefrontal cortex during sleep contributes to the long-term consolidation of new memories. Inducing slow oscillation-like potential fields by low-frequency (0.75 Hz) transcranial alternating current stimulation (tACS) during early nocturnal slow wave sleep enhanced the retention of hippocampus-dependent declarative memories in healthy

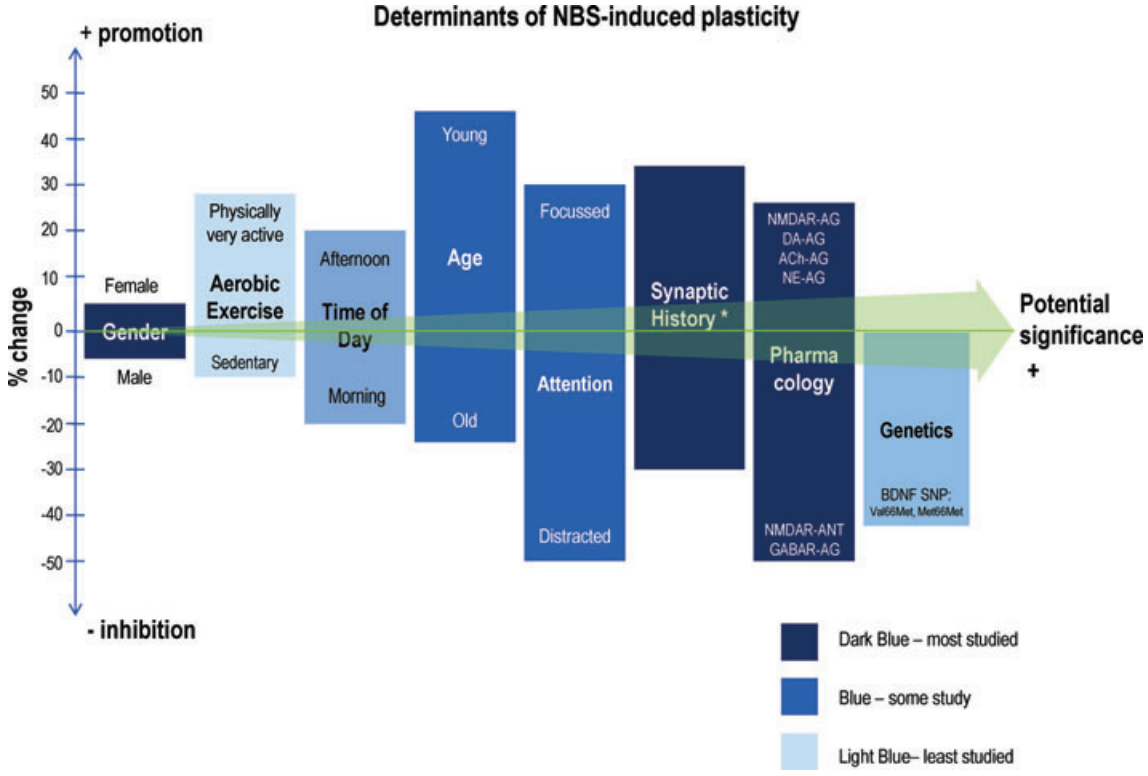

Figure 1. Schematic diagram demonstrating relative magnitude (*y*-axis) and potential significance (*x*-axis) of determinants on neuroplasticity induction by NBS with some examples indicated
Potential significance is a somewhat subjective estimation of the authors of the future potential of the various determinants to play a role in purposefully modulating direction and magnitude of NBS-induced plasticity. Zero (*y*-axis) represents the level of NBS-induced plasticity that would be expected in a random adult subject group. Colours of bars (see inserted legend) indicate the level of evidence from the number and consistency of the available studies. *Note that with one form of NBS (QPS), significantly larger priming effects have been reported in one study. Abbreviations: AG, agonist; ANT, antagonist; DA, dopamine; Ach, acetylcholine; NE, noradrenaline.

humans (Marshall *et al.* 2006). The tACS protocol induced an immediate increase in slow wave sleep, endogenous cortical slow oscillations and slow spindle activity in the frontal cortex. In contrast, higher frequency (5 Hz) tACS decreased slow oscillations but left declarative memory unchanged. These findings indicate for the first time that endogenous slow potential oscillations have a causal role in the sleep-associated consolidation of memory, and that this role can be purposefully up-regulated by enhancing these endogenous brain oscillations with a frequency-matching tACS protocol. These findings also suggest, given that NBS can influence endogenous brain oscillatory activity, that brain oscillations may in turn influence the response to NBS protocols.

Basic neuroscience research in hippocampal slices indicated that the direction of long-term synaptic plasticity is significantly influenced by the timing of stimulation: LTP was induced when given during the peaks of cholinergically induced theta oscillations, but LTD of previously potentiated synapses occurred when applied during the troughs (Huerta & Lisman, 1995). These results suggest that the similar bursts observed during theta rhythm *in vivo* may be a natural stimulus for inducing LTP/LTD. However, this hypothesis still awaits confirmation in humans by using EEG triggered NBS protocols.

Summary

In this review we have shown that a multitude of determinants influence the magnitude and direction of NBS induced plasticity of the human brain (Table 1). These factors play an important role in explaining the known substantial inter-individual variability of the NBS response (Fig. 1). Also, it is important to consider that a number of these factors (e.g. genetic profile and age) may interact with each other resulting in a complex multifactorial influence on neuroplasticity induction. Taking into account these determinants will help to predict better an individual plasticity response. In addition, the fascinating perspective is raised that one can make use of several of these determinants to sculpt the NBS response, for instance to purposefully enhance therapeutic NBS efficacy.

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