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Review article

Variability in non-invasive brain stimulation studies: Reasons and results

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ABSTRACT

Non-invasive brain stimulation techniques (NIBS), such as Theta Burst Stimulation (TBS), Paired Associative Stimulation (PAS) and transcranial Direct Current Stimulation (tDCS), are widely used to probe plasticity in the human motor cortex (M1). Although TBS, PAS and tDCS differ in terms of physiological mechanisms responsible for experimentally-induced cortical plasticity, they all share the ability to elicit long-term potentiation (LTP) and depression (LTD) in M1. However, NIBS techniques are all affected by relevant variability in intra- and inter-subject responses. A growing number of factors contributing to NIBS variability have been recently identified and reported. In this review, we have readdressed the issue of variability in human NIBS studies. We have first briefly discussed the physiological mechanisms responsible for TBS, PAS and tDCS-induced cortical plasticity. Then, we have provided statistical measures of intra- and inter-subject variability, as calculated in previous studies. Finally, we have reported in detail known sources of variability by categorizing them into physiological, technical and statistical factors. Improving knowledge about sources of variability could lead to relevant advances in designing new tailored NIBS protocols in physiological and pathological conditions.

1. Introduction

Over the last two decades, an increasing number of researchers have applied a variety of non-invasive brain stimulation (NIBS) techniques to probe plasticity processes in the human primary motor cortex (M1). In NIBS studies, plasticity commonly refers to changes in cortical excitability, assessed by measures such as motor evoked potential (MEP) amplitudes, outlasting brain stimulation by minutes. Less frequently, interference or improvements in behavioural and motor learning tasks have been used to assess the impact of NIBS protocols. Most NIBS techniques are promising and are met with enthusiasm; the newly-described methods are effective and reliable, but nonetheless are followed by reports of variability in intra-subject and inter-subject response (for a detailed review see [1]). Consequently, the issue of variability has raised concerns about the reliability of NIBS as a therapeutic approach for applications such as neurorehabilitation but also its validity as an experimental tool. We provide an opinionated review with the aim of analysing and discussing critically the issue of variability in NIBS studies. In the first part of this review we describe the physiological mechanisms activated by the currently used NIBS protocols, such as Theta Burst Stimulation (TBS), Paired Associative Stimulation (PAS) and transcranial direct current stimulation (tDCS). We then provide quantitative measures of intra- and inter-subject variability reported in previous studies. Finally, we review specific known factors leading to intra- and inter-subject variability in NIBS.

2. Common NIBS protocols

Among NIBS protocols, TBS consists of rhythmic gamma bursts (50 Hz), repeated at theta frequencies (5 Hz) at low intensity over M1. The initial report by Huang et al. [2] demonstrated that the intermittent application of TBS (iTBS) could potentiate MEPs, whereas the continuous form (cTBS) could reduce MEP amplitudes for about 30–60 min. The putative mechanism is long-term potentiation (LTP) and long-term depression (LTD)-like plasticity, as human TBS was designed to resemble TBS protocols used to trigger LTP or LTD in brain slices [2,3].

Another NIBS technique widely used in human studies is PAS. PAS combines repetitive electrical nerve stimulation with transcranial magnetic stimulation (TMS) over the contralateral M1 at specific interstimulus intervals (ISIs). PAS at 25 ms ISI increases MEP amplitudes, whereas PAS at 10 ms ISI is thought to decrease MEP amplitudes for 30–60 min [4]. PAS is considered to elicit a Hebbian form of spike-

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timing-dependent plasticity (STDP) in humans [5–7]. PAS may induce LTP/LTD-like phenomena in M1, through repetitive activation of specific sensorimotor circuits within a narrow time window [4,8]. Several modified PAS protocols have also been developed: for instance, PAS protocols implying afferent inputs to M1 other than somatosensory (e.g. visual, auditory or nociceptive stimuli, etc.) or protocols consisting of paired TMS pulses delivered over different cortical areas (e.g. cortico-cortical PAS) [8–11].

Another NIBS protocol currently used in plasticity studies in humans is the transcranial direct current stimulation (tDCS). During tDCS, when the anode is commonly placed over M1 and the cathode over the contralateral frontal pole (anodal tDCS), MEPs tend to increase in amplitude, whereas when polarity is inverted (cathodal tDCS), MEPs tend to decrease in amplitude for 30–60 min. tDCS is thought to mimic protocols of cortical polarisation described in early studies in rats showing that cortical polarisation resulted in a long-term modification of the amplitude of somatosensory evoked potentials elicited by electric stimuli delivered over the skin of the contralateral forepaw [12,13]. Accordingly, tDCS may work through whole-brain polarisation and is believed to induce depolarization or hyperpolarization of the resting membrane potential leading to LTP/LTD-like mechanisms [14]. In particular, tDCS-induced after-effects seem to be mostly related to intracellular calcium dynamics and N-methyl-D-aspartate (NMDA) receptor activity [1,15,16]. Gamma-aminobutyric acid (GABA) neurotransmission down-modulation can also play a role [17,18].

3. Intra- and inter-subject variability in NIBS studies

Reliability is defined as the extent to which measurements can be replicated. A number of studies have consistently demonstrated that the response to all NIBS protocols are rather variable in healthy humans with a substantial portion of subjects considered as non-responders. We have summarized the previously published data concerning intra-subject and inter-subject variability for TBS, PAS and tDCS protocols used in healthy subjects in Tables 1–3. Studies are arranged by the NIBS technique, and then by protocol (intensity and duration). Several quantitative measures, including Intraclass Correlation and the grand average of the percentage of responders, are provided to evaluate intra- and inter-subject variability.

The Intraclass Correlation Coefficient (ICC) is a widely used reliability index in test-retest, intra-rater, and inter-rater reliability analyses. ICC measures are an excellent way to describe test-retest (intra-individual) variability as it reflects both degree of correlation (like Pearson's Correlation) and agreement between measurements (like *t*-tests or Bland Altman plots). Based on the 95% confident interval of the ICC estimate, values less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and higher than 0.90 are indicative of poor, moderate, good, and excellent reliability, respectively.

When considering intra-subject variability in NIBS studies described in Table 1, one might be tempted to estimate the ICC of the most commonly applied tDCS protocol (e.g. 1 mA, 10 min) to be moderate (0.5-0.75) in the first 30 min after stimulation. This appears to improve if the duration of stimulation is longer (e.g. 15 min), but an increase in stimulation strength over a shorter duration does not appear to improve matters. iTBS and cTBS using standard parameters also show moderate ICC but there are no sham-controlled studies to date. Concerning PAS, a poor ICC has been estimated (Table 1).

When considering inter-subject variability (Tables 2–3), two descriptive statistical approaches are common: arithmetic means (statistical tests such as T-Tests or ANOVA on averages of all measures at individual time points or grand averages of all measures at all time points) and Clustering. The use of clustering has grown in popularity with the recognition that the arithmetic mean of a measure with high response variability between subjects is not a good summary descriptor of the data. Clustering methods like k-means minimize within-cluster variance, thus producing clusters (groups within the larger data set). The approach taken can affect the interpretation of the data. For example, Lopez-Alonso et al. [19] and Hinder et al. [20] reported results on variability in response to iTBS that support very different conclusions. Hinder et al. [20] reported 73% of responders (based on grand mean average) while Lopez-Alonso et al. [19] reported 43% of responders (based on clustering) to the technique.

4. Factors leading to intra- and inter-subject variability in NIBS studies

A large number of factors have been identified and reported to explain a variable amount of intra- and inter-subject variability in NIBS studies. We summarize here known factors contributing to intra- and inter-variability by dividing them into three main categories: physiological, technical and statistical aspects.

4.1. Physiological state

This category refers to non-modifiable and modifiable factors that influence the physiological state at the time when the NIBS protocol is applied. Most of these physiological factors have been reported in detail in previous consensus papers [1,3,8,21]. In brief, non-modifiable factors refer to those subjective characteristics which are immutable for all practical purposes in experimental sessions: age, gender, handedness and genetics. Physiological aging, for instance, has been related to a decline in M1 plasticity [22–24], but differences in response to NIBS have also been reported depending on gender, handedness and, particularly for tDCS, skull conformation and skin condition [25–28]. The most studied genetic factor influencing the direction and amount of response to NIBS protocols has been the Brain-Derived Neurotrophic Factor (BDNF) genotype. Although not consistently replicated [29–31], it has been demonstrated that M1 plasticity is impaired in individuals expressing the Val66Met polymorphism [32–34]. Adversely, carrying specific allelic variants in NMDA receptor subunits enhances the iTBS-induced LTP-like plasticity [35].

When considering modifiable factors of variability in NIBS, intake of medical and non-medical substances is an important consideration. Caffeine, nicotine and alcohol [36–38], as well as Antidepressants, Benzodiazepines and Antiepileptic drugs influence brain excitability and plasticity [39]. Quality of sleep and arousal also modulates NIBS effects: sleep deprivation the night before and reduced alertness or attention during the execution of protocols influence the results [40–42]. Moreover, the state of motor system activation should be considered since prolonged physical activity and/or motor pre-activation prior, during or immediately after NIBS can alter LTP/LTD-like plasticity [43–45]. On a related note, the state of M1 intracortical excitability at the time of applying a NIBS protocol can influence the outcome. The strength of short-interval intracortical inhibition (SICI) at baseline can predict the effectiveness of PAS [19,46]. Also, the level of short latency afferent inhibition (SAI) inversely correlates with LTP-like PAS-induced effects, accounting for ≈40% of the inter-individual variability in response [47]. Hormones and their cyclic fluctuations are also well known factors of intra- and inter-subject variability. In particular, female hormones, such as progesterone, and circadian variations of cortisol blood levels modulate cortical excitability [48–50].

4.2. Technical factors

A failure of a technology (like a specific NIBS protocol) can be conceived as being due to several factors. Technical factors include those operator-dependent methodological details that need to be carefully controlled while conducting the NIBS experiment. For instance, different elements of M1 are preferentially activated by using different TMS coil orientations and the optimal position for eliciting late I-waves is posterior-to-anterior [51]. Precise and continuous stimulation of the targeted area is obviously needed to induce effective neuromodulation

Table 1
Intra-subject variability for various NIBS techniques.

Protocol	N	N sessions	Control	Variability estimates					References
				Outcome		Intra-individual variability			
				MEP amplitude (range/epoch)	Other	ICC	% maintaining response	Correlation	
tDCS									
Anodal									
0.2 mA/10′	29	2		0–30′/10′		−0.50			[59]
1 mA/7′	12	3	Sham	0′,15′,30′		0.545 0.789*			[60]
1 vs 2 mA/10′	20	2†		0–20′/5′		No reliability (negative values)			[61]
1 mA/10′	14	3	Sham	0–30′/5′		0.062			[62]
1 mA/13′	45	2				0.530			[63]
				0–30′/5′		−0.028			
				35–60′/5′		−0.092–0.430*			
					SICI 6′	0.465			
					SICI 46′	0.147			
1 mA/15′	7	3	Sham	0–30′/5′		0.738			[64]
				60–120′/30′		0.642 −0.050–0.780*			
1 mA/15′	15	2		0′,10′,30′	Area (0′,10′,30′)	0.63–0.88* 0.67–0.93*			[65]
1 vs 2 mA/20′	20	2†		0–20′/5′		0.40–0.59			[61]
1,5 mA/10′ vs 20′	54	2†		0–30′/5′			66		[34]
1.5 mA/10′ vs 20′	33	2†		0–30′/5′			45	$r = -0.040$, $p = 0.826$	[66]
2 mA/7′	12	3	Sham	0′,15′,30′		0.076 0.535*			[60]
2 mA/20′	10	4	Sham		IO Curve 20′	0.276			[67]
Cathodal									
1 mA/10′	14	3	Sham	0–30′/5′		0.055			[62]
2 mA/20 min	10	4	Sham		IO curve 20′	0.137			[67]
TBS									
iTBS									
600 stimuli	33			0–30′/5′			48	r = −0.214, p = 0.233	[66]
80% AMT									
600 stimuli	30	2		0–36′/3′		0.534	83.3	r = 0.55; p = 0.001	[20]
80% AMT									
cTBS									
600 stimuli 70% RMT	18	3		0′,15′,30′		0.538–0.539			[68]
PAS									
PAS_{N20} 140 pair stimuli	16				SEP N20-P25		56.2		[41]
PAS_{25} 140 pair stimuli	18			0′		−0.003–0.005	27.8		[69]

*ICC of single time points (range or best response); †comparison of sessions with different conditions.
SEP − Somatosensory Evoked Potentials.

[52,53]. Moreover, type (monophasic/biphasic), intensity (low/high), frequency and total number of TMS stimuli significantly affect the direction and amounts of NIBS after-effects [1,8,54]. Regarding tDCS, differences in electrodes size, intensity used, montages, targeted area and duration of stimulation can be responsible of intra- and inter-individual variability. International guidelines are already available for many of these factors (see for example [27]). For others a consensus statement is still lacking (e.g. use of a neuronavigation system to ensure the precise stimulation of the same “spot” throughout the neuromodulation protocol and during the MEPs’ serial measurements post-intervention), even though several recommendations for best technical practice have been recently proposed for PAS studies [8]. However, there is little or no consensus on minimum requirements for training and assessment of technical competency, in a field where the majority of literature is produced by graduate or postgraduate students with variable experience, expertise and supervision. Technical factors generally affect both inter- and intra-subject variability, and explicit standards for training as well as consensus guidelines for individual NIBS protocols are necessary for reliable, reproducible results. A final comment involves the consideration that each NIBS protocol can be thought of as a technology. The most obvious factor in this category is an intrinsic lack of efficacy. However, factors other than raw efficacy should be considered to minimize variability. Technology can fail to benefit when it is improperly applied (technical factors) and if a particular technology is difficult to apply, failures are more common. Some factors, such as basing stimulation intensity on a factor open to inter-

Table 2
Inter-subject variability after Transcranial Direct Current Stimulation.

Protocol	N	Control	Variability estimates					References
			Outcome		% of Responders			
			MEP amplitude (range/epochs)	Other	Grand Average	Clustering	Other measures	
tDCS								
Anodal								
0.2 mA/10′	29		0–30′/10′		34.5**	41		[59]
0.5 mA/10′	29		0–30′/10′		17.2–24.1**			[59]
0.5 mA/15′	20	Sham	0–30′/5′		75			[64]
1 mA/7′	29		0–30′/5′				79.3* (single best timepoint MEP)	[70]
1 mA/7′	12	Sham	0′,15′,30′		33.3***VBTs	25		[60]
1 mA/9′	59		0′,5′,10′,20′,30′,40′		61	41†		[71]
1 mA/10′	29		0–30′/10′		20.7**			[59]
1 mA/10′	20		0–20′/5′		35$			[61]
1 mA/10′	14	Sham	0–30′/5′		57.1–64.3			[62]
					28.6–42.8**			
1 mA/13′	56		0–60′/5′	SICI (6′,46′)	50	45	56.3	[19]
1 mA/13′	45		0–60′/5′		51.1–64.4			[63]
1 mA/13′	30		0′,5′,10′,20′,30′		66			[72]
					55*			
					21****			
1 mA/15′	20	Sham	0–30′/5′		90			[64]
1 mA/20′	26	Sham	0′,5′		35.7			[73]
1 mA/20′	20		0–20′/5′		35$			[61]
1,5 mA/10′	54		0–30′/5′		64	42		[34]
1.5 mA/10′	33		0–30′/5′		55*			[66]
1.5 mA/15′	20	Sham	0–30′/5′		75			[64]
1.5 mA/20′	54		0–30′/5′		62	42		[34]
1.5 mA/20′	33		0–30′/5′		52*			[66]
2 mA/7′	12	Sham	0′,15′,30′		52.8***VBTs	47.2		[60]
2 mA/10′	53		0–30′/5′		74	47.2		[74]
2 mA/10′	29		0–30′/10′		38**	21		[59]
2 mA/10′	20		0–20′/5′		20$			[61]
2 mA/15′	20	Sham	0–30′/5′		85			[64]
2 mA/20′	20		0–20′/5′		35$			[61]
Cathodal								
1 mA/9′	59		0′,5′,10′,20′,30′,40′		53	49†		[71]
1 mA/10′	14	Sham	0–30′/5′		21.4–42.9			[62]
					0–28.6**			
2 mA/10′	53		0–30′/5′		41	52.8		[74]
2 mA/20′	20		10′,20′	SLP_{ac} 20′,40′	42**		40	[75]

SICI − Short-interval intracortical inhibition; SLP_{ac} − Silent-period latency during active contraction; VBTs − Sham Variability-Based Threshold.
Grand average cut-off is 100% unless *110, **120, ***130, ****150, $Other cut-offs.
Clusters → 2Steps unless † Agglomerative hierarchical clustering or other clustering methods.

observer variability such as Active Motor Threshold, may be intrinsic to a particular NIBS protocol.

4.3. Statistical aspects

The very first step of any study should be to formulate a priori hypothesis and, only after that, to design the experiment [55]. A publish-or-perish mentality and editorial preference for 'positive' studies can result in data mining and publication bias. It is also important to state that most NIBS studies have been conducted on relatively low numbers of subjects (see Tables 1–3). Using an inadequate sample size is a relevant methodological issue contributing to variability and affecting the reliability of NIBS studies. Finally, the variance can result not just from the NIBS intervention itself, but also the assay utilized to assess outcomes [55]. The most common assay is the MEP, which is collected and averaged in most TMS studies in blocks of 15–30 MEPs per time point (see for example [20,56,57]). Accuracy and reliability of TMS measures are definitely low when averaging a reduced number of trials [58]. Moreover, time dynamics of neuromodulatory interventions cannot be adequately described when testing a low number of time points. A final consideration concerns the statistical analysis used for evaluating NIBS after-effects. It should be taken into account that the use of parametric tests is allowed only when the assumptions of normality of the distributions are satisfied. Using parametric tests for evaluating data that is not normally-distributed might influence the reliability of NIBS studies [55]. The same effect can be caused by arbitrary outlier rejection and use of inappropriate summary statistics.

5. Conclusions

In the present review, we have readdressed the issue of intra- and inter-subjects variability and tabulated the pertinent published literature for the commonest NIBS protocols. We have also summarized the known factors contributing to variability in human studies. At the very least, this should confirm the growing consensus that variability in response to NIBS is a consistent and significant research issue. However, although a number of factors contributing to intra- and inter-subject variability have been identified, their relative importance is uncertain. Moreover, it is unlikely that we have already discovered all possible sources of variability in response to NIBS. Hence, further studies aimed to identify response modifiers and to quantify their relative importance are warranted. While the issue of variance in outcomes of NIBS protocols continues to attract criticism about the relevance, reliability and the science of NIBS from some quarters, we believe they should be considered an opportunity. A more detailed understanding of the sources of variability is fundamental to understand the basis for altered

Table 3
Inter-subject variability after Theta Burst Stimulation and Paired Associative Stimulation.

Protocol	N	Control	Variability estimates					References
			Outcome		% of Responders			
			MEP amplitude (range/epoch)	Other	Grand Average	Clustering	Other measures	
TBS								
iTBS								
300 stimuli @ 80% AMT	33		0–30′/5′		58*	No Cluster		[66]
600 stimuli @ 80% AMT	56		0–60′/5′	SICI (6′,46′)	46.4	43	54.6	[19]
600 stimuli @ 80% AMT	52		0–30′/5′		52			[56]
600 stimuli @ 70% RMT	40		5′,15′,25′		47.5			[76]
600 stimuli @ 80% AMT	38		0′,5′,10′,20′		56–65			[77]
600 stimuli @ 80% AMT	33		0–30′/5′		55*	52		[66]
600 stimuli @ 80% AMT	30		0–36′/3′		73 60*			[20]
600 stimuli @ 80% AMT	16	Sham	0′		44*			[78]
600 stimuli @ 80% AMT	16		0–60′/10′		62.5			[79]
cTBS								
600 stimuli @ 80% AMT	52		0–30′/5′		42			[56]
600 stimuli @ 70% RMT	36		0–30′/5′		72**			[44]
600 stimuli @ 70% RMT	34		0′/20′		35.3–47.1			[80]
600 stimuli @ 80% AMT	31		5′,10′,20′,30′,40′,50′		19.3			[81]
600 stimuli @ 80% AMT	24		10′,25′,40′		62.5			[82]
600 stimuli @ 80% AMT	21		5′,10′,15′,20′,30′,40′,50′			57.1†		[83]
600 stimuli @ 70% RMT	18		0′,15′,30′		61–78			[68]
600 stimuli @ 70% RMT	10		0–30′/5′		70**			[44]
600 stimuli @ 80% RMT	8		0′,20′		38–75			[84]
PAS								
Different protocols	190				53			[85]
PAS_{25} 200 paired stimuli Ulnar nerve	56		0–60′/5′	SICI (6′,46′)	39	53.6	46.5	[19]
PAS_{25} 180 paired stimuli Median nerve	37		1′,8′,15′		63			[42]
PAS_{25} 180 paired stimuli Ulnar nerve	30		0′,5′,10′,20′,30′		79 72* 47****			[72]
PAS_{25} 200 paired stimuli Median nerve	18				63			[46]
PAS_{25} 200 paired stimuli Ulnar nerve	16		0–60′/10′		75			[79]
PAS_{N20} 140 paired stimuli Median nerve	16			Somatosensory Evoked Potentials N20-P25			56.2	[41]
PAS_{20} 225 paired stimuli Median nerve	14		0–30′/5′		52			[86]

Grand average cut-off is 100% unless *110, **120, ***130, ****150.
Clusters → 2Steps unless † Agglomerative hierarchical clustering or other clustering methods.

response to NIBS in several neurological disorders (e.g. [3,8]). More importantly, a better understanding of sources of variability would also allow the design of new tailored interventions making the neuromodulation strategies as effective as possible.

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