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Neurobiological after-effects of non-invasive brain stimulation

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ABSTRACT

Background: In recent years, many studies have evaluated the effects of noninvasive brain stimulation (NIBS) techniques for the treatment of several neurological and psychiatric disorders. Positive results led to approval of NIBS for some of these conditions by the Food and Drug Administration in the USA. The therapeutic effects of NIBS have been related to bi-directional changes in cortical excitability with the direction of change depending on the choice of stimulation protocol. Although after-effects are mostly short lived, complex neurobiological mechanisms related to changes in synaptic excitability bear the potential to further induce therapy-relevant lasting changes.
Objective: To review recent neurobiological findings obtained from *in vitro* and *in vivo* studies that highlight molecular and cellular mechanisms of short- and long-term changes of synaptic plasticity after NIBS.
Findings: Long-term potentiation (LTP) and depression (LTD) phenomena by itself are insufficient in explaining the early and long term changes taking place after short episodes of NIBS. Preliminary experimental studies indicate a complex scenario potentially relevant to the therapeutic effects of NIBS, including gene activation/regulation, de novo protein expression, morphological changes, changes in intrinsic firing properties and modified network properties resulting from changed inhibition, homeostatic processes and glial function.
Conclusions: This review brings into focus the neurobiological mechanisms underlying long-term after-effects of repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) recently obtained from *in vitro* and *in vivo* studies, both in animals and humans.

1. Introduction

Throughout the past three decades, noninvasive brain stimulation (NIBS) techniques have been widely used for studying the physiology of the central nervous system (CNS) [1,2]. They offered a new way to identify the functional role of specific human brain structures and, more recently, to explore large-scale neural network dynamics.

The rationale for NIBS applications as clinical/therapeutic tools resides in the maintenance of the after-effects that outlast the time of stimulation. Accordingly, repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) have been found to be a promising noninvasive treatment for a variety of neuropsychiatric disorders [3–10].

Despite this wide and growing clinical and therapeutic value, mechanisms underlying the efficacy of NIBS remain essentially unexplained.

Emerging evidence suggests that in addition to changes in neural excitability, several other mechanisms may contribute to the lasting effects of NIBS [11] and likely reflect forms of short-lived activity-dependent modulation of synaptic efficacy. Thus, it is conceivable that the explanation for long term effects resides in other mechanisms that need to be further elucidated and appear to be related to changes in cortical synaptic transmission [12], in a

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manner being biologically similar to the long term potentiation/long term depression (LTP/LTD) process [13], and additional regulatory mechanisms from cellular to brain networks level.

Hitherto, little is known about the cellular processes directly influenced by NIBS. This review brings into focus the neurobiological mechanisms underlying long-term after-effects of rTMS and tDCS recently obtained from *in vitro* and *in vivo* studies, both in animals and humans.

2. Noninvasive brain stimulation (NIBS) techniques

2.1. rTMS and tDCS protocols

Several rTMS protocols have been widely used in basic and clinical research [14]. We usually distinguish *simple protocols*, realized by single stimuli repeated at fixed inter-stimulus intervals (ISIs, fixed frequency), and *patterned protocols* that use a combination of different ISIs. The duration of the effects produced by different stimulation protocols is influenced by several variables including stimulus frequency and intensity, shape of the magnetic pulse, duration of the application period, and the total number of stimuli [15].

The most widely used NIBS technique is the rTMS: application of simple rTMS to a target brain area for several minutes induces after-effects that outlast the period of stimulation in a frequency-dependent manner. Low frequency (≤1 Hz) rTMS reduces cortical excitability whereas high-frequency (5–20 Hz) rTMS does the opposite [16,17].

Among patterned rTMS protocols, theta-burst stimulation (TBS) (bursts of three pulses at 50-Hz repeated at 200-ms intervals) induces longer-lasting effects with shorter application time than conventional rTMS paradigms. *Continuous* TBS (cTBS) (a single train of burst lasting 20–40 s) has primarily an inhibitory effect on corticospinal excitability, while *intermittent* TBS (iTBS) (the burst train is split up into twenty 2 s-sequences repeated every 10 s), has an excitatory effect [18–20].

I-wave TMS (ITMS) [21] using two pulses delivered at ISI 1.5 ms or 2 ms, repeated every 5 s, was shown to induce bidirectional changes in excitability with high temporal fidelity [22,23]. Later adaptations showed that delivery of four subthreshold pulses (quadripulse stimulation, QPS) at 1.5 ms [24] or longer intervals [25] could induce bidirectional plastic changes on a broader temporal scale.

Alternatively, tDCS can be used to induce changes in cortical excitability, by applying a weak constant current (1–2 mA) to the brain for 5–20 min using a pair of saline-sponged electrodes [26].

One of them, the specific electrode, is placed onto the scalp above the cortical area to be modulated, while the other electrode is placed distantly [27]. Opposite effects on cortical excitability can be achieved when changing the polarity of the current. Anodal tDCS (a-tDCS) usually raises cortical excitability, likely by depolarizing neuronal compartments closer to the electrode, while cathodal tDCS (c-tDCS) diminishes excitability [28].

The duration, magnitude and polarity of excitability changes induced by NIBS protocols varies according to various parameters such as stimulation duration [27] and intensity [29].

tDCS, in summary, represents a plasticity-inducing NIBS technique that produces a polarity-dependent changes of cortical excitability. Different to TMS, the induced membrane depolarization is well below the threshold to elicit action potentials.

A direct link between DCS and synaptic plasticity has been established by several experimental studies that demonstrated a-tDCS-induced LTP in mouse motor cortex [30] and a polarity-specific modulation of LTP induction in the rat hippocampus [31]. Recently, extracellular recordings in viable rat hippocampus brain slices after brain stimulation showed that 30 min of brain anodal tDCS in rats induced a robust enhancement and persistence of synaptic plasticity [32] confirming that such effects are mediated by an LTP-like mechanism [33].

3. Synaptic plasticity

The continuous remodeling of brain function in response to external stimuli, through short- and long-term modifications of interneural connections, represents one of the major adaptive properties of the CNS and it is called *synaptic plasticity* [34].

Plasticity occurs at different levels, from the ultrastructure to brain networks level and it is accompanied by transient or lasting changes in Ca^{2+} dynamics, neurotransmitter release, protein expression and gene activity [35].

The exact mechanisms underlying these plastic changes vary, depending on the activity of pre- and post-synapses, the circuits in which they operate and the temporal relationship between pre- and post-synaptic activity that determines whether LTP or LTD is induced [12].

The latter property is called *spike timing-dependent plasticity* (STDP) and represents the major strategy to synchronize neuronal firing and thus to increase synaptic strength, a process that is dependent on the activation of post-synaptic NMDAr [36].

Changes in excitatory synaptic strength needs to be maintained over time. Early LTP is related to modifications of synaptic strength usually lasting for 30–60 min and reflects post-transcriptional modifications of pre-existing proteins, such as protein phosphorylation. By contrast, changes in gene and protein expression are thought to be responsible for the late components of LTP, persisting for hours, days and even months.

The term *metaplasticity* refers to a higher order form of plasticity, in other words a "plasticity of synaptic plasticity", whereby the history of synaptic or cellular activity influences the direction and degree of synaptic plasticity that may be induced by a subsequent protocol [37]. Metaplasticity can permit or inhibit plasticity induction, stabilize synapses, homeostatically regulate cellular activity and, in the extreme, it can act as a braking mechanism which avoids excessive synaptic strengthening or weakening [38]. Maintaining a relatively stable equilibrium of neural activity over time is defined as *homeostatic synaptic plasticity*. Homeostatic plasticity can be achieved by regulating synaptic strength or factors of intrinsic excitability, thereby adjusting the balance between synaptic input and neuronal firing [39].

Metaplasticity regulates synaptic plasticity in space and time (from minutes to days) and serves to prolong the time-window for associative interactions between neural events (associative plasticity), thereby likely increasing information encoding during repeated tasks [40].

The effect of conditioning stimulation cannot be predicted unless the history of stimulation of the neural network is known. It is important to keep in mind that plasticity and metaplasticity share some mechanisms (both NMDAr and metabolic glutamate receptors seem to play a role) being potentially operative at the same time. Accordingly, it is very difficult to distinguish between changes in synaptic efficacy related to "conventional synaptic" plasticity and metaplasticity [41].

4. Neurobiological after-effects of NIBS

Therapeutic NIBS application requires the induction of long-lasting changes. In humans, both NMDAr- and Ca^{2+}-dependent modifications induced by NIBS protocols point to synaptic