Modulation of laser-evoked pain perception and event-related potentials with non-invasive stimulation of the motor cortex

Ph.D. Thesis

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Summary

In the last two decades new techniques of non-invasive brain stimulation have been introduced that enable relatively long-lasting and reversible facilitation or inhibition of distinct cortical areas by modulating the excitability of underlying neurons. Among these methods, repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) are the most widespread ones. To date, both have been successfully used to modulate various perceptual, cognitive and motor functions in healthy subjects and several diseases, including chronic pain. Their efficacy regarding acute pain perception in healthy subjects, however, is still not well-established.

The aims of our studies were to investigate the effects of a novel rTMS paradigm, called continuous theta-burst stimulation (cTBS), and tDCS on laser-induced acute pain perception and laser-evoked potentials (LEPs) when applied to the motor cortex of healthy adult volunteers. In two psychophysical and two electrophysiological experiments, we have compared the effects of real cTBS and two tDCS protocols (anodal and cathodal) to those of sham stimulations.

We have shown for the first time that cTBS over the motor cortex significantly alleviated laser-induced pain on both hands, accentuating on the contralateral limb. The effect of cTBS was accompanied by reduced N2-P2 LEP amplitudes in the case of medium intensity pain. In the tDCS experiments, cathodal stimulation of the motor cortex reduced mild pain contralateral to the side of stimulation. Moreover, cathodal tDCS attenuated N2-P2 LEP components, without modulating thresholds of medium intensity pain. On the contrary, anodal tDCS facilitated laser-induced warm sensation contralateral to the side of tDCS, without affecting either pain sensation or LEPs.

Our results indicate that non-invasive stimulation of the motor cortex causes antinociceptive effects that depend on the parameters of stimulation and are probably due to excitability changes in remote pain-related areas such as the operculoinsular region and the anterior cingulate cortex. These findings further strengthen the application of cTBS and tDCS in pain research, which might contribute to a more efficient manipulation of brain plasticity for therapeutic purposes.

Összefoglalás

Bevezető

A fájdalomcsillapítás mindig is az orvostudomány legnagyobb kihívásai közé tartozott, ám a gyógyszeripari erőfeszítések ellenére sok kórképben a mai napig nem megoldott a tartós fájdalommentes állapot elérése. Az elmúlt 40 év során számos olyan műtéti beavatkozás terjedt el, amely a központi idegrendszer működésének modulálásával eredményesen csökkentette a krónikus, farmakorezisztens fájdalmat.

Az invazív módszerek közül az elsődleges mozgatókéreg tartós elektromos ingerlését tartják a leghatékonyabbnak. Ennek ellenére, a beavatkozás pontos hatásmechanizmusa a mai napig nem ismert. A jelenleg legelfogadottabb értelmezés szerint, a mozgatókéregből induló kortikotalamikus rostok a talamusz aktiválásával egyrészt a fájdalom affektív komponensét csökkentik a cinguláris/orbitofrontális kéreg befolyásolása által, másrészt pedig az agytörzsi periakveduktális szürkeállományból a gerincvelői hátulsó szarvhoz leszálló pályák a nociceptív ingerek belépését és felszállását gátolják, feltehetően ópiáterg mechanizmus által (Garcia-Larrea és Peyron, 2007). Mivel azonban a beavatkozás sokszor igen komoly kockázattal és számos mellékhatással járhat, a mai napig nagy az igény hasonló hatékonyságú, ám sokkal biztonságosabb módszerek iránt.

A kilencvenes években megjelent több olyan nem-invazív agyi ingerlési módszer, amelyek lehetővé tették viszonylag körülírt kérgi agyterületek működésének átmeneti serkentését vagy gátlását. Ezen eljárások az agy elektromos ingerlésén alapulnak, és az alkalmazás helyétől valamint az ingerlési paraméterektől függően mindezidáig eredményesen alkalmazták őket a perceptuális, kognitív és motoros folyamatok befolyásolására mind egészséges személyekben, mind különféle kórképekben. A két legelterjedtebb nem-invazív agyi ingerlési módszer az ismételt transzkraniális mágneses ingerlés (repetitive transcranial magnetic stimulation, rTMS) és a transzkraniális egyenáram-ingerlés (transcranial direct current stimulation, tDCS).

Az rTMS alapja az elektromágneses indukció, amely során egy feltekercselt vezetéket helyeznek a fejre az ingerelni kívánt agyterület fölé, majd nagyon rövid ideig, rendkívül erős mágneses mezőt hoznak létre ismétlődő jelleggel. A mágneses térerő-változás elektromos térerő-változást indukál a kéregben, és az ingerlés idejét meghaladó, de teljes mértékben

reverzibilis facilitációt vagy inhibíciót okoz a kérgi neuronokban. Mindezidáig számos, krónikus fájdalommal járó kórképben alkalmazták sikeresen a magas frekvenciájú (10-20 Hz-es) rTMS kezelést. Hasonlóan az invazív eljárásokhoz, az rTMS esetében is a mozgatókéreg ingerlése bizonyult a leghatékonyabbnak (Lefaucheur és mtsai, 2001a; Khedr és mtsai, 2005; André-Obadia és mtsai, 2006). Ennek ellenére, egészséges személyek esetében a mai napig ellentmondásos, hogy mely rTMS protokollok eredményeznek hatékony fájdalomcsillapítást (Summers és mtsai, 2004; Yoo és mtsai, 2006; Mylius és mtsai, 2007; Valmunen és mtsai, 2009). Pár éve egy speciális rTMS protokoll, a folyamatos théta frekvenciájú sorozatingerlés (continuous theta-burst stimulation, cTBS) erőteljes, mozgatókérget gátló hatásáról számoltak be (Huang és mtsai, 2005), amely bíztató lehet számos kórkép hatékonyabb rTMS kezelése szempontjából.

A tDCS során két, viszonylag nagyméretű elektródát (egy anódot és egy katódot) helyeznek a fejre, amelyek között egy elemmel működtetett ingerlő segítségével általában 1-2 mA-es egyenáram áramlik az anód felől a katód felé legfeljebb 20 percen keresztül. Ennek hatására az ingerlési paraméterektől függően viszonylag hosszan, akár 1 órán át megváltozik az ingerelt kéregrész neuronjainak ingerlékenysége. Az anodális ingerlés depolarizálja, míg a katodális hiperpolarizálja a neuronokat, ezáltal modulálván az idegsejtek akciós potenciáljainak frekvenciáját. A tDCS esetében a mozgatókéreg anodális ingerlékenysége bizonyult mindezidáig a legerőteljesebb fájdalomcsillapító hatásúnak krónikus fájdalom szindrómákban (Fregni és mtsai, 2006a; 2006b; Antal és mtsai, 2010), de kevés adat áll rendelkezésünkre a módszer hatékonyságáról egészséges személyeknél.

Jelen disszertáció két cTBS és két tDCS kísérlet eredményeit ismerteti, amelyekkel az eljárások mozgatókéreg feletti alkalmazásának fájdalomcsillapító és Tm:YAG lézer által kiváltott eseményfüggő potenciálokra (LEP-ekre) gyakorolt hatásait vizsgáltuk egészséges személyekben.

Módszerek

Mindegyik kísérlet során a valós cTBS illetve tDCS ingerlés hatásait a placebo ingerlésnél tapasztalt változásokhoz hasonlítottuk. Egy kísérleten belül a valós és placebo ingerlést ugyanazon személyeknél végeztük; a négy kísérletben különböző személyek vettek részt. Az első, viselkedéses kísérletben a cTBS fájdalomcsillapító hatását mértük fel három

lézerintenzitás (gyenge, közepes és magas) esetén 13 személynél. A második, elektrofiziológiai kísérletben 10 személynél a cTBS előtt és után LEP-eket regisztráltunk, miközben arra törekedtünk, hogy minden esetben közepes erősségű fájdalmat váltsunk ki. A harmadik, viselkedéses kísérletben 16 személynél hasonlítottuk össze a katodális, anodális és placebo tDCS hatását egy küszöbalatti (melegérzet) és három fájdalmas (enyhe, közepes, erős) tartományban. A negyedik kísérletben 10 személynél vetettük össze a három tDCS ingerlés LEP amplitúdókra gyakorolt hatását.

A cTBS során a bal motoros kéreg felett alkalmaztunk 40 másodperces ingerlést. Az impulzusok intenzitását az ú.n. aktív motoros küszöb 80 %-ában határoztuk meg, az ingerlési mintázat a következő volt: 3 ingerből álló, gyors (50 Hz-es) sorozatingerek, amelyek théta (5 Hz) frekvenciával (azaz 200 milliszekundumonként) követték egymást. A placebo ingerléshez speciális tekercset használtunk.

A tDCS során az egyik elektródát a bal motoros kéreg fölé helyeztük (a C3-as elektródapozíció a Nemzetközi 10-20-as rendszer alapján meghatározva)., míg a másik elektróda a jobb szem feletti területre került. Az ingerlés 1 mA intenzitású és 10 perc hosszú. A placebo helyzetben csak pár másodpercre kapcsoltuk be az ingerlőt.

Eredmények

Az 1. kísérletben a cTBS szignifikáns mértékben fájdalomcsillapító hatásúnak bizonyult mindkét kéz ingerlése során. Az ellenoldali kéz esetében a hatás kifejezettebb volt közepes és magas lézerintenzitások esetén, és közvetlenül a cTBS után jelentkezett. Az azonos oldali kéz esetében csak az ingerlést követően 30 perccel észleltünk fájdalomcsillapító hatást, ugyancsak közepes és magas lézerintenzitások mellett.

A 2. kísérletben a cTBS az ellenoldali kéz ingerlése során csökkentette a fájdalomérzetet, miközben a placebo ingerléshez képest szignifikáns N2-P2 LEP amplitúdócsökkenést eredményezett. Ezt a változás elsősorban az N2 komponens csökkenése okozta. Meglepetésünkre, a P2 komponens csökkenése a placebo helyzetben volt nagyobb mértékű.

A 3. kísérletben a katodális tDCS megemelte az enyhe fájdalom küszöbét az ellenoldali kéz ingerlése során, de nem volt hatással a közepes és erős mértékű fájdalomérzetre. Az

anodális tDCS ezzel ellentétben a melegérzet küszöbét csökkentette az ellentétes oldalon, de nem befolyásolta a fájdalomküszöböket.

A 4. kísérletben a katodális tDCS csökkentette mind az N2, mind a P2 amplitúdókat az ellenoldali kéz ingerlése esetén, de nem befolyásolta a fájdalomérzetet. Az anodális tDCS hatása nem különbözött a pacebo ingerléstől.

Diszkusszió

Vizsgálatainkkal sikerült kimutatnunk, hogy mindkét általunk vizsgált nem-invazív ingerléses eljárás befolyásolja a lézer-kiváltotta akut fájdalomérzetet egészséges személyekben. Bár a fájdalomcsillapító hatás jellege különbözött a befolyásolt fájdalomtartomány, a hatás lateralizációjának és időbeli jelentkezésének tekintetében is, eredményeink bíztatóak a két módszer klinikai felhasználását illetően. A tény, hogy mind a cTBS, mind a tDCS csökkentette az N2-P2 LEP amplitúdókat arra utal, hogy az ingerlés feltehetően a mozgatókéreg kapcsolatai által távoli agyi struktúrák, többek között az operkuloinzuláris és az elülső cinguláris kéreg ingerlékenységét is befolyásolta.

Mindkét eljárással kapcsolatos eredményeink részben ellentmondanak más kutatócsoportok krónikus fájdalomban szenvedő betegek körében észlelt eredményeinek, hiszen a betegeknél sokkal inkább a motoros kéreg ingerlékenységének serkentése (magas frekvenciájú rTMS és anodális tDCS) okozott fájdalomcsillapító hatást. E jelenség értelmezéséhez figyelembe kell vennünk, hogy krónikus fájdalom szindrómák esetén megváltozik egyes agyterületek makro- és mikroszkópos struktúrája, neurokémiai profilja és neuronjainak válaszkészsége. Ugyanakkor számos kutatás igazolta, hogy mind az rTMS, mind a tDCS ugyancsak tartós változásokat idéz elő az agyi mikroarchitektúrában, befolyásolja több neurotranszmitter felszabadulását és a neuronok ingerlékenységét. Ez a hatás egészséges személyekben neurofarmakonokkal vagy más nem-invazív módszerekkel kombinálva lényegesen befolyásolható, sőt, akár ellentétes is lehet. Elképzelhető tehát, hogy a két beavatkozás merőben eltérő hatásokat idéz elő egészséges személyekben és különböző betegpopulációkban.

Mindezek tükrében eredményeink hozzájárulhatnak újabb ingerlési protokollok kidolgozáshoz, amelyekkel még erőteljesebb és tartósabb fájdalomcsillapító hatás érhető el számos betegség esetében.

List of abbreviations

ACC: anterior cingulate cortex

AMT: active motor threshold

CL: central lateral nucleus of the thalamus

cTBS: continuous theta-burst stimulation

DLPFC: dorsolateral prefrontal cortex

EEG: electroencephalography

EFNS: European Federation of Neurological Societies

fMRI: functional magnetic resonance imaging

LEP: laser-evoked potential

LTD: long-term depression

LTP: long-term potentiation

M1: primary motor cortex

MCS: motor cortex stimulation

MDvc: ventrocaudal part of the medial dorsal nucleus

MEG: magnetoencephalography

MEP: motor-evoked potentials

NAS: numeric analogue scale

NMDA: N-methyl-D-aspartate

PAG: periaqueductal gray matter

PBN: parabrachial nucleus

PET: positron emission tomography

Pf: parafascicular nucleus

rTMS: repetitive transcranial magnetic stimulation

RVM: rostral ventromedial medulla

S1: primary somatosensory cortex

S2: secondary somatosensory cortex

tDCS: transcranial direct current stimulation

TBS: theta-burst stimulation

TMS: transcranial magnetic stimulation

VMpo: posterior part of the ventromedial nucleus

VPI: ventral posterior inferior nucleus

VPL: ventral posterior lateral nucleus

VPM: ventral posterior medial nucleus

Introduction

According to the International Association for the Study of Pain (Merksey and Bogduk, 1994), pain is 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage'. This definition nicely emphasizes the highly subjective nature of pain perception that depends on far more factors than the mere parameters of the physical stimulus. In fact, pain sensation and corresponding activation of pain-related brain areas can be evoked by hypnosis, without physical stimulation (Raij et al., 2005), and conversely, pain can be significantly alleviated by methods such as biofeedback (deCharms et al., 2005) or diverting attention (Bantick et al., 2002). In this respect, understanding the complex neural mechanisms of pain perception in terms of emotional and cognitive factors is extremely important, which could help developing new methods of pain reduction and eventually help hundreds of thousands of patients suffering from chronic pain worldwide.

Chronic pain is a debilitating condition the prevalence of which is around 19% in Europe and Israel (Breivik et al., 2006). Moreover, it is estimated that approximately 45% of the population suffers from persistent pain in some period of their lives in the United States (Fregni et al., 2007). This condition is associated with a poorer quality of life (Hagg et al., 2003) and a higher risk of anxiety, depression or even suicide (Gureje et al., 1998; Tang and Crane, 2006). In addition, it causes substantial loss of function and productivity, posing an economic burden both to the individual and the society (Juniper et al., 2009). Therefore, achieving rapid, effective and long-lasting pain relief is still one of the greatest challenges of modern medicine. Despite the use of various potent pharmacological agents, only 30-40% of patients with chronic neuropathic pain reach >50% pain relief with pharmacotherapy (Cruccu et al., 2007). Perhaps the most promising alternative methods to pharmacological pain reduction are based on electrical or magnetic stimulation of the nervous system. Several such techniques are known, ranging from transcutaneous electrical nerve stimulation (Thorsteinsson et al., 1977) to invasive stimulation of thalamic nuclei (Hosobuchi et al., 1973) or certain areas in the brainstem (Richardson and Akil, 1977) with implanted electrodes. These methods not only differ in their target sites and invasiveness, but the magnitude and length of their effects also show great variability, depending on stimulation parameters and most importantly, on the patient population (Cruccu et al., 2007). Hence, none of these methods can be regarded as

optimal, and novel techniques are still required that enable reliable, robust and long-lasting pain reduction, while minimizing the risk of treatment and the cost of the therapy.

Functional anatomy of pain perception

The anatomical and physiological summary of pain perception is based on recent reviews and book chapters (Treede et al., 1999; Basbaum and Jessel, 2000; Price, 2000; Schnitzler and Ploner, 2000; Apkarian et al., 2005; Brooks and Tracey, 2005; Vogt, 2005; Tracey and Mantyh, 2007; May, 2008). The anatomy of pain pathways and structures involved in pain perception are presented in Figure 1.

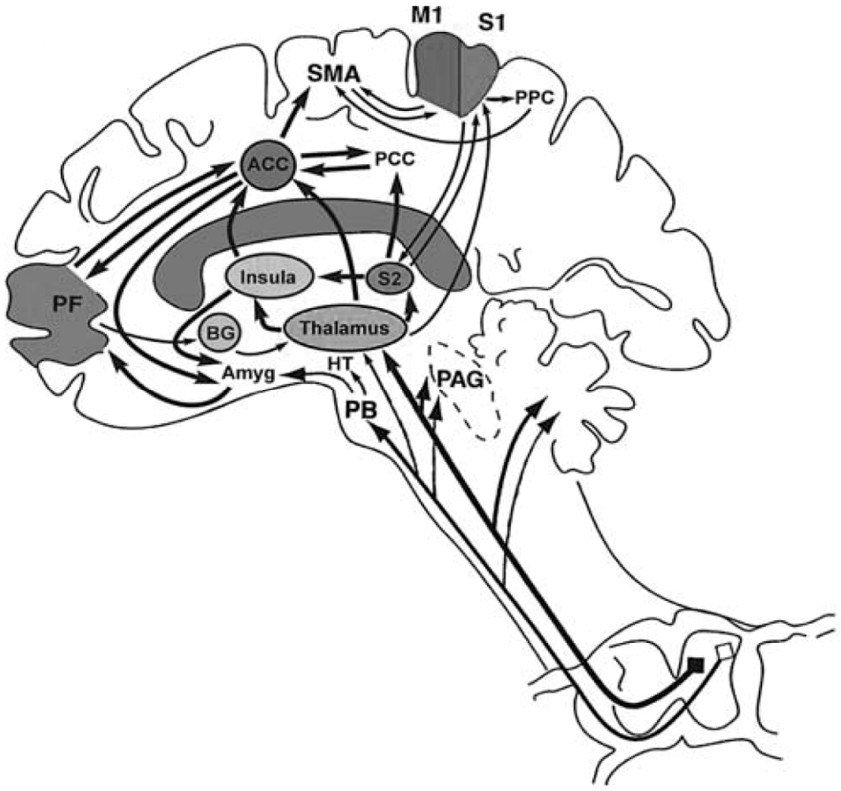

Figure 1. Anatomical summary of pain perception. Abbreviations: ACC - anterior cingulate cortex, AMYG - amygdala, BG - basal ganglia, HT - hypothalamus, M1 - primary motor cortex, PAG - periaqueductal gray matter, PB - parabrachial nuclei, PCC - posterior cingulate cortex, PF - prefrontal cortex, PPC - posterior parietal cortex, S1 - primary somatosensory cortex, S2 - secondary somatosensoros cortex, SMA - supplementary motor area. With permission from Apkarian et al., 2005. For detailed description of pain processing in the central nervous system see the text below.

Subcortical pain processing

Peripheral pain receptors or nociceptors are free nerve endings in the skin, mucosa and deep tissues that are excited by mechanical, thermal and various chemical stimuli. Painful thermal and mechanical stimuli excite small, myelinated Aδ-fiber endings, which are responsible for intensive pain with a rapid onset (‘sharp’ or ‘first pain’). On the other hand, polymodal (mechanical, thermal and chemical) nociceptors convey nerve impulses via unmyelinated C-fibers and are related to slowly developing and more prolonged ‘burning’ or ‘dull’ pain (‘second pain’). These nerve endings are activated or sensitized by endogenous substances released by damaged cells (such as bradykinin, histamine, prostaglandines, leukotrienes, substance P, etc.) and they are involved in hyperalgesia caused by skin injury or inflammation (Perl, 1996). According to results of CO_2 laser stimulation of the human skin, the number of C-afferent terminals are higher those of Aδ-endings (Bragard et al., 1996).

Nociceptive axons terminate in the **dorsal horn of the spinal cord** or in the spinal trigeminal nucleus in the brainstem. In the dorsal horn, Aδ-axons terminate in both superficial (lamina I) and deeper (lamina V) layers, whereas C-fibers project to lamina II and connect to second-order nociceptive neurons in lamina I via interneurons. Most of neurons is lamina I respond exclusively to noxious stimuli (nociceptive-specific neurons), while the so-called wide dynamic range neurons are predominantly found in lamina V, have large receptive fields and respond to both painful and non-painful mechanical stimulation, the latter conveyed by large-diameter myelinated Aβ-fibers. Lamina I also contains non-nociceptive neurons that receive modality-specific information related to thermoreception (Craig and Andrews, 2002). Laminae VII and VIII also contain neurons that respond to noxious stimuli, but their response properties are much more complex, probably due to the fact that primarily lamina VII neurons receive polysynaptic inputs from superficial layers. The axons of all projecting neurons in the dorsal horn cross the midline at the anterior white commissure in the spinal cord and ascend towards higher brain regions forming multiple pathways: the spinothalamic, cervicothalamic, spinoreticular, spinomesencephalic and spinohypothalamic tracts.

The **spinothalamic tract** is the major ascending pathway, which is located in the anterolateral part of the spinal cord white matter. It is formed by axons of both nociceptive-specific and wide dynamic range neurons. The lesion of this pathway causes severe reductions in

nociception and thermoreception. Based on their anatomy, functional properties and phylogenetic appearance, this pathway can be traditionally divided into the so-called neospinothalamic and paleospinothalamic pathways. The phylogenetically more recent neospinothalamic pathway is located ventrally in the white matter of the spinal cord and contains axons of neurons situated in laminae I and V of the dorsal horn that project to lateral complex of thalamic nuclei. This pathway is primarily responsible for the sensory-discriminative aspects of pain (i.e. coding of location, intensity and duration of pain). The phylogenetically older paleospinothalamic pathway is highly developed in non-primate mammals as well. It is located laterally in the white matter of the spinal cord and projects to the medial nuclear group of the thalamus. These nuclei and connected structures are considered to be involved in the motivational-emotional reactions to painful stimuli.

In the head region, nociceptive information is primarily conveyed by the trigeminal nerve (and to a much lesser degree by cranial nerves VII, IX and X) to the spinal trigeminal nucleus in the lower brainstem. This nucleus is considered to be the upper continuation of superficial layers of the spinal dorsal horn. From here, axons forming the **anterior trigeminothalamic tract** decussate and project to lateral and medial thalamic nuclei.

The **cervicothalamic tract** is composed of axons of neurons in lateral cervical nuclei in the upper two cervical segments that project to certain thalamic nuclei.

The **spinoreticular tract** is another important ascending nociceptive pathway in the spinal cord. It is located in the anterior portion of the white matter, contains both ipsi- and contralateral fibers from laminae VII and VIII and terminates in the medial reticular formation. This pathway contributes to vegetative reactions to painful stimuli, such as controlling cardiovasular responses or arousal changes. Moreover, by its connections with other brainstem nuclei (see below), the reticular formation is also believed to contribute to descending pain modulation.

Many supraspinal regions exhibit control over pain perception, the majority of which modulate the activity of the ascending nociceptive pathways that primarily arise from wide dynamic range neurons in deeper layers of the spinal cord. These structures form the **descending pain modulatory system**, which may produce both pro- and antinociceptive effects.

The main destination of axons forming the **spinomesencephalic tract** is the **periaqueductal gray matter** (PAG) in the mesencephalon. Given that the PAG contains a high concentration of μ-opioid receptors and that its electrical stimulation induces profound analgesia and inhibits the firing of nociceptive neurons in laminae I and V, the PAG is considered as one of the key structures of the endogenous antinociceptive system of the brain. This effect is not primarily due to direct connections with neurons in the dorsal horn, but rather established via serotoninergic neurons in the **nucleus raphe magnus** and noradrenergic neurons in the **locus coeruleus**. The nucleus raphe magnus might not only inhibit ascending pain-related activity, but also facilitate it via different serotonin receptors, which points towards the complex neuropharmacological properties of these brainstem nuclei (for review see Millan, 2002).

In addition to the PAG, some fibers of the spinomesencephalic tract project to the superior part of the reticular formation and to the **parabrachial nucleus** (PBN) of the pons. The PBN has dense connections with the amygdala and the hypothalamus, and therefore it is thought to integrate pain-related information with autonomic, homeostatic and affective functions. Also, the PBN sends axons to superficial laminae of the dorsal horn, and suppresses the responses of these neurons to both nociceptive and non-noxious stimulation (Yoshida et al., 1997).

Another important nucleus of the descending pain modulatory system, the **rostral ventromedial medulla** (RVM) receives extensive direct inputs to both superficial and deep layers of the dorsal horn. It is implicated in both facilitation and inhibition of pain perception, probably via serotoninergic and opioidergic mechanisms.

Finally, the **nucleus tractus solitarius** and the **dorsal reticular nucleus** are located in the medulla oblongata, interconnected with hypothalamic and other brainstem nuclei and both send direct efferents to the dorsal horn. They are regarded as interface nuclei between somatosensory and visceral stimuli (Millan, 2002) and also involved in both descending facilitation and inhibition.

The **hypothalamus** receives direct spinal input via the **spinohypothalamic tract** that originates from laminae I, V and VIII of the dorsal horn. As a major centre of autonomic regulation, it is believed to be involved in vegetative and affective responses to painful stimuli and mediating the descending control of pain, which is partially based on the finding that some hypothalamic nuclei (including the medial preoptic nucleus) send axons to the PAG and RVM.

The **thalamus** is an extremely complex structure subserving many functions ranging from processing and relaying of sensory and motor information to cognitive functions such as language or memory (Schmahmann, 2003). The neospinothalamic and paleospinothalamic pathways (and their trigeminothalamic equivalents) project to the lateral and medial thalamic nuclei respectively. The lateral nuclear group is composed of the ventral posterior medial (VPM), ventral posterior lateral (VPL), ventral posterior inferior (VPI) nuclei and the posterior part of the ventromedial nucleus (VMpo). Lesion of these nuclei can produce severe central pain with allodynia contralateral to the site of lesion (Montes et al., 2005). The medial nuclear group comprises the central lateral nucleus (CL) of the intralaminar complex, the ventrocaudal part of the medial dorsal nucleus (MDvc) and the parafascicular nucleus (Pf). Based on the projection sites from lateral and medial thalamic nuclei to cortical areas, pain-related brain regions have been divided into the lateral and medial pain systems (Albe-Fessard et al., 1985). In the lateral system information is conveyed to the primary and secondary somatosensory cortices and it is considered to be involved in processing of sensory-discriminative aspects of pain. The medial system on the other hand is responsible for affective-cognitive and motor components of pain perception, and is composed of the medial nuclear group of the thalamus along with the anterior cingulate cortex and certain regions of the prefrontal cortex. Due to its complex response properties to painful stimulation, the insular cortex is regarded as a region that occupies the space between the lateral and medial pain systems. Although this dichotomy is probably an oversimplification, it was introduced to provide an overview of the distributed brain regions that appear to have similar roles in pain perception.

The **amygdala** complex of the limbic system is a group of nuclei implicated in the recognition of aversive stimuli (Adolphs, 2002), episodic emotional memory and fear conditioning (LaBar and Cabeza, 2006). It has dense connections with thalamic nuclei, the ventral striatum and medial frontal regions such as the cingulate cortex, but also receives spinal nociceptive input via the PBN (Bernard et al., 1996). Neuroimaging studies reported increased right amygdala hemodynamic response to experimentally evoked vascular pain (Schneider et al., 2001), while in another study amygdala responses were reported not only to noxious, but also to not noticed, subthreshold stimuli, which according to the authors' interpretation, might indicate the role of amygdala in coding stimulus uncertainty (Bornhövd et al., 2002).

Cortical pain processing

For many decades it was questionable whether specific pain-related cortical sites exist. In their pioneering observations, Head and Holmes noted that even the damage of the primary somatosensory cortex did not alter pain sensation (Head and Holmes, 1911). Also, Penfield and Bolderey could only rarely elicit pain sensations by direct cortical stimulation (Penfield and Boldrey, 1937). With the advent of neuroimaging and electrophysiological studies from the early 1990's it became obvious that certain cortical regions are commonly activated by noxious stimuli, including the primary and secondary somatosensory cortices (S1 and S2), the anterior cingulate cortex, the insular cortex and certain prefrontal regions. These are frequently referred to as the 'pain matrix' (Ingvar, 1999).

Although it was debated for a long time, several streams of evidence support the role of **S1** in acute pain perception. According to recent views of nociception (Treede et al., 1999), the S1 is part of the lateral pain system and receives input from lateral thalamic nuclei, predominantly from the VPL (Gingold et al., 1991) and VMpo (Montes et al., 2005). Nociceptive cells in S1 are much less frequent than those responding to tactile stimuli, and are found at the borders of Brodmann areas 3b-1 and 1-2. These neurons are somatotopically arranged and their activity correlates with the duration and intensity of painful stimulation (Chudler et al., 1990). In line with this, a positron emission tomography (PET) study described somatotopic organisation of signal intensity evoked by intracutaneous injections of capsaicin to the hand and foot (Andersson et al., 1997), while another study reported regional blood flow changes to be proportionate to the intensity of noxious stimuli (Coghill et al., 1999), which was later confirmed by a functional magnetic resonance imaging (fMRI) study (Bornhövd et al., 2002). Moreover, in a patient with unilateral postcentral stroke, painful laser stimulation produced unpleasant sensations with the inability to localise the area of stimulation (Ploner et al., 1999a). Along with other findings, these studies all confirm the role of S1 in intensity coding and spatial discrimination of painful stimuli.

Along with the posterior insular cortex, the **S2** is the only cortical region the direct electrical stimulation of which elicits pain sensation in humans (Mazzola et al., 2006). This region is localised in the parietal operculum and receives direct thalamic afferentation from the VPI nucleus. This direct thalamic input to S2 and the fact that activations of S1 and S2 after painful

stimulation occur nearly simultaneously (Ploner et al., 1999b) indicate that unlike tactile processing, the S1 and S2 activate in a parallel rather than sequential manner. Neurons in S2 have large, bilateral receptive fields, show closely spaced somatotopy and seem to encode stimulus intensity to a lesser degree than S1 neurons (Dong et al., 1994). Some of the S2 neurons have multimodal response properties and they respond to threatening visual stimuli as well (Dong et al., 1994). The S2 is one of the cortical areas most frequently found to be activated during noxious stimulation in human neuroimaging studies. Due to their close proximity, hemodynamic responses in the S2 are difficult to separate from the pain-sensitive regions of the insula, and hence they are commonly referred to as the parasylvian or operculoinsular cortex/region. Nociceptive neurons in the S2 project to temporal limbic structures and it has been hypothesized that the S2 plays an important role in pain-related learning and memory (Friedman et al., 1986). Furthermore, since lesions of the parietal operculum along with the posterior insula has been reported to cause elevated pain thresholds (Greenspan et al., 1999) and deficits in recognition of the painful nature of stimulation (Ploner et al., 1999a), the S2 is regarded as a key structure of the lateral, sensory-discriminative pain system.

Modern views on the function of the **insular cortex** emphasize its central role in monitoring and integration of interoceptive and exteroceptive information and representing all feelings of the body, which is essential for the emergence of self awareness (Craig, 2009). The insula receives direct thalamic input from the VMpo nucleus and also from S2 (Craig, 2002). The posterior granular portion is primarily related to auditory, visual and tactile perception, whereas the anterior agranular part is involved in olfactory, gustatory and pain perception and also is linked to limbic-autonomic functions (Augustine, 1996). Nociceptive insular neurons show multimodal characteristics (Hanamori et al., 1998) and are spatially separated from tactile neurons. Insular hemodynamic responses correlate with pain intensity (Coghill et al. 1999; Bornhövd et al., 2002) and they are modulated by the side of stimulation and attention (Brooks et al., 2002). Functional connectivity analysis revealed that activation in the anterior insula is coupled with the medial pain system (medial prefrontal areas) and to the S2, whereas the posterior insula is functionally related to the S1 and the primary motor cortex (Peltz et al., 2011). In line with this, lesion of the anterior insula reduces affective evaluation of pain without causing alterations of pain threshold (Greenspan et al., 1999). Insular lesions were also

found in patients with pain asymbolia, a condition characterized by lack of motor responses to painful stimulation and inadequate or no emotional reactions (Berthier et al., 1988). Activation of the anterior insula has consistently been reported in chronic pain patients (Apkarian et al., 2005), but has also been associated with the development of anxiety, depression and somatisation (Craig, 2009). Since this region is connected to structures of the descending pain modulatory system (such as the PAG, PBN and RVM), the insula is likely to be involved in the co-occurrence of affective symptoms and chronic pain in many patients.

It has been recognised for a long time that anterior cingulotomy causes a marked reduction in the unpleasantness of intractable pain, but does not affect the detection of noxious stimuli (Ballantine et al., 1967). According to the four region model of the **cingulate cortex**, it can be divided into the anterior, mid-, posterior and retrosplenial cingulate cortex (Vogt, 2005). The anterior cingulate cortex (ACC) has been implicated in several neural processes ranging from endocrine and autonomic functions to regulation of emotion and goal-directed behaviour (Devinsky et al., 1995). This region is also one of the cortical areas found to be most frequently activated in pain studies (Treede et al., 1999; Schnitzler and Ploner, 2000). In line with this, nociceptive neurons with large receptive fields have been found in the rabbit (Sikes and Vogt, 1992) and in the human ACC (Hutchison et al., 1999). It has been also shown that Brodmann area 24 of the ACC receives thalamic afferents from nuclei implicated in nociception (mainly nuclei CL, Pf and MDvc; Vogt, 2005). The ACC is most frequently divided into the sub- and perigenual regions. The subgenual part has projections to the amygdala, PAG and PBN and it is perhaps the most promising candidate for the deep brain stimulation treatment of pharmacoresistant depression (Mayberg et al., 2005). On the contrary, activity in the perigenual region has been found to correspond to C-fiber mediated second pain (Ploner et al., 2002). In an fMRI study, separate regions of the perigenual ACC were found to encode stimulus and subjective pain intensity, whereas hemodynamic changes in the anterior portion of the ACC were related to attention to pain and working memory (Büchel et al., 2002). There is ample evidence that hemodynamic responses to pain in ACC regions are modulated by contextual and attentional factors. Activation of the ACC was increased by paying selective attention to painful stimulation (Peyron et al., 1999), but also in studies of pain illusion (Craig et al., 1996) and empathy for pain (Singer et al., 2004). Also, regional blood flow in the ACC correlated with the magnitude of hypnotic modulation of pain unpleasantness in a PET study

(Rainville et al., 1997), while activity in the perigenual ACC region has been reported to be proportionally reduced to the magnitude of placebo-induced pain relief (Wager et al., 2004). Covariations between ACC and brainstem activity were reported for placebo- and distraction-related antinociceptive effects (Petrovic et al., 2002; Valet et al., 2004), further supporting the role of ACC in the cognitive-affective evaluation of pain and top-down regulation of ascending nociceptive pathways. Indeed, high binding of opioid ligands has also been shown in the ACC (Jones et al., 1991). In contrast to perception of acute pain, the activation of the ACC has been reported to be rather decreased in patients with chronic pain, which has been suggested to be secondary to maladaptive changes characteristic of chronic pain (Peyron et al., 2000).

The **dorsolateral prefrontal cortex** (DLPFC) is a cortical region commonly associated with working memory and executive control (Miller and Cohen, 2001). However, it has been recently emphasized that this area is also important in cognitive-emotional integration (Pessoa, 2008). In line with this, greater hemodynamic changes in the DLPFC during anticipation of experimental pain correlated with the degree of placebo-related pain relief in an fMRI study (Wager et al., 2004). Also, activity in the DLPFC correlated with the magnitude of cognitive control over experimental pain (Lorenz et al., 2003) and predicted the intensity of suggestion-induced pain (Raij et al., 2009). Interestingly, reduced pain sensation in patients suffering from Alzheimer's disease has been linked to deterioration in frontal lobe functions (Benedetti et al., 2006) and reduced functional connectivity between the right DLPFC, the hypothalamus and the PAG, measured with fMRI (Cole et al. 2011). Several streams of evidence support the view that the DLPFC is also involved in the development of chronic pain. Reduced gray matter densities in bilateral DLPFC and the right thalamus were reported in patients with chronic back pain (Apkarian et al., 2004), while activity in the DLPFC and ACC was also shown to be significantly increased in patients suffering from sympathetically mediated chronic pain (Apkarian et al., 2001). These findings highlight the role of the frontal cortex and cognitive functions in the evaluation of pain and development of chronic pain syndromes.

During the last two decades, the function of the **primary motor cortex** (M1) has been implicated in non-motor processes such as social cognition (Gallese et al., 2004; but see also: Jacob and Jeannerod, 2005) and language comprehension (Pulvermuller and Fadiga, 2010). Regarding pain perception, neuroimaging studies with healthy subjects have shown that experimentally induced pain increases hemodynamic responses in the M1 (Gelnar et al., 1999;

Tracey et al., 2000; Nash et al., 2010), although it has been hypothesized that this activation was rather a pain epiphenomenon related to movement suppression than a correlate of pain evaluation *per se* (Apkarian et al., 2005).

The use of laser-evoked potentials for studying pain perception

Electroencephalography (EEG) and magnetoencephalography (MEG) are non-invasive methods suitable for direct recording of neural activity with excellent temporal resolution. The resulting signal is a complex waveform composed of several frequency bands reflecting changes of the extracellular electromagnetic field that is caused by postsynaptic potentials of cortical neurons. In order to evaluate neural activity evoked by experimental events, the signal-to-noise ratio has to be improved, which is most usually achieved by averaging single-trial responses. The resulting event-related potentials (in EEG) and fields (in MEG) are informative of the temporal evolution of synchronized cortical responses and hence they can be used to assess several physiological and pathological processes.

In the field of pain research, numerous methods are suitable to experimentally evoke pain. These include intensive thermal or electrical stimulation, induction of transient ischemia, intradermal injection of capsaicin or other irritating substances and even mechanical distension of viscera such as the rectum. The problem with the above techniques is that they either stimulate somatosensory Aβ-fibers as well or the onset and length of stimulation can hardly be controlled. Lasers on the other hand provide short and selective stimulation of Aδ and C nociceptive nerve endings and hence they are more suitable for the investigation of pain-related neural processes. The most frequently used lasers are CO_2, Tm-YAG or argon lasers, all of which stimulate the skin or mucosa in a controlled manner. Depending on the type of the laser, stimulation, duration varies between 1-100 milliseconds, and the intensity is usually 1.5-2 times the pain threshold (which is around 10-14 mJ/mm^2). The most common side effect is a mild and harmless redness of the skin that heals completely within few days (Treede et al., 2003).

When combined with EEG, laser evoked potentials (LEPs) can be recorded from central and temporal scalp locations that are informative of the peripheral conduction of pain-evoked nerve impulses and their processing in distributed structures of the pain matrix. Based on their latencies, LEP waveforms can be divided into early (N1/P1), late (N2 and P2) and ultra-late

LEPs. Their peak latency depends on the site and duration of stimulation, while their amplitude reflects the activity of underlying cortical structures. The peak latency of the early N1/P1 potential is around 160 milliseconds. The N1 typically appears at bitemporal scalp sites (electrodes T3 and T4, referenced to Fz), while its positive counterpart, the P1 component has a maximum at the vertex. Their brain generators are most probably in the operculoinsular region (Garcia-Larrea et al., 2003). Although early potentials are usually regarded as pre-perceptual, there is evidence that the N1 amplitude correlates with subjective pain intensity (Iannetti et al., 2005). The late N2 and P2 potentials are maximal at the vertex (electrode Cz referenced to linked mastoids), with latencies of 210/330 and 250/380 milliseconds in the case of hand and foot stimulation respectively. Generators of the N2 potential lie in the operculoinsular cortices bilaterally and in the S1 contralateral to the site of stimulation (Valeriani et al., 1996; Ohara et al., 2004). Regarding the P2 potential, most source localizing studies reported it to be generated in the ACC (Garcia-Larrea et al., 2003). Although the peak-to-peak N2-P2 amplitude correlates with the reported intensity of perceived pain (Garcia-Larrea et al., 1997), this is more likely due to modifications of the N2 amplitude (Iannetti et al., 2005). In addition to the perceived intensity of pain, the N2-P2 complex is also modulated by changes in arousal and by spatial attention (Beydoun et al., 1993; Garcia-Larrea et al., 1997; but see also: Towell and Boyd, 1993).

While early and late LEP components reflect Aδ-fiber-mediated neural responses, ultra-late LEPs appearing 700-1300 milliseconds post-stimulation are associated with C-fiber stimulation. These components are usually not detectable with conventional LEP techniques, probably because of central inhibitory effects induced by Aδ-afferents (Treede et al., 2003). Several special techniques have been reported to be useful for recording these components (Bragard et al., 1996; Kakigi et al., 2003).

Invasive motor cortex stimulation

Deep brain stimulation is an effective method for alleviating symptoms of chronic neurological disorders such as Parkinson's disease (Krack et al., 2003) and dystonia (Vidailhet et al., 2005). Stimulation of the PAG or the lateral nuclei of the thalamus has been successfully used for reducing central pain in post-stroke patients (Owen et al., 2006), and the intervention is

recommended by the European Federation of Neurological Societies (EFNS) for the treatment of pain after peripheral lesions such as phantom limb pain (Cruccu et al., 2007).

In the case of cortical stimulation it might seem surprising that the M1 is the main target in the invasive treatment of chronic neuropathic pain. Twenty years ago Tsubokawa and colleagues discovered that the electric stimulation of the cat motor cortex reduces the hyperactivity of thalamic neurons after spinothalamic deafferentation (Tsubokawa et al., 1991). This finding lead to the introduction of motor cortex stimulation (MCS) for pain control in humans, which is still one of the most powerful invasive interventions for alleviating certain forms of chronic pain (Tsubokawa et al., 1993). During the procedure a grid of epidural electrodes is implanted over the central sulcus, predominantly above the precentral gyrus (Brown and Barbaro, 2003). The intactness of the somatosensory cortex is not necessary for successful treatment (Peyron et al., 1995). According to recent EFNS guidelines, MCS is recommended in central post-stroke pain and neuropathic facial pain (Cruccu et al., 2007).

Despite its efficacy, the underlying mechanism of MCS is still not understood. Early PET studies did not find changes in regional blood flow under the site of stimulation or in the somatosensory cortex (Garcia-Larrea et al., 1999). The authors also reported stable somatosensory event-related potentials during stimulation, which also confirms that the effect of MCS is not due to excitability changes in the S1. Nevertheless, significant blood flow increases were reported in the ipsilateral ventral-lateral and medial thalamus, perigenual ACC, orbitofrontal cortex, anterior insula and the upper brainstem. While blood flow increase in the thalamus evolves relatively quickly, it only develops slowly in the perigenual ACC and may last up to 30 minutes post-stimulation (Garcia-Larrea et al., 1999). The signal increase in the ACC and orbitofrontal cortex was found to be higher in patients with good therapeutic responses, suggesting that these regions are key areas of pain relief. In line with this, functional connectivity analysis revealed that blood flow changes in the ACC correlate with those measured in the PAG, basal ganglia and lower pons (Peyron et al., 2007). According to the current model of its mechanism of action, MCS triggers rapid activation of thalamic nuclei, which in turn leads to reduced affective evaluation of pain by modulating ACC and orbitofrontal activities and causing top-down facilitation of the PAG and the descending pain modulatory system (Garcia-Larrea and Peyron, 2007). This antinociceptive effect is probably

due to increased opioid release in the anterior portion of the midcingulate gyrus, the lateral prefrontal cortex, the PAG and the cerebellum (Maarrawi et al. 2007).

Non-invasive methods of brain stimulation

Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) as a tool for non-invasive modulation of cortical excitability was introduced in 1985 (Barker et al., 1985). The method is based on electromagnetic induction, according to which a rapid, high-voltage alternating current is generated in a coil, producing a strong magnetic field that lasts for a few milliseconds. In the case of the TMS stimulator, the coil is located in a metal housing that is placed above the cortical area to be stimulated. Figure-of-eight coils are the most widely used coils since they produce maximal magnetic fields at the intersection of the two loops and hence enable relatively precise targeting and focal stimulation. The generated magnetic field penetrates the scalp, the skull, meninges and the cerebrospinal fluid and induces electric currents in the cortex. This current stimulates the axons of neurons rather than cell bodies (which have higher stimulation thresholds), and generates action potentials. Although the magnetic field does not reach subcortical structures, several cortical and subcortical regions might be stimulated indirectly via these axons. Therefore, the anatomical connectivity of a cortical region must always be taken into account when interpreting the results of the stimulation.

The single-pulse TMS is not suitable for inducing long-lasting excitability changes in the brain. Repetitive TMS (rTMS) on the other hand is appropriate for this purpose, where the induced effects largely depend on the parameters of stimulation, such as stimulation intensity, orientation of the coil, frequency of stimulation, number of applied pulses and the repetition of stimulation for days or even weeks. While single-pulse TMS produces quantifiable changes (e.g. TMS applied above the M1 causes involuntary muscle contractions in the corresponding muscles), for safety reasons, rTMS pulses are of subthreshold intensity. The intensity is frequently determined as the ratio of the passive or active motor threshold, measured at rest or voluntary muscle contraction respectively. Depending on the protocol, the after-effects of rTMS might outlast the period of stimulation for hours or even be additive when applied for consecutive days. The frequency of stimulation is a central parameter as low frequencies

(usually at 1Hz) exhibit inhibitory effects, while 10Hz or higher frequencies facilitate the excitability of the stimulated area. This is believed to be due to the lower stimulation threshold of inhibitory GABAergic interneurons that are excited at 1Hz rTMS, whereas stimulation at higher frequencies recruits excitatory pyramidal cells as well (Ridding and Rothwell, 2007).

The molecular mechanisms of rTMS are not entirely clear, but are probably related to long-term potentiation (LTP) or depression (LTD), depending on the pattern of stimulation (Chen et al., 1997; Pascual-Leone et al., 1998). In line with this, N-methyl-D-aspartate (NMDA) glutamate receptor agonists such as dextromethorphan or memantine block the after-effects of rTMS (Stefan et al., 2002; Huang et al., 2007).

The efficacy of rTMS was first demonstrated on the motor cortex: high frequency stimulation resulted in larger motor-evoked potential (MEP) amplitudes (Pascual-Leone et al., 1994a), whereas 25 minutes of 1Hz rTMS reduced MEP amplitudes for 30 minutes after stimulation (Chen et al., 1997). The efficacy of rTMS was demonstrated on sensory (Amassian et al., 1993) and cognitive functions as well (Pascual-Leone 1994b).

In addition to rTMS at constant frequencies, new stimulation protocols have been described recently, such as certain forms of theta-burst stimulation (TBS). This stimulation pattern is based on the observation that four consecutive pulses presented rapidly (at 100Hz) repeated at theta frequency (5Hz) induces LTP in the CA1 region of the hippocampus (Larson et al., 1986). Huang and colleagues adapted this protocol to rTMS and introduced continuous and intermittent theta-burst stimulation that have been reported to cause robust excitability changes when applied to the M1 (Huang et al., 2005). In particular, continuous TBS (cTBS) caused significant inhibition for up to 60 minutes post-stimulation, which was much longer than those of standard low frequency rTMS protocols.

Risk and safety guidelines for TMS have been published more than a decade ago (Wassermann et al., 1998). Single pulses are accompanied by a relatively loud sound and a tactile stimulus under the coil, which is caused by the direct stimulation of scalp muscles. Most common side effects are transient, mild headache and nausea; epileptic seizures were also reported at high frequency rTMS. The use of TMS is absolutely contraindicated in people with intracranial magnetisable metal implants and cardiac pacemakers, while certain conditions (e.g. pregnancy, taking drugs that reduce seizure threshold) should be subject of careful consideration.

As for every medical intervention, placebo effects should always be considered when interpreting the results. Pain perception is special in this regard, because it is well-known that evaluation of pain can be modified by expectation, emotional state and attention. Therefore, laboratory and clinical studies should evaluate the effect of real rTMS in comparison to a sham stimulation. Given that TMS pulses cause both audible and tactile stimuli, the application of sham stimulation is not easy. The two most widespread methods are either the rotation of the coil by 90 degrees (i.e. its plane becomes perpendicular to the scalp surface) or by using a sham coil with a thicker metal housing that significantly reduces the intensity of the magnetic field (Lisanby et al., 2001).

rTMS has been successfully used to alleviate symptoms of depression, Parkinson's disease, dystonia, stroke or epilepsy (for review see: Ridding and Rothwell, 2007). Given that MCS was already known to exhibit antinociceptive effects, the first rTMS studies in patients with chronic pain have also been carried out by the stimulation of M1 contralateral to the site of the pain. Although only one of the two patients responded to the stimulation (Migita et al., 1995), the applied frequency in that study was very slow (0.2Hz), and later studies confirmed the efficacy of 10Hz or 20Hz rTMS both in central and peripheral neuropathic pain syndromes (Lefaucheur et al., 2001a; Khedr et al., 2005; André-Obadia et al., 2006). High frequency stimulation of M1 does not occur immediately after stimulation: the majority of studies reported antinociceptive effects only after 2-4 days of daily sessions of rTMS. The duration of therapeutic effects can be remarkably long, lasting from weeks to more than a year (Lefaucheur et al., 2001b; 2004). Interestingly, while MCS is effective by stimulating the cortical area corresponding to the painful site, in the case of rTMS, areas adjacent to the cortical representation of the painful region seem to be the most effective target sites (Lefaucheur et al., 2006a).

In the case of experimentally induced acute pain the effects of low and high frequency rTMS over the M1 seem to depend on the type of noxious stimulation. C-fiber mediated acute pain induced by intradermal capsaicin administration could be attenuated by 1Hz rTMS over the motor cortex (Tamura et al., 2004a), whereas it increased Aδ fibre-mediated laser-induced pain in another study (Tamura et al., 2004b). Similarly, controversial effects were observed after 10Hz and 20Hz rTMS (Summers et al., 2004; Yoo et al., 2006; Mylius et al., 2007; Valmunen et al., 2009). This discrepancy is probably due to differences in the applied parameters of

rTMS, techniques of pain induction, methods for evaluation of subjective pain and ways of determining the exact site of motor cortex stimulation.

Transcranial direct current stimulation

TMS is regarded as both a neurostimulatory and neuromodulatory technique because depending on stimulation parameters it can both generate action potentials in the axons and change the excitability of cortical neurons for a longer time period. In contrast, transcranial direct current stimulation (tDCS) is a sole neuromodulatory technique.

The first animal experiments investigating the effects of cortical stimulation with weak direct currents were carried out in the 1960's (Bindman et al., 1964; Purpura and McMurtry, 1965). These studies revealed that depending on the parameters of stimulation, direct current can cause shifts in the membrane potentials of neurons and consequently change their excitability. The application of this technique in humans was rediscovered by two independent research groups, who showed that electrodes placed on the scalp above the motor cortex reduce MEP amplitudes evoked by single-pulse TMS (Priori et al., 1998; Nitsche and Paulus, 2000). During the procedure, two electrodes (sized between 20-35 cm^2) connected to a stimulator are inserted into a wet sponge pouch and placed on the scalp. The intensity of the current is usually 1-2 mA, which is flowing from the anode towards the cathode for maximally 20 minutes. Although some degree of the current shunts through the scalp, the majority enters the brain (Miranda et al., 2006) and causes excitability changes that can last up to an hour, depending on the parameters of stimulation (Nitsche and Paulus, 2000; 2001). The effect can even be longer if the stimulation is repeated on a daily basis for 1-2 weeks. Anodal stimulation depolarizes, while cathodal hyperpolarizes the stimulated neurons, hence modulating the frequency of action potentials and causing facilitation or inhibition respectively. NMDA-receptor antagonists prevent the induction of long-lasting effects of both anodal and cathodal stimulation, and the effect of anodal stimulation can also be reduced with the voltage-dependent sodium channel blocker carbamazepine and the calcium channel blocker flunarizine (Nitsche et al., 2003). According to a recent study, the D2-receptor blocker sulpride completely abolished the effects of anodal and cathodal tDCS, while the D2-receptor agonist pergolide exhibited opposite effects (Nitsche et al., 2006). The effects of anodal tDCS can be enhanced with the $GABA_A$-receptor agonist lorazepam (Nitsche et al., 2004a), the NMDA-receptor

partial agonist D-cycloserine (Nitsche et al., 2004b) and the catecholamine re-uptake blocker amphetamine (Nitsche et al., 2004c). The above findings point towards a very complex mechanism of action in which the proper functioning of ion channels is essential and the long-term effects are probably due to LTP- and LTD-like neuroplastic changes.

Various tDCS protocols have been shown to efficiently modulate the excitability to motor (Nitsche and Paulus, 2001), visual (for a review see Antal and Paulus, 2008) and somatosensory cortical areas (Matsunaga et al., 2004), but tDCS also facilitates probabilistic classification learning (Kincses et al., 2004) and working memory (Fregni et al., 2005) when applied over the prefrontal cortex. When compared to TMS, tDCS provides a much less focal stimulation, which is due to the large surface of the electrodes and to the fact that the applied current flows from one electrode towards the other and therefore stimulates all the cortical regions in between. Moreover, regardless of the electrode montage used, one electrode exhibits facilitatory, while the other inhibitory effects.

tDCS is a safe method of brain stimulation; there are no known long-lasting adverse effects. The most common side-effect is a mild burning-itching sensation on the skin under the electrodes, which lasts only for a few minutes. Intensive and/or long stimulation might cause burning of the skin, but fatigue, headache, nausea and insomnia have also been reported (Poreisz et al., 2007).

Sham stimulation is much more easily applied than in the case of TMS, since tDCS is not accompanied by intensive tactile and somatosensory stimuli. Electrodes are placed on the scalp in the same manner as for real stimulation and the current is switched on for a few seconds to provide the initial itching feeling and is turned off afterwards.

As for rTMS, tDCS has also been investigated for its potential antinociceptive effects in patients with chronic pain. One electrode is most commonly placed above the motor cortex contralateral to the side of pain or above the dominant side, while the other electrode is placed above the supraorbital region contralateral to the stimulated motor cortex. The initial studies have investigated the efficacy of anodal stimulation in patients with spinal cord injury, fibromyalgia (Fregni et al., 2006a; 2006b), and in a heterognous group of chorinc pain patients (Antal et al., 2010). In these studies the current intensity of 1-2 mA was applied for 20 minutes on five consecutive days. Patients reported reduced pain sensation from the second treatment

day and the effect of stimulation proved to be additive on subsequent treatment days. Other studies have shown that anodal tDCS over the motor cortex alleviates pharmacoresistant visceral pain (Silva et al., 2007), chronic pelvic pain (Fenton et al., 2009), neuropathic pain in patients with multiple sclerosis (Mori et al., 2010) and pain in a mixed group of patients with post-stroke pain, trigeminal neuralgia, back pain or fibromyalgia (Antal et al., 2010).

Aims of the studies

The aim of our first study was to investigate the effect of cTBS applied above the motor cortex on laser-induced acute pain perception and LEP parameters in healthy adult volunteers. Given the controversial antinociceptive effect of low and high frequency rTMS in Aδ-fiber mediated pain in healthy subjects, we chose the most effective TBS protocol (cTBS), which exhibits robust and relatively long-lasting inhibition of the motor cortex. We hypothesized that when compared to sham stimulation, cTBS would alter pain thresholds and LEP amplitudes on the hand contralateral to the side of motor cortex stimulation. For this purpose, we first carried out a psychophysical experiment (Experiment 1), where laser intensity was systematically increased and decreased. This enabled us to examine the efficacy of cTBS for three different laser intensities, evoking warm sensation, mild and moderate pain respectively. In the second experiment (Experiment 2), we aimed at inducing moderate pain in order to record LEPs before and after real and sham stimulation.

The second study was designed to assess the efficacy of anodal and cathodal tDCS of the motor cortex on laser-induced pain thresholds and LEP amplitudes. As for cTBS, we hypothesised that tDCS (primarily anodal stimulation) would significantly modulate pain thresholds, which would also be reflected in reductions of LEP amplitudes. We carried out a psychophysical experiment (Experiment 3) for assessing the effects of anodal and cathodal tDCS on warm sensation, mild, moderate and intensive pain and an EEG experiment (Experiment 4) during the course of which always moderate pain was induced to evoke reliable LEPs for analysis.

Methods

Subjects

Thirteen healthy right-handed volunteers (8 male and 5 female) between 18 and 30 years took part in Experiment 1, while 10 right-handed subjects (5 male and 5 female) aged between 20-30 years participated in Experiment 2. Regarding the tDCS experiments, 16 (5 male and 11 female) subjects took part in Experiment 3, while 10 volunteers (5 male and 5 female) participated in Experiment 4. There was no overlap between the participants of the four experiments. All participants were informed about all aspects of the experiments and only those who signed an informed consent were included in the study. We conformed to the Declaration of Helsinki and the experimental protocol was approved by the Ethics Committee of the University of Göttingen. None of the participants suffered from chronic pain syndromes nor took any medication regularly. None had a history of neurological or psychiatric illness. All participants participated in the two cTBS (one sham and one real stimulation) or the three tDCS (one sham, one anodal and one cathodal stimulation) sessions within the experiment that were separated by at least 5 days in order to avoid the effect of interference. The order of the real and sham sessions was counterbalanced across subjects.

Laser stimulation and pain evaluation

Pain was elicited using a Tm:YAG laser system (WaveLight Laser Technologie AG, Erlangen, Germany). The thulium laser emits near-infrared radiation (wavelength 2000 nm, pulse duration 1 ms, laser beam diameter 7 mm) with a penetration depth of 360 μm into the human skin. It also allows the emitted heat energy to be precisely restricted to the termination area of primary nociceptive afferents without affecting the subcutaneous tissue. The distal handpiece of the laser was positioned 30 cm from the radial part of the dorsal surface of the hand. The skin temperature of the stimulated area was checked prior to every switch of hands and corrected with a heating lamp when below 35°C. We stimulated slightly different spots in a 5x5 cm square for each measurement to reduce receptor fatigue or sensitization through skin overheating (Treede et al., 2003). The ears of the subjects were always plugged and white noise was presented during the measurements to avoid auditory artefacts due to laser stimulation. In

both experiments the right hand was stimulated first in half of the cases; in the other half we started with the left hand.

We used a numeric analogue score (NAS) to assess the subjective intensity of pain. We instructed the subjects to pay attention to the laser stimuli and to rate the perceived pain verbally with numbers (in Experiments 1 and 2: 0 for warm, 1-9 from mildest to most intensive pain; in Experiments 3 and 4: 1 for warm, between 1.1 and 1.9 from mildest to most intensive pain) about 2–3 seconds after each stimulation. Inter-stimulus interval varied between 8 and 15 seconds. In order to obtain reliable pain rating scores, the subjects were trained to get accustomed to the NAS. Prior to the experimental stimulation sessions they were presented a series of laser stimuli from 200 mJ to 800 mJ during which they had to evaluate the intensity of pain.

In the psychophysical experiments (**Experiments 1 and 3**) we applied two series of stimuli for each hand before (cTBS and tDCS), immediately after (cTBS and tDCS) and 30 minutes after stimulation (cTBS). We systematically increased laser intensity from 200 mJ (5.2 mJ/mm^2) in 50 mJ steps until subjects reported moderate pain. Then the laser energy was decreased from that intensity again in 50 mJ steps. This stimulation protocol was repeated twice. Hence we obtained four pain rating scores for each laser intensity prior to and following cTBS or tDCS for each hand and simulation type.

In the electrophysiological experiments (**Experiments 2 and 4**) we first determined the pain thresholds of both hands by applying laser stimuli from 200 mJ in 50 mJ steps. Pain threshold was defined at a laser energy level where subjects consistently perceived painful sensation between NAS scores 1.1–1.3. However, in order to get reliable LEP waveforms, we aimed to induce medium intensity pain by adapting the bioadaptive approach designed by Weiss and colleagues (Weiss et al., 1997). Therefore, we started with a laser intensity of 1.5–1.6 times of the threshold level during EEG recording and adjusted laser energy manually in order to keep the magnitude of pain between NAS scores 1.4–1.6. We delivered 40 laser pulses to each hand before and after cTBS and tDCS.

Theta-burst stimulation

In **Experiments 1 and 2**, real and sham cTBS was applied over the hand area of the left M1. In Experiment 1, we used a standard, figure-of-eight-coil (MC-B70 Butterfly Coil, Magventure

A/S, Farum, Denmark) and MagPro stimulator (Medtronic, Denmark) with an outer half-coil radius of 75 mm, while a figure-of-eight-coil with an outer half-coil radius of 90 mm (Dantec S.A., Skovlunde, Denmark) and a Magstim Super Rapid stimulator (Magstim Company Ltd., Whitland, Wales, UK) were used in Experiment 2. In both experiments, we applied a posterior-anterior current flow in the coil. Stimulus intensity was 80% of the active motor threshold (AMT) (Huang et al., 2005). For AMT determination, the coil was placed tangentially onto the scalp, with the handle pointing backwards and laterally 45° from the midline. MEPs of the right abductor digiti minimi muscle were recorded by Ag–AgCl electrodes in a belly-tendon-montage. This procedure was repeated before both real and sham stimulation conditions. The signals were amplified and filtered (1.59Hz–1kHz, sampling rate of 5kHz), digitalized with a micro-1401 AD converter (Cambridge Electronic Design, Cambridge, UK) and recorded by a computer using SIGNAL software (Cambridge Electronic Design, Cambridge, UK). AMT was defined as the minimum intensity at which at least 3 out of 6 consecutive stimuli elicited a MEP of a superior size (approximately 200 μV in amplitude) when compared to spontaneous moderate muscular activity. The pattern of cTBS consisted of bursts containing 3 pulses at 50Hz which were repeated at 200 ms intervals (i.e. 5Hz) for 40 seconds (resulting 600 pulses in total) continuously. In accordance with earlier studies (Huang et al., 2005), we stimulated above the position of electrode C3, which is situated above the primary motor cortex (Homan et al., 1987; Steinmetz et al., 1989). In a separate experimental session, sham stimulation was applied with the same cTBS protocol using a sham coil (MC-P-B70, Magventure A/S, Farum, Denmark) held at the same position. The participants were blinded concerning the type of the magnetic stimulation (real or sham).

Direct current stimulation

In **Experiments 3 and 4**, we applied anodal, cathodal and sham tDCS by a battery driven constant current stimulator (NeuroConn, Ilmenau, Germany) using a pair of rubber electrodes placed in a 5 x 7 cm synthetic water-soaked sponge. One electrode was placed at position C3 (according to the international 10–20 system), while the other was situated above the right eyebrow. We chose this position as it is situated over motor areas (Homan et al., 1987; Steinmetz et al., 1989), and recent studies found that tDCS over this area is not only effective in reducing chronic pain (Fregni et al., 2006a, 2006b), but also improved muscle endurance in

patients with neuromuscular fatigue (Cogiamanian et al., 2007). The electrodes were orientated approximately parallel to the central sulcus and the eyebrow. This montage had been already proven to be the most effective in modulating motor cortex excitability (Nitsche and Paulus, 2000). The type of stimulation (anodal or cathodal) refers to the polarity of the electrode above motor cortex. The current was applied for 10 minutes with an intensity of 1.0 mA, while for sham stimulation it was turned on only for a few seconds to provide the slightly itchy sensation at the beginning of the stimulation. Subjects were not aware of the polarity and type of tDCS.

Electroencephalographic recording

In **Experiment 2**, EEG was recorded using a 64-channel montage with ring electrodes (inner diameter: 6 mm, outer diameter: 12 mm; EasyCap; Falk and Minow Gmbh, Munich, Germany). In **Experiment 4**, we could only use a five channel montage (Fz, Cz, Pz, T3 and T4) to enable the placement of the large tDCS electrodes. The EEG electrodes were placed in accordance with the extended international 10-20 system. The impedance was kept below 5 kOhm. In Experiment 2, Fz was used as reference, the ground was placed 2 cm anterior to the tragus of the right ear. In Experiment 4, we used the connected mastoids as reference and the ground electrode was positioned on the forehead. Data were always collected with a sampling rate of 1000Hz using the BrainAmp system (Brain Products GmbH, Munich, Germany) and were analysed off-line. All EEG data in the cTBS experiment (Experiment 2) and EEG recorded on channels Fz, Cz and Pz in the tDCS experiment (Experiment 4) were re-referenced to connected mastoids, while channels T3 and T4 in Experiment 4 were re-referenced to Fz (Treede et al., 2003). A low-cutoff filter of 0.5Hz and a high-cutoff filter of 30Hz were used. After automatic artefact detection (200 μV amplitude criterion), all epochs were visually inspected, and those containing eye blinks or muscle movement artefacts were excluded. All recordings consisted of at least 35 artefact-free epochs. Baseline correction was performed on the basis of the 100 ms pre-stimulus interval. In Experiment 2, we assessed LEPs according to the scalp distribution of the analysed peaks. The N2–P2 components are larger over the lateral temporal and frontocentral areas, and therefore LEP analysis was performed with regard to the three scalp regions: central (FCz, Cz, Pz), left (FC3, FC5, C3, C5, CP3, CP5) and right (FC4, FC6, C4, C6, CP4, CP6). In Experiment 4, we analysed baseline N1 amplitudes on channels T3 and T4, while N2 and P2 amplitudes were measured on all five channels.

Data analysis

In **Experiment 1**, NAS values were divided into three groups according to the intensity of the laser stimulation, resulting in low intensity 200–399 mJ (5.2–10.4 mJ/mm^2), medium intensity 400–599 mJ (10.4–15.6 mJ/mm^2) and high intensity 600–800 mJ (15.6–20.8 mJ/mm^2) groups. In either group, NAS values obtained for both hands of every participant were separately averaged with regard to TIME (before, immediately after and 30 minutes after tDCS) and CONDITION (cTBS and sham). A repeated measures analysis of variance (ANOVA) was calculated with the mean NAS values across participants and was entered as dependent variables, while HAND (left vs. right), CONDITION (sham vs. cTBS), TIME (before, after and 30 minutes after) and the INTENSITY of laser stimulation (low, medium and high) served as independent variables. For post-hoc comparison Tukey's HSD test was used.

In **Experiment 2**, we normalized the data by dividing pain rating scores (1–9) by the actual laser energy (J). These values were compared for both hands separately with repeated-measures ANOVA, where CONDITION (real and sham cTBS) and TIME (before and after cTBS) served as within-subject factors. N2 and P2 baseline amplitudes and the N2–P2 peak-to-peak amplitude were entered into repeated-measures ANOVA for both hands separately. CONDITION (real cTBS and sham cTBS) and TIME (before cTBS and after cTBS) were within-subject factors, whereas scalp REGION was a between-subject factor.

In **Experiment 3**, we grouped the obtained pain rating scores into four perceptual categories: warm sensation (NAS = 1), mild (NAS between 1.1–1.3), moderate (NAS between 1.4-1.6) and intensive pain (NAS between 1.7-1.9). We used this kind of classification because pain perception shows high inter-individual variability even among healthy subjects (Fillingim et al., 2005) and therefore subjective ratings do not necessarily correlate linearly with the laser energy applied. Given that there were different numbers of laser stimuli belonging to the same perceptual category before and after tDCS, we applied a 2-way ANOVA for each hand and perceptual category in order to determine whether there was a significantly different change in laser energies (mJ) before and after cathodal vs. sham, anodal vs. sham, and cathodal vs. anodal tDCS. Since we were interested whether the change in laser energies was dependent on the tDCS conditions that had been used, we always examined the interaction of the TIME (before and after tDCS) and CONDITION (tDCS comparison pairs) factors.

In **Experiment 4**, laser energies necessary to induce moderate pain were compared with repeated-measures ANOVA for both hands, where CONDITION (anodal, cathodal and sham tDCS) and TIME (before and after tDCS) were entered as within-subject factors. Regarding LEPs, N1, N2, and P2 baseline amplitudes were entered into repeated-measures ANOVA for both hands separately. For each LEP component, pre- and post-stimulation values (TIME factor), tDCS condition pairs (CONDITION) and electrodes (ELECTRODE factor) were entered into the statistical analyses. Here, we considered an amplitude change dependent on the tDCS condition only if the time x type interaction was significant. Furthermore we investigated whether this effect was dependent on the electrode positions by calculating the TIME x CONDITION x ELECTRODE interaction. Student's *t*-test was used to compare the baseline amplitudes between different conditions.

Results

cTBS experiments

In **Experiment 1**, low intensity laser stimulation (200–399 mJ) was either unperceivable or caused only a warm sensation in most of the participants. In the case of medium intensity laser stimulation (400–599 mJ) mean NAS values indicated pain sensation around threshold. High-intensity laser stimulation (600–800 mJ) resulted in a moderate-to-intensive pain sensation in all participants. The repeated measures ANOVA showed significant main effect of HAND [F=9.44; p=0.01], TIME [F=9.04; p=0.001] and INTENSITY [F=204.32; p<0.001], whereas there was a marked, but insignificant effect of CONDITION [F=4.51; p<0.055]. We found a significant HAND x CONDITION [F=4.28; p=0.026] and HAND x CONDITION x TIME [F=6.94; p=0.004] interaction as well. Other interactions were not significant (p>0.05).

As the main effect of HAND and the interaction of HAND x CONDITION x TIME were significant, we repeated the ANOVAs for both hands separately. In the case of right hand stimulation ANOVA showed a significant main effect of CONDITION [F=5.44; p=0.038], TIME [F=6.52; p=0.005] and INTENSITY [F=227.39; p<0.001] as well. Additionally, only the CONDITION x TIME interaction was significant [F=3.69; p=0.04]. In the case of left hand stimulation, the effect of CONDITION was not significant [F=3.38; p=0.09], but the effect of TIME [F=6.67; p=0.005] and INTENSITY [F(2,24)=5.94; p<0.001] were significant.

Furthermore, only the CONDITION x TIME interaction was significant [F=5.94; p=0.008]. The post-hoc analysis revealed no significant difference between the before values for neither the right hand nor the left hand laser stimulation (p>0.9).

Regarding right hand laser stimulation, there was a significant effect of TIME in the medium intensity group if real cTBS was applied (before vs. immediately after: p=0.02; before vs. 30 minutes after: p=0.02). In the high intensity group there was a significant decrease in NAS only immediately after cTBS (before vs. immediately after: p=0.04). We found a significance difference between conditions (cTBS vs. sham) only in the medium-intensity group (cTBS immediately after vs. sham immediately after: p=0.03) (Figure 2a). All of the other comparisons were not significant (p>0.07).

In case of left hand stimulation there was a significant effect of TIME only in the high intensity group 30 minutes after cTBS (before vs. 30 minutes after: p=0.01).

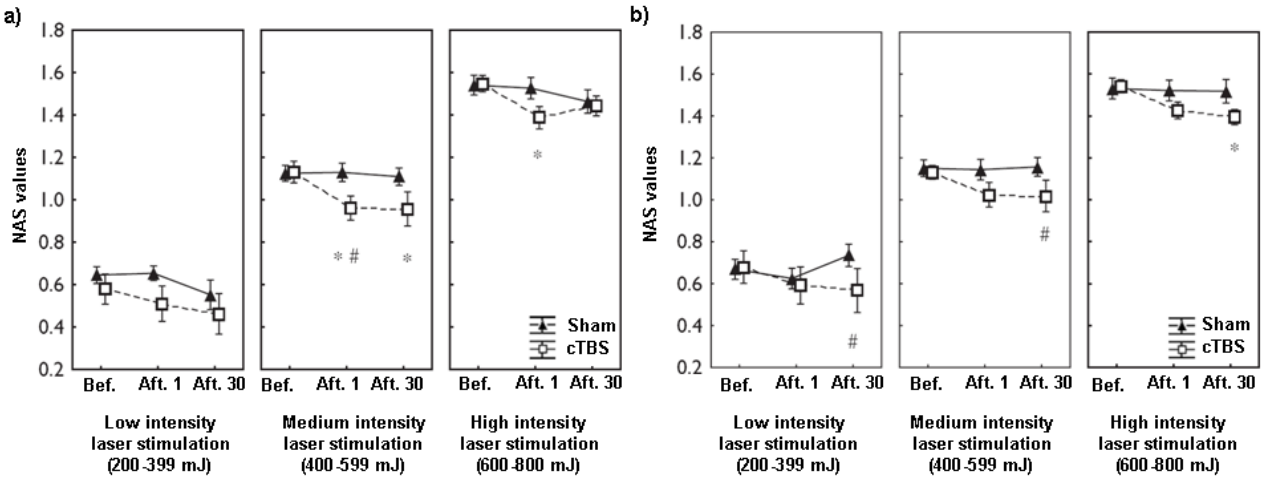

Figure 2. Mean NAS values from all 13 participants in case of right-hand (a) and left-hand (b) laser stimulation before (bef), immediately after (aft1) and 30 minutes after (aft30) 40 seconds of cTBS or sham stimulation. The ANOVA revealed significant CONDITION x TIME interaction (right hand: p=0.04, left hand: p=0.008). For post-hoc comparison Tukey's HSD test was used (p<0.05). Please note that * marks significant differences compared with before values within the same condition (results of the Tukey's HSD test: p<0.05), and # shows significantly different values between the conditions at the same time point (results of the Tukey's HSD test: p<0.05).

Comparing the two conditions (cTBS vs. sham) there were significant differences 30 minutes post-stimulation in the low-intensity group (cTBS 30 minutes after vs. sham 30 minutes after:

p=0.003) and in the medium-intensity group (cTBS 30 minutes after vs. sham 30 minutes after: p=0.03; Figure 2b).

In **Experiment 2**, the repeated-measures ANOVA revealed a significant decrease in normalized pain rating scores in both hands (F=97.94; p<0.001 for the right hand and F=29.90; p<0.001 for the left hand; Figure 3 and Table 1). This indicates a robust habituation to laser stimuli, despite the bioadaptive design used. However, the interaction with CONDITION was significant only in the right hand laser stimulation condition (F=25.14; p<0.001 for the right hand and F=0.91; p=0.34 for the left hand), for real cTBS causing almost four times larger decrease in pain sensation compared with sham stimulation (Figure 3 and Table 1).

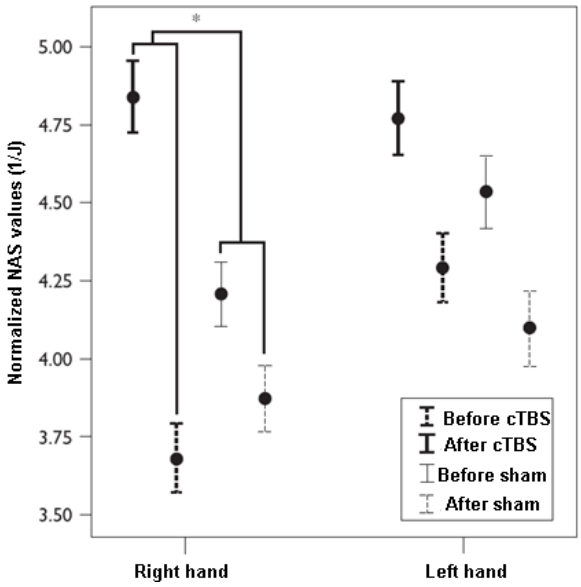

Figure 3. Mean normalized NAS values (1/J) for both cTBS conditions and hands. The * marks significant (p<0.001) difference for right (contralateral) hand stimulation between real cTBS and sham cTBS.

The laser stimulation induced a pricking pain in all individuals and biphasic N2–P2 components were clearly identified in all LEP measurements (Figure 4). The N2 amplitude decreased in both cTBS conditions for both hands after stimulation (F=191.14; p<0.001 for the right side and F=76.78; p<0.001 for the left side; Table 1). The interaction with the CONDITION (real vs. sham cTBS) was also significant for both hands (F=12.52; p<0.001 for the right hand and F=4.00; p=0.04 for the left hand), indicating that after real cTBS the N2 amplitude reduction was larger than after sham stimulation.

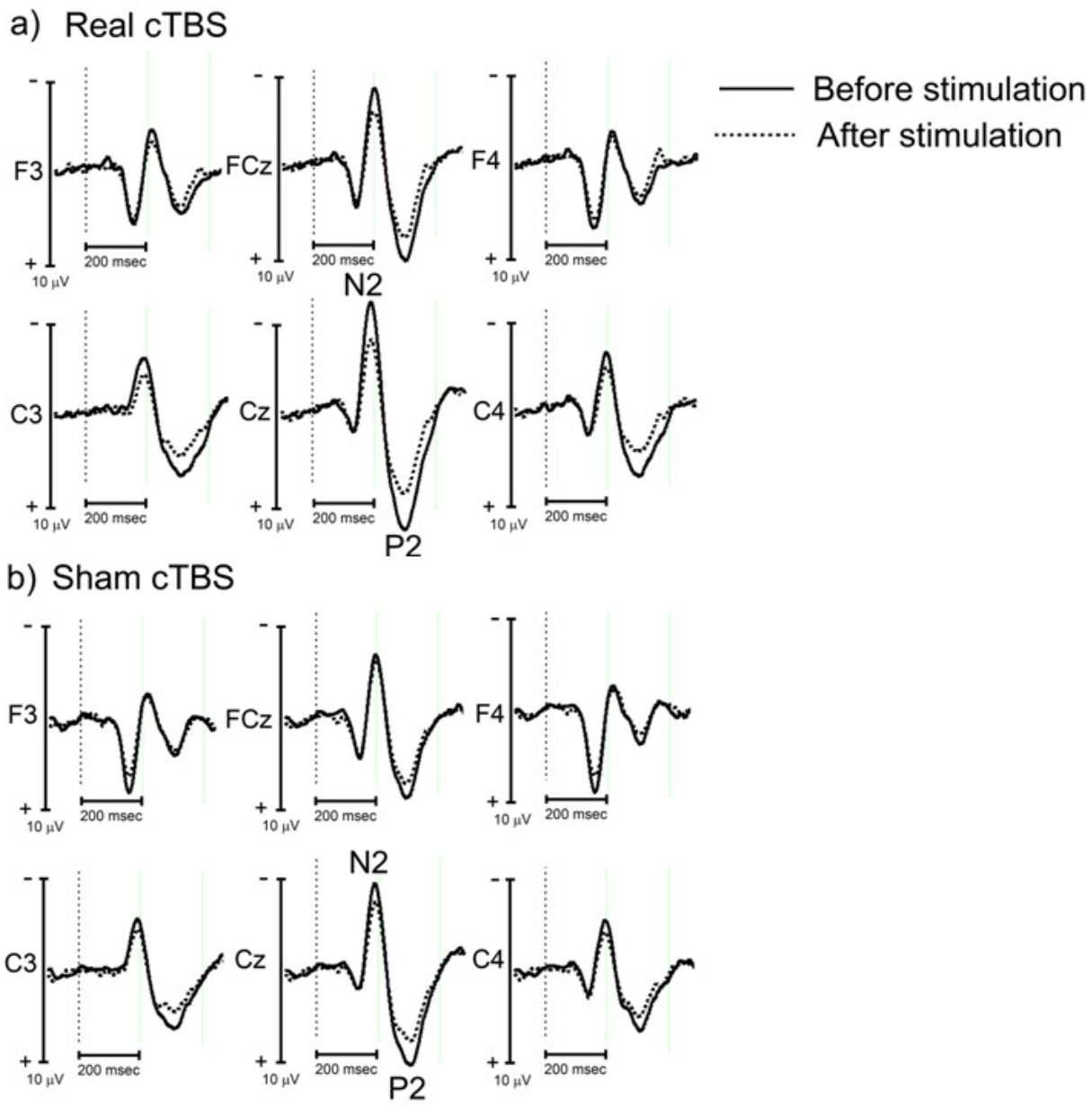

Figure 4. Grand averages of laser-evoked potentials at six scalp electrodes obtained for right-hand laser stimulation in the (a) real cTBS and (b) sham conditions. Note that there is a greater N2–P2 reduction for real cTBS (most prominent at electrode Cz), which is mainly because of smaller N2 amplitudes after motor cortex stimulation.

Separate analysis of the N2 component over the three regions revealed that in cases of left hand stimulation, the change was independent of the scalp distribution ($F=0.712$; $p=0.40$ for the central region, $F=1.280$; $p=0.25$ for the right region and $F=1.749$, $p=0.18$ for the left region), whereas for right hand stimulation, a significant effect of condition (real vs. sham) over the

central (F=3.984; *p*=0.05) and left hemisphere (F=5.227; *p*=0.02), but not for the right hemisphere (F=0.104; *p*=0.74) was found.

Table 1. Characteristics of psychophysical and electrophysiological data for both hands, time periods and cTBS conditions

		Right hand		**Left hand**	
		Real cTBS	**Sham cTBS**	**Real cTBS**	**Sham cTBS**
Rating / energy (1/J)	Before	4.81±0.10	4.21±0.10	4.76±0.10	4.53±0.11
	After	3.67±0.08	3.87±0.10	4.32±0.09	4.10±0.12
	Before - After	1.14±0.09	0.32±0.12	0.44±0.13	0.43±0.14
	Real cTBS – Sham cTBS	0.83±0.17		0.02±0.22	
N2-P2 amplitudes (µV)	Before	17.87±0.65	17.09±0.73	16.27±0.74	14.79±0.72
	After	13.44±0.50	13.49±0.66	13.06±0.61	10.78±0.55
	Before - After	4.42±0.42	3.59±0.26	3.21±0.34	4.01±0.32
	Real cTBS – Sham cTBS	0.83±2.81		-0.80±3.69	
N2 amplitudes (µV)	Before	7.31±0.26	6.88±0.32	6.51±0.32	5.14±0.31
	After	5.31±0.20	5.45±0.28	5.06±0.27	4.20±0.22
	Before - After	2.00±0.18	1.40±0.14	1.45±0.22	0.91±0.17
	Real cTBS – Sham cTBS	0.60±2.51		0.55±3.59	
P2 amplitudes (µV)	Before	8.59±0.31	8.34±0.31	7.99±0.32	8.15±0.31
	After	6.71±0.26	6.60±0.28	6.62±0.25	5.70±0.26
	Before - After	1.88±0.20	1.74±0.12	1.37±0.14	2.45±0.17
	Real cTBS – Sham cTBS	0.14±2.84		-1.08±2.58	

With regard to the P2 amplitude, there was also a significant modulation for both hands and cTBS CONDITION (F=225.38; p<0.001 for the right hand and F=239.65; p<0.001 for the left hand), but there was a significant TIME x CONDITION interaction only for left-hand laser stimulation (F=0.69; p=0.40 for the right hand and F=22.50; p<0.001 for the left hand). Interestingly, for the left hand, the magnitude of amplitude change was significantly less when real cTBS was used (Table 1), showing that in this case real cTBS caused dishabituation. Analysis of scalp REGION showed a significant effect for CONDITION over the left and right

hemispheres (F=6.95; p<0.01 for the left region, F=18.448; p<0.001 for the right region and F=2.085; p=0.15 for the central region). As for the separate LEP components, the N2–P2 peak-to-peak amplitudes also decreased significantly regardless of hand or cTBS CONDITION (F=224.21; p<0.001, for the right hand and F=241.24; p<0.001 for the left hand). The TIME x CONDITION interaction was significant only for right-hand laser stimulation (F=4.53; p=0.03 for the right hand and F=1.64; p=0.20 for the left hand), regardless of the scalp REGION (Figure 4).

tDCS experiments

In **Experiment 3**, we found a significantly reduced warm threshold for the right hand after anodal tDCS, when compared to the effects of sham stimulation (F=4.34; *p*=0.038; Figure 5a).

On the contrary, cathodal stimulation significantly increased laser intensities that were needed to induce mild pain when compared with sham or anodal stimulation as shown in Figure 5b (F=4.83; *p*=0.028 for cathodal-anodal and F=7.63; *p*<0.01 for cathodal-sham comparison). A similar but not significant effect was found for strong pain when it was compared to sham stimulation condition (F=3.82; *p*=0.051; Fig. 5d). Neither of the tDCS types modified the laser energy values necessary to induce moderate pain as shown in Figure 5c.The F and *p* values for all three tDCS comparison pairs, both hands and both perceptual categories are presented in Table 2.

Table 2. Statistical results of Experiment 3. Stars mark significant changes between the tDCS conditions.

Hand	tDCS comparison	Warm	Mild	Moderate	Strong
RIGHT HAND	**Cathodal - Sham**	F=0.75, *p*=0.38	F=4.83, *p*=0.02*	F=0.10, *p*=0.752	F=3.82, *p*=0.051
	Anodal - Sham	F=4.34, *p*=0.038*	F=0.30, *p*=0.58	F=0.31, *p*=0.57	F=1.39, *p*=0.23
	Cathodal - Anodal	F=1.65, *p*=0.20	F=7.63, *p*<0.01*	F=0.78, *p*=0.37	F=0.55, *p*=0.45
LEFT HAND	**Cathodal - Sham**	F=1.12, *p*=0.29	F=1.60, *p*=0.20	F=0.39, *p*=0.53	F=0.01, *p*=0.90
	Anodal - Sham	F=0.30, *p*=0.58	F=3.63, *p*=0.057	F=0.28, *p*=0.59	F<0.01, *p*=0.94
	Cathodal - Anodal	F=0.22, *p*=0.63	F=0.41, *p*=0.51	F=0.01, *p*=0.92	F=0.04, *p*=0.83

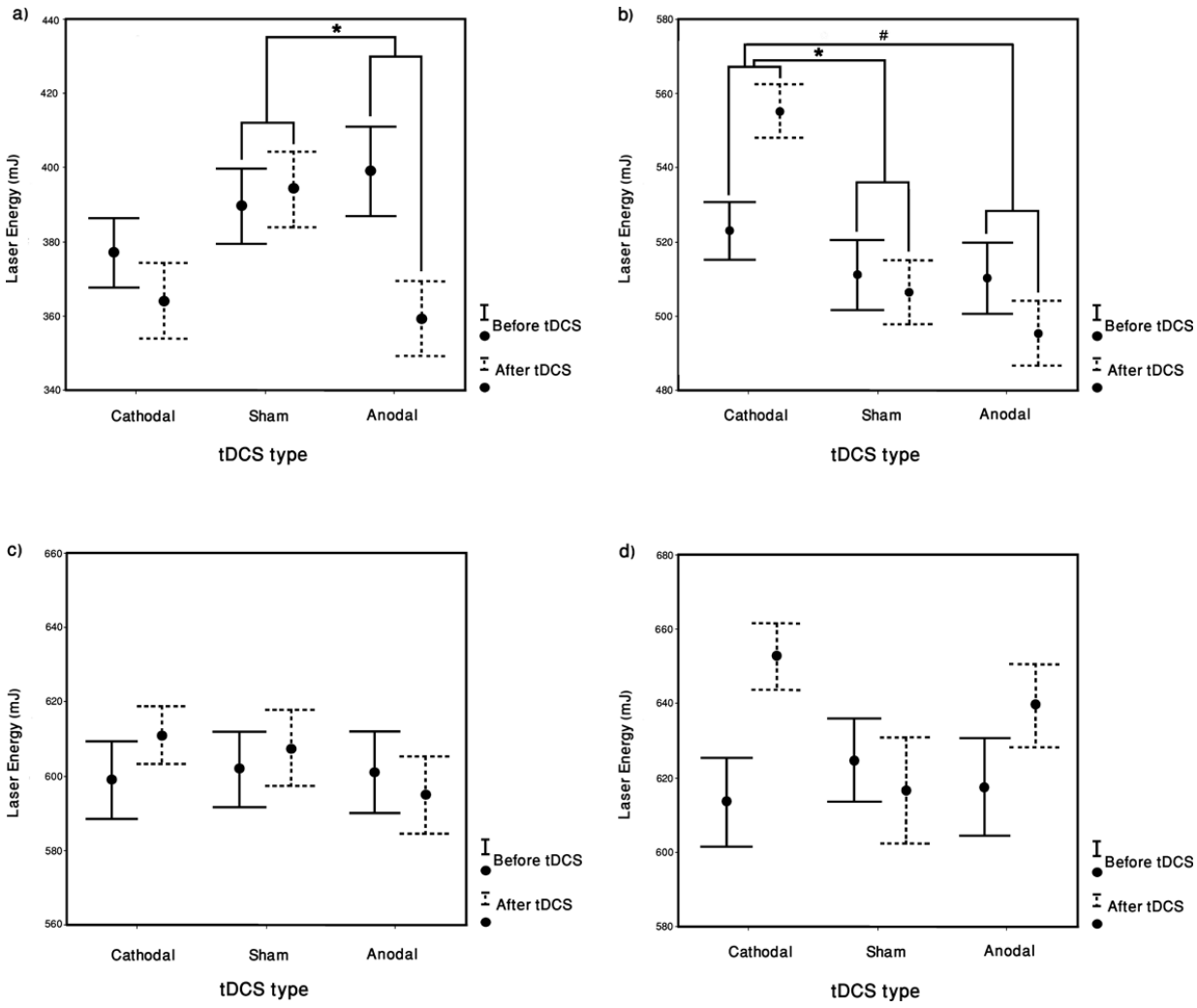

Figure 5. Laser energy changes in the four perceptual categories (warm sensation (a), mild (b), moderate (c) and strong pain (d)) before and after cathodal, sham and anodal tDCS in the case of the right hand. The star on panel a) marks significant difference between the changes of laser energy in the anodal and sham conditions necessary to induce warm sensation. The star on panel b) represents significant difference between the changes of laser energy in the cathodal-sham, while the double cross shows significant difference between cathodal and anodal tDCS in the mild pain category.

In **Experiment 4**, moderate pain was always induced in order to get reliable LEP components. However, we did not get any significant difference among the different conditions with regard to the laser intensities necessary to induce moderate pain ($F<0.38$; $p>0.37$ for all conditions and both hands).

The laser stimulation induced a pricking pain in all subjects and a N1 and a biphasic N2-P2 component was clearly identified in all LEP measures (Figure 6). In case of the N1 component

the amplitudes recorded at T3 and T4 channels were analyzed separately for each hand. There was no significant main effect of type of stimulation on channel T3 (right hand: F=2.07; *p*=0.12; left hand: F=1.18; *p*=0.3) and on channel T4 (right hand: F=1.18; *p*=0.21; left hand: F=0.2; *p*=0.8). The time factor was also not significant on channel T3 (right hand: F=0.9; *p*=0.4; left hand: F=0.03; *p*=0.9) and on channel T4 (right hand: F=1.47; *p*=0.3; left hand: F=1.82; *p*=0.2). Similarly, there was no significant TIME x CONDITION interaction on T3 (right hand: F=0.21; *p*=0.8; left hand: F=0.21; *p*=0.8) and on channel T4 (right hand: F=0.7; *p*=0.6; left hand: F=0.5; *p*=0.6).

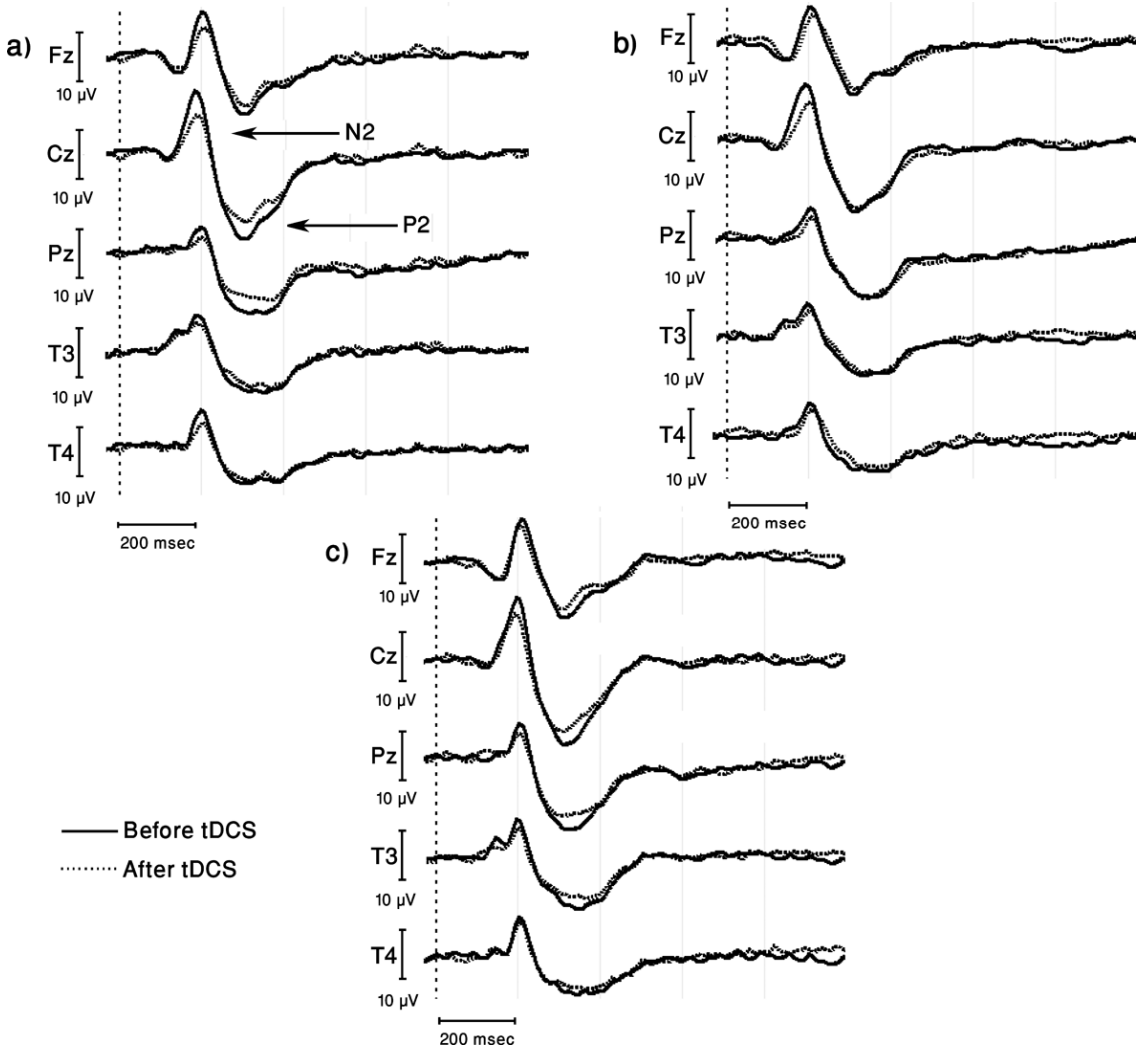

Figure 6. Grand averages of LEPs obtained by right hand laser stimulation for five scalp electrodes. The solid line shows LEPs before and the intermittent line after cathodal (a), sham (b) and anodal (c) tDCS. Please note that a greater amplitude reduction of the N2 and P2 components for cathodal tDCS is observed when compared with sham tDCS (most prominent on electrode Cz).

In the case of the N2 component, we found a significant TIME x CONDITION interaction only when the right hand was stimulated. When compared with sham and anodal tDCS, cathodal stimulation significantly diminished the N2 amplitude (F=6.02; p=0.018 for cathodal-sham and F=4.58; p=0.038 for cathodal-anodal comparison). The interaction with electrode position (TIME x CONDITION x ELECTRODE) was not significant though (F=0.39; p=0.81 and F=0.42; p=0.79). In contrast to cathodal stimulation, anodal stimulation did not affect the N2 amplitude when compared with sham tDCS (F=0.08; p=0.77). The amplitude differences of the N2 component for all three tDCS conditions and both hands are shown in Figure 7a.

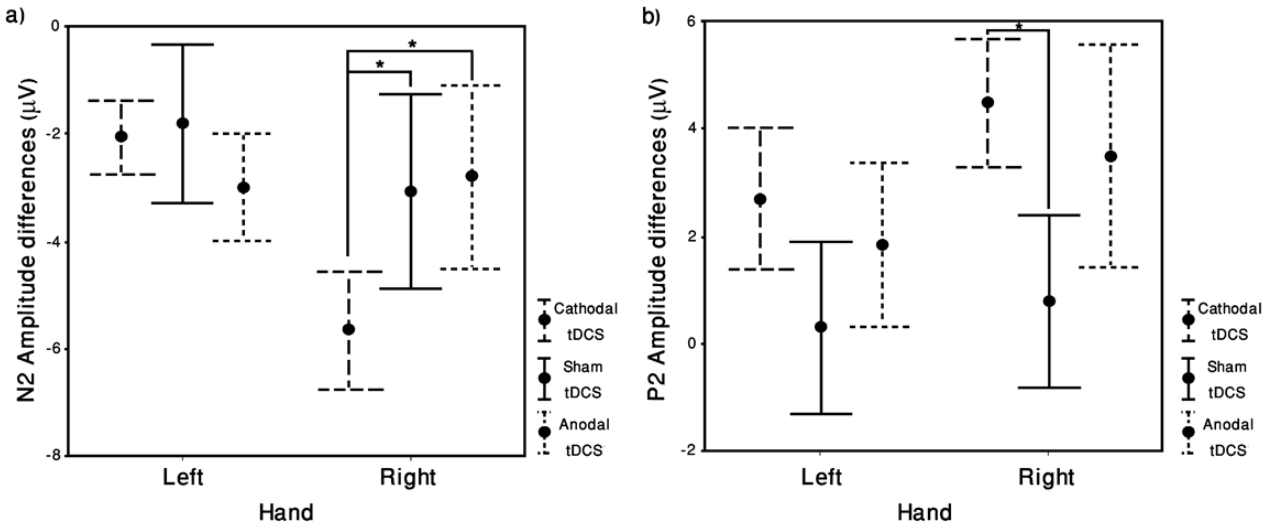

Figure 7. LEP component amplitude differences (before tDCS—after tDCS) in the three tDCS conditions for the N2 (a) and P2 (b) waveforms at the Cz electrode for both hands. The stars mark significant differences for the N2 component in the case of the right hand laser stimulation between cathodal-sham and cathodal-anodal tDCS conditions (p<0.03). They also mark significant differences for the P2 component in the case of the contralateral hand between cathodal-sham tDCS conditions (p<0.01). Please note that in the case of the N2 wave a negative difference represents a decrease in the amplitude, while in the case of the P2 component the more positive the value is, the greater the amplitude reduction was caused by tDCS.

With regard to the P2 amplitude, we found a greater reduction after cathodal tDCS when the left hand was laser stimulated (from 18.31 μV to 15.62 μV and from 16.26 μV to 15.95 μV); however, the TIME x CONDITION interaction was not significant (F=0.01; p=0.7). In the case of the contralateral right hand, the repeated-measures ANOVA revealed a significant modulatory effect of tDCS. The interaction of TIME and CONDITION was significant for the cathodal-sham (F=9.86; p<0.01), marked but not significant for the anodal-sham (F=3.00;

p=0.09) and not significant for the anodal-cathodal (F=0.78; *p*=0.37) comparison (Figure 7). There was no significant interaction with electrode position (F=1.08; *p*=0.37; F=0.56; *p*=0.69 and F= 0.06; *p*=0.99). The amplitude differences of the P2 component for all three tDCS conditions and both hands are shown in Figure 7b. The means and standard deviations for both hands, LEP components and tDCS pairs are presented in Table 2.

Discussion

The effects of cTBS

In Experiments 1 and 2, we have shown that cTBS applied above the motor cortex alleviates experimentally induced acute pain and corresponding LEP amplitudes. In Experiment 1, the reduction of the pain perception was dominantly contralateral to the side of the cTBS (for medium and high intensity laser stimulation) and was observed immediately post-stimulation for medium-level laser intensity (around pain threshold). Furthermore, it remained stable for up to 30 min after stimulation (Figure 2a). In case of the left hand, the significant effect of the cTBS appeared to be delayed (Figure 2b). In Experiment 2 we have also shown that parallel to the contralateral-sided reduction of moderate intensity pain sensation after cTBS, real stimulation reduces the N2-P2 LEP complex, which is primarily due to smaller N2 amplitudes.

Regarding the utility of rTMS in chronic pain patients, the consensus of previous studies using M1 stimulation is that high frequency stimulation is more effective than 1Hz stimulation (Khedr et al., 2005; André-Obadia et al., 2006, Lefaucheur et al., 2006a). Considering that high frequency rTMS is believed to enhance M1 excitability (Quartarone et al., 2005a), current findings pointing towards the efficacy of the suppressive cTBS seems to be contradictory. One possible explanation is that in chronic pain patients the neuronal excitability is altered compared to healthy participants and high frequency facilitatory stimulation might lead to inhibition via plastic changes (e.g. by excitatory of inhibitory pathways), which has been observed in another study (Quartarone et al., 2005a). In line with this, abnormal intra-cortical GABAergic inhibition in M1 has been found in patients with chronic pain, which was normalized by 10Hz facilitatory rTMS (Lefaucheur et al., 2006b). The other possible explanation may be that the underlying mechanisms of cTBS and rTMS are probably not simply inhibitory or facilitatory. Possibly both of them involve many of the basic elementary mechanisms described previously in the LTP/LTD literature. Indeed, the results of the

experiments using single trains of cTBS suggest that this kind of stimulation produces a mixture of facilitation and inhibition on synaptic transmission, with a facilitatory effect building up faster and saturating earlier than the inhibitory effects (Huang et al., 2004). The more complex effect of TBS protocols is further supported by the finding that two TBS paradigms of supposedly opposite effects (cTBS and intermittent TBS) were reported to alter the N2 LEP component is a similar manner after the stimulation of S1 (Poreisz et al., 2008). These findings highlight the importance of considering both the molecular/electrophysiological effects of stimulation and the underlying pathological changes when interpreting results of rTMS protocols.

The fact that cTBS over the motor cortex attenuated the amplitude of the N2-P2 complex points towards the fact that remote effects should also be considered, since these LEP components reflect the activities of the bilateral operculoinsular and ACC regions (Garcia-Larrea et al., 2003). Given that the motor cortex is intensively interconnected with the ACC (Morecraft and van Hoesen, 1992) and that invasive stimulation of M1 induced regional blood flow changes in several pain-related structures (Garcia-Larrea et al., 1999), it seems plausible that cTBS in Experiments 1 and 2 caused similar changes in these regions, which manifested in reduced LEP components.

Finally, although we have observed more prominent contralateral effects both in the case of subjective pain ratings and LEP components. cTBS modulated pain perception on the ipsilateral hand at all three laser intensities in Experiment 1, albeit these effects built up with a longer latency. Also, both the N2 and P2 components were affected by cTBS during the stimulation of the ipsilateral hand. As noted earlier, the affective-cognitive pain system is bilaterally organized, and transcallosal connections between somatomotor areas should also be taken into account in TBS studies, as it was previously shown in the case of intermittent TBS (Mochizuki et al., 2007).

With regard to the ipsilateral side, the decreased habituation of the P2 amplitude after real cTBS is a rather puzzling finding of the study. A similar phenomenon was reported in the visual modality as well: local inhibition of the visual cortex by applying 900 pulses of 1Hz rTMS significantly reduced the habituation to pattern reversal visual-evoked potential amplitudes in healthy volunteers, an effect which was prolonged to several weeks after five daily rTMS sessions in another study (Fumal et al., 2006). The reduced P2 habituation in our

study could be a consequence of a generalized dishabituation that evolves on the grounds of local inhibition of the ipsilateral motor cortex. Whatever the exact underlying mechanisms are, changes of the P2 component did not affect the N2–P2 amplitudes, the magnitude of which is considered to be an electrophysiological index of subjective pain experience (Garcia-Larrea et al., 1997). In addition, this ipsilateral effect did not influence subjective pain rating scores during left-hand stimulation.

The effects of tDCS

In Experiments 3 and 4, cathodal tDCS over the motor cortex resulted in diminished perception of mild pain and LEP components, while anodal tDCS facilitated warm sensation. All these effects were only present when the hand contralateral to the side of tDCS was stimulated with the Tm:YAG laser.

In the psychophysical experiment, we found a significant reduction of mild pain perception after cathodal tDCS when the contralateral hand was stimulated with laser, which seems to contradict to findings of studies where anodal tDCS applied over the motor cortex successfully alleviated pain in chronic pain symptoms (e.g. Fregni et al., 2006a, 2006b; Antal et al., 2010). As previously noted in regard to our cTBS results, one possible explanation for this discrepancy is that we examined experimentally induced acute pain in healthy subjects, while in the above studies the motor cortex was stimulated in chronic pain syndromes. Such chronic pathological states are characterized by both functional (i.e., reorganization of synaptic transmission) (Flor et al., 1995) and structural (Apkarian et al., 2004) alterations in cortical and subcortical areas, probably also leading to cortical and subcortical excitability changes (Dettmers et al., 2001). The efficacy of cathodal tDCS in healthy subjects was also confirmed in a study where only inhibitory stimulation of S1 exhibited antinociceptive effects and diminution of the N2 LEP component (Antal et al., 2008). Another important issue is that in the first studies (Fregni et al., 2006a, 2006b), antinociceptive effects were reached with 20 minute-long daily sessions of 2 mA strong anodal tDCS; that is with twice as long and strong stimulation parameters, as the one applied in our study. Interestingly enough, even by using this protocol they did not observe significant changes immediately after tDCS, but only the following day, indicating that the effect of tDCS developed much slower than one would expect it in any other modality among healthy subjects.

The effect of anodal stimulation on warm thresholds was to some extent opposite to that of cathodal tDCS. Anodal tDCS facilitated warm sensation without influencing pain sensation or LEP amplitudes. Regarding the differential effect of anodal tDCS on warm and pain sensation, there is some evidence that certain brain regions are differentially involved into processing of warm and painful stimuli. In a PET study, increased regional blood flow was found for mild painful stimulation but not for warm perception in the contralateral insular cortex, bilateral prefrontal cortex, bilateral inferior parietal cortex, and the ipsilateral premotor area (Derbyshire et al., 1997). Additionally, different blood oxygen level-dependent signal change in the contralateral operculoinsular region was reported for painful and warm stimulation as revealed by fMRI (Bornhövd et al., 2002). Thus, we might speculate that the modulation of all or some of these regions (namely the insular, motor/premotor cortex or the right frontopolar area, where the reference electrode was placed) could contribute to the observed shift of warm threshold and manifest in thermal hyperesthesia. The discrepancy between the effect of anodal tDCS on warm and pain perception could also be explained by the different peripheral receptors involved in the two processes. Warm perception is mediated by C-fiber nociceptors, while painful thermal stimuli trigger both A-δ and C-afferents. The N2 and P2 late LEP components reflect A-δ activation, and albeit they remain unchanged after anodal tDCS, this might not be true for the so-called ultralate LEPs that reflect C-fiber activity. However, the analysis of ultralate LEP components requires special techniques and was not subject of our study.

Regarding LEP components in Experiment 4, we found significantly decreased N2 and P2 amplitudes after cathodal tDCS on the contralateral hand. One possible explanation for the observed effects is that the diminution of both LEP components after cathodal tDCS reflects a modulation of neural excitability in the S1, the operculoinsular region and/or the ACC. Indeed, in a PET study, changes in regional cerebral blood flow in several brain regions was reported while the investigators used exactly the same stimulation protocol for modifying motor cortex excitability of 16 healthy subjects as we did in our study (Lang et al., 2005). Concerning pain related regions, cathodal tDCS significantly changed regional blood flow in the right cingulate cortex and the right thalamus. As these areas are situated far from the cortical convexity, it is not likely that tDCS could have directly modulated this structure. However, secondary modification of excitability via motor cortex efferents could be a possible explanation of our results.

General conclusions

In our studies we have demonstrated that both cTBS and tDCS reduce subjective pain intensity and attenuate late LEP components when applied over the motor cortex. Although we found differences between cTBS and tDCS regarding the intensity of pain that was modulated, the laterality of effects and the manner to which the N2 and P2 components were changed, both stimulation techniques exhibited significant effects when compared to sham conditions. Our better understanding of the molecular effects of rTMS paradigms and tDCS is essential to further improve such antinociceptive effects. In fact, since the publication of our studies, it has been shown in the same laboratory that the D2-receptor agonist pergolide prolonged the antinociceptive and N2 amplitude reducing effects of cathodal tDCS when applied over the M1 (Terney et al., 2008). These are very promising findings, which hopefully will facilitate the development of novel techniques for alleviating chronic pain.

The following conclusions can be drawn on the basis of the experiments presented in this thesis:

1. We have shown for the first time that 40 seconds of cTBS over the motor cortex resulted in significantly reduced laser-induced pain perception on both hands.
2. The effects on the hand contralateral to the side of cortical stimulation were more robust: both mild and moderate-to-intensive pain thresholds were elevated.
3. The effects on the hand ipsilateral to the side of cortical stimulation were milder for each laser intensity category, but their onset latency was prolonged to 30 minutes post-stimulation.
4. We have shown that the antinociceptive effect of cTBS over the motor cortex is accompanied by reduced N2-P2 LEP amplitudes in the case of medium-intensity pain.
5. Separate analysis of N2 and P2 amplitude changes revealed that the antinociceptive effect is primarily due to reductions of the N2 component.
6. On the contrary, attenuation of the P2 LEP component was smaller for real cTBS than in the sham condition when the hand ipsilateral to the side of cTBS was stimulated with laser.

7. We have shown for the first time that cathodal and not anodal tDCS over the motor cortex reduces laser-induced mild pain contralaterally to the side of stimulation in healthy subjects.
8. In the case of moderate pain, cathodal tDCS over the motor cortex reduced both the N2 and the P2 LEP components, when the hand contralateral to the side of tDCS was stimulated with laser. This LEP reduction was not accompanied by modulations of pain thresholds.
9. We have shown that anodal tDCS over the motor cortex facilitated laser-induced warm sensation contralaterally to the side of tDCS.

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Appendix

I. Poreisz C, **Csifcsák G**, Antal A, Levold M, Hillers F, Paulus W. Theta burst stimulation of the motor cortex reduces laser-evoked pain perception. Neuroreport. 2008; 19:193–196. *Impact factor: 1.904*

II. **Csifcsák G**, Antal A, Hillers F, Levold M, Bachmann CG, Happe S, Nitsche MA, Ellrich J, Paulus W. Modulatory effects of transcranial direct current stimulation on laser-evoked potentials. Pain Med. 2009; 10:122–132. *Impact factor: 2.393*

III. **Csifcsák G**, Nitsche MA, Baumgärtner U, Paulus W, Treede RD, Antal A. Electrophysiological correlates of reduced pain perception after theta-burst stimulation. Neuroreport. 2009; 20:1051–1055. *Impact factor: 1.805*

IV. **Csifcsák G**, Antal A. Non-invasive brain stimulation for relieving acute and chronic pain. Ideggy Szle. Accepted for publication.

Theta burst stimulation of the motor cortex reduces laser-evoked pain perception

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Repetitive transcranial magnetic stimulation over the primary motor cortex (MI) was recently introduced to modulate pain perception. The aim of this double-blind cross-over study was to investigate the effect of a modified rTMS paradigm, called cTBS on experimentally induced laser-evoked pain applied over the left MI. cTBS inhibits the cortical excitability of the MI for approximately 1 h. Subjective pain was measured using the verbal analogue scale prior to, immediately after and 30 min post-stimulation. cTBS, and not the sham stimulation resulted in a significant decrease in pain perception on both hands, accentuated on the right hand. Further studies are needed using motor cortex TBS in chronic pain to pave the way towards a therapeutic tool. *NeuroReport* 19:193–196

Keywords: laser stimulation, motor cortex, pain, theta burst transcranial magnetic stimulation

Introduction

Noninvasive cortical stimulation of the primary motor cortex (M1) for the treatment of certain kinds of chronic and experimentally induced pain has recently attracted much interest. Among several noninvasive methods, repetitive transcranial magnetic stimulation (rTMS) is possibly the most frequently used. In general, high-frequency (5 Hz or more) rTMS increases motor cortex excitability [1] whereas low frequency rTMS decreases it [2]. Regarding the M1, both low-frequency and high-frequency rTMS has been reported to reduce subjective pain perception and has been used experimentally to reduce chronic pain perception ([3–5] for review see Refs [6,7]). In the case of experimentally induced acute pain the effects of low-frequency and high-frequency rTMS over the M1 seem to depend on the type of noxius stimulation [8–11]. C-fibre-mediated acute pain induced by intradermal capsaicin administration could be attenuated by 1 Hz rTMS over the motor cortex [8], whereas it increased Aδ-fibre-mediated laser-induced pain in another study [9]. Similarly, controversial effects were observed after 20 Hz rTMS [10,11]. In contrast, 10 Hz rTMS over the M1 increased electrically induced Aδ-fibre-mediated pain threshold [12], but others found that 10 Hz rTMS has only an effect on the unpleasantness of the pain without any exerting effect on pain threshold ([13], for review see Refs [6,7]).

Recently, Huang *et al.* [14] developed a specific rTMS paradigm to modulate human M1 excitability using low-intensity, repetitive bursts of magnetic stimuli. The authors distinguished three stimulation patterns which were proven to have different effects on M1 activity, monitored by the amplitude of motor-evoked potentials (MEPs). Continuous TBS (cTBS) caused a significant reduction in MEP amplitudes which was probably because of the inhibition of specific excitatory circuits (I1-wave inputs to corticospinal neurons), as was confirmed later by another study [15]. In contrast, intermittent TBS (iTBS) facilitated M1 activity and produced increase in MEP amplitudes. Interestingly, intermediate TBS (imTBS) had no effect at all. Besides M1, TBS has also been shown to influence the excitability of the human premotor [16] and visual cortices [17].

The aim of our placebo-controlled, cross-over double-blind study was to investigate the possible antinociceptive effect of cTBS when applied over the left M1 in healthy participants.

Methods

Participants

Thirteen healthy right-handed volunteers between 18 and 30 years were informed about all aspects of the experiments and only those who signed an informed consent were included in the study. We conformed to the Declaration of Helsinki and the experimental protocol was approved by the Ethics Committee of the University of Göttingen. None of the participants suffered from chronic pain syndromes nor took any medication regularly. None had a history of neurological or psychiatric illness. All participants received the cTBS and sham stimulations.

TBS stimulation

TBS was applied over the hand area of the left M1 using a standard, figure-of-eight-coil (MC-B70 Butterfly Coil) and MagPro stimulator (Medtronic, Denmark) with an outer

half-coil radius of 75 mm, with a posterior-anterior-posterior current flow in the coil. Stimulus intensity was 80% of the active motor threshold (AMT) [14].

For AMT determination, the coil was placed tangentially onto the scalp, with the handle pointing backwards and laterally 45° from midline. MEPs of the right abductor digiti minimi muscle (ADM) were recorded by Ag–AgCl electrodes in a belly-tendon-montage. This procedure was repeated before both, real and sham stimulation conditions. The signals were amplified and filtered (1.59 Hz–1 kHz, sampling rate of 5 kHz), digitalized with a micro-1401 AD converter (Cambridge Electronic Design, Cambridge, UK) and recorded by a computer using SIGNAL software (Cambridge Electronic Design, version 2.13). AMT was defined as the minimum intensity at which at least 3 out of 6 consecutive stimuli elicited a MEP of a superior size ($\sim$200 μV in amplitude) when compared to spontaneous moderate muscular activity.

The pattern of cTBS consisted of bursts containing 3 pulses at 50 Hz which were repeated at 200 ms intervals (i.e. 5 Hz) for 40 s (600 pulses) continuously. In separate experimental sessions, sham stimulation was applied with the same cTBS protocol using sham coil (MC-P-B70) held at the same position. The experimental sessions were separated from each other by at least 5 days. The participants as well as the investigators who applied the laser stimulation, were blinded as to the type of the magnetic stimulation. The order of the sessions was randomized across participants.

Laser stimulation

A Tm:YAG laser system (WaveLight Laser Technologie AG, Erlangen, Germany) was used to induce painful stimulation. The thulium laser emits near-infrared radiation (wavelength 2000 nm, pulse duration 1 ms, laser beam diameter 7 mm) with a penetration depth of 360 μm into the human skin and allows a precise restriction of the emitted heat energy to the termination area of primary nociceptive afferents without affecting the subcutaneous tissue [18,19]. The distal handpiece of the laser was positioned 30 cm from the radial part of the dorsal surface of the hand and laser stimuli were delivered at each interval to a slightly different spot in a 5×5 cm on the dorsum of the hand in order to reduce receptor fatigue or sensitization by skin overheating [18]. A train of laser stimulations was presented on both hands at the beginning of each session by applying laser stimuli of increasing intensities from 200 mJ in 50 mJ steps to allow the participants to feel the nature of painful stimulation. During measurements, each laser stimulus was delivered with increasing intensity in steps of approximately 50 mJ from 200 to 800 mJ and back to 200 mJ two times. Skin temperature of the stimulated area was checked prior to every switch between hands, and corrected with a heating lamp if it fell below 35°C. In both conditions, the right hand was stimulated first in half of the cases and the left hand was stimulated first in the other half.

Psychophysical evaluation

We used the verbal analogue score (VAS) to assess the subjective intensity of pain. The participants were instructed to pay attention to the laser stimuli and to rate the perceived pain verbally (1 warm, 1.1 smallest pain, 1.9 most intense pain) about 2–3 s after each stimulation. The ears of the participants were plugged during the measurements to avoid auditory artefacts accompanying laser stimulation. VAS was measured before TBS (bef), immediately after (aft1) and 30 min after (aft30) cTBS.

Data analysis

VAS values were divided into three groups according to the intensity of the laser stimulation applied, resulting in a low-intensity 200–399 mJ (5.2–10.4 mJ/mm^2), medium-intensity 400–599 mJ (10.4–15.6 mJ/mm^2) and high-intensity 600–800 mJ (15.6–20.8 mJ/mm^2) group. As the laser intensity showed variability in a range from $\pm$5% of the given value, the number of data points in each individual sample was varied between 7 and 19. In either group (low, medium, high intensity), VAS values obtained for both hands of every participant were separately averaged with regard to session (bef, aft1, aft30) and condition (cTBS and sham). A repeated measures ANOVA was calculated with the mean VAS values across participants and was entered as dependent variables and HAND (left vs. right), CONDITION (sham vs. cTBS), TIME (before, after and 30 min after) and the INTENSITY of laser stimulation (low, medium and high) as independent variables. For post-hoc comparison Tukey HSD test was used.

Results

Low-intensity laser stimulation (200–399 mJ) was either unperceivable or caused only a warm sensation in most of the participants. In the case of medium-intensity laser stimulation (400–599 mJ) mean VAS values indicated pain sensation around threshold. High-intensity laser stimulation (600–800 mJ) resulted in a moderate-to-intensive pain sensation in all participants.

The repeated measures ANOVA showed significant main effect of HAND [$F(1,12)=9.44$; $P=0.01$], TIME [$F(2,24)=9.04$; $P=0.001$] and INTENSITY [$F(2,24)=204.32$; $P<0.001$], whereas there was a marked, but insignificant effect of CONDITION [$F(1,12)=4.51$; $P=0.055$]. We found a significant HAND $\times$ CONDITION [$F(2,24)=4.28$; $P=0.026$] and HAND $\times$ CONDITION $\times$ TIME [$F(2,24)=6.94$; $P=0.004$] interaction as well. The other interactions were not significant ($P>0.05$).

As the main effect of HAND and the interaction of HAND $\times$ CONDITION $\times$ TIME were significant, we repeated the ANOVAs for both hands separately.

In the case of right-hand stimulation ANOVA showed a significant main effect of CONDITION [$F(1,12)=5.44$; $P=0.038$], TIME [$F(2,24)=6.52$; $P=0.005$] and INTENSITY [$F(2,24)=227.39$; $P<0.001$] as well. Additionally, only the CONDITION $\times$ TIME interaction was significant [$F(2,24)=3.69$; $P=0.04$]. In the case of left-hand stimulation, the effect of CONDITION was not significant [$F(1,12)=3.38$; $P=0.09$], but the effect of TIME [$F(2,24)=6.67$; $P=0.005$] and INTENSITY [$F(2,24)=5.94$; $P<0.001$] was significant. Furthermore, only the CONDITION $\times$ TIME interaction was significant [$F(2,24)=5.94$; $P=0.008$].

The post-hoc analysis revealed no significant difference between the before values for neither the right-hand nor the left-hand laser stimulation ($P>0.9$).

Regarding right-hand laser stimulation, there was a significant effect of time in the medium-intensity group if cTBS was applied (bef vs. aft1 $P=0.02$; bef vs. aft30 $P=0.02$). In the high-intensity group there was a significant decrease in VAS only immediately after cTBS (bef vs. aft1 $P=0.04$).

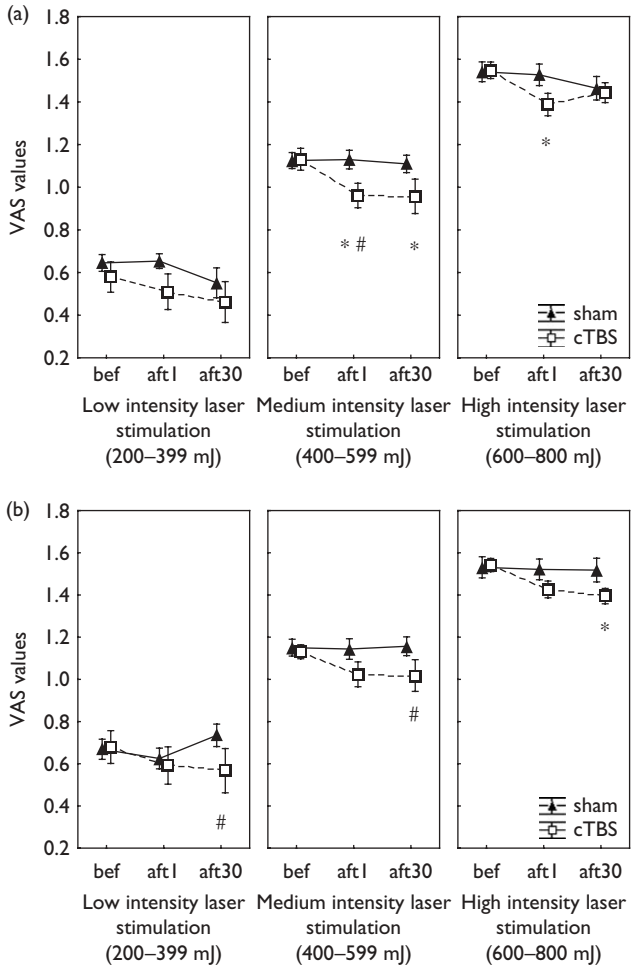

Fig. 1 Subjective pain perception in case of right-hand laser stimulation. The figure shows the mean VAS values from all 13 participants in case of right-hand (a) and left-hand (b) laser stimulation before (bef), immediately after (aft1) and 30 min after (aft30) 40 s cTBS or sham stimulation. The ANOVA showed significant CONDITION × TIME interaction (right hand: $P=0.04$, left hand: $P=0.008$). For post-hoc comparison Tukey HSD test was used ($P<0.05$). Please note that *marks significant differences compared with before values within the same condition (results of the Tukey HSD test, $P<0.05$), and # shows significantly different values between the conditions at the same time point (results of the Tukey HSD test, $P<0.05$).

A significance difference was there between conditions (cTBS vs. sham) only in the medium-intensity group (cTBS aft1 vs. sham aft1 $P=0.03$) (Fig. 1a). All of the other comparisons were not significant ($P>0.07$).

In case of left-hand stimulation there was a significant effect of time only in the high-intensity group 30 min after cTBS (bef vs. aft30 $P=0.01$). Comparing the two conditions (cTBS vs. sham) there were significant differences 30 min poststimulation in the low-intensity group (cTBS aft30 vs. sham aft30 $P=0.003$) and in the medium-intensity group (cTBS aft30 vs. sham aft30 $P=0.03$) (Fig. 1b).

Discussion

Our results demonstrate the reduction of experimentally induced subjective pain perception, evoked by laser stimulation of the dorsum of the hands, after 40 s cTBS stimulation over the left M1. The reduction of the pain perception was dominantly contralateral to the side of the cTBS and was not detectable after sham stimulation. The antinociceptive effect of cTBS was observed immediately poststimulation if the right hand was laser stimulated, and if the laser intensity was on the medium level (around pain threshold). It remained stable for up to 30 min after stimulation (Fig. 1a). In case of the left hand, the significant effect of the cTBS appeared to be delayed (Fig. 1b).

Functional imaging studies suggest the bilateral nature of pain processing [20,21]. As the M1 is highly interconnected within the pain-related cortical network [7,8], it is likely that the unilateral modulation of this region might modulate pain perception evoked from both hands. Moreover, an indirect effect on the contralateral (right) M1 or primary somatosensory cortex (S1) via transcallosal connections, as was previously shown in the case of intermittent TBS (iTBS), [22] could also alleviate pain on either body side. Regarding our experiment, however, a more detailed discussion of the possible underlying electrophysiological or functional mechanisms of M1 stimulation would be rather speculative at this stage in the research. The consensus of previous rTMS and electrical stimulation studies using M1 stimulation in chronic pain is that high-frequency stimulation is more effective than 1 Hz stimulation [6,7,23]. Considering that high-frequency rTMS is believed to enhance M1 excitability [1], the current findings suggesting a suppressive effect of cTBS seems to be contradictory. It is important to mention, however, that these findings have been observed in chronic pain patients and not in healthy participants during experimentally induced pain condition. The modification of the experimentally induced acute pain perception appears to depend on the type of noxius stimulation [8–11]. One possible explanation is that in chronic pain patients the neuronal excitability is altered compared to healthy participants and the high-frequency facilitatory stimulation might lead to inhibition via metaplasticity-like mechanisms observed in other studies [24]. The other possible explanation may be that there is a principal difference in activation between distinct cortical circuits when using different types of stimulation (TMS, electrical stimulation) and different stimulation patterns (rTMS, TBS). In summary, the underlying mechanisms of the effects of TBS and rTMS are probably different. Possibly both of them, however, involve many of the basic elementary mechanisms described previously in the LTP/LTD literature.

The neuronal mechanism of the theta burst paradigm is still speculative. The results of the experiments using single trains of cTBS suggest that this kind of stimulation produces a mixture of facilitation and inhibition on synaptic transmission, with a facilitatory effect building up faster and saturating earlier than the inhibitory effects [25]. Recordings of descending corticospinal volleys evoked by single pulse TMS show that cTBS preferentially suppresses the I1-wave, indicating that the synaptic input responsible for the initial discharge of corticospinal neurons is reduced [15]. Additionally, the effect of cTBS is abolished by memantine, a drug that interferes with transmission at NMDA receptors [26]. A recent study implies that, cTBS activates the circuitry responsible for I1 inputs to corticospinal neurons and that this pattern of stimulation reduces the excitability of its excitatory glutamatergic synapses, possibly via an LTD-like mechanism [27].

Conclusion

Our results suggest that the short, 40 s cTBS of the M1 resulted in comparable effects to those previously described by rTMS [6,7]. Pain tolerance improved after cTBS but not after sham stimulation. One limitation of our study is that the time course of any after-effect was not observed for a sufficiently long time period. The other limitation is the unilaterality of the cTBS stimulation which gives cause for speculation when interpreting the results regarding the slower development of antinociceptive effect after left-hand laser stimulation. Further studies are needed to investigate the time course of the effect, the parameters of stimulation (e.g., reverse current direction, right M1 stimulation), the intensity of the stimulation [27] and whether cTBS has an antinociceptive effect in chronic pain.

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Original Research Articles

Modulatory Effects of Transcranial Direct Current Stimulation on Laser-Evoked Potentials

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ABSTRACT

Objective. Invasive stimulation of the motor cortex has been used for years to alleviate chronic intractable pain in humans. In our study, we have investigated the effect of transcranial direct current stimulation (tDCS), a noninvasive stimulation method, for manipulating the excitability of cortical motor areas on laser evoked potentials (LEP) and acute pain perception.

Designs and Settings. The amplitude of the N1, N2, and P2 LEP components of 10 healthy volunteers were evaluated prior to and following anodal, cathodal, and sham stimulation of the primary motor cortex. In a separate experiment subjective, pain rating scores of 16 healthy subjects in two perceptual categories (warm sensation, mild pain) were also analyzed.

Results. Cathodal tDCS significantly reduced the amplitude of N2 and P2 components compared with anodal or sham stimulation. However, neither of the tDCS types modified significantly the laser energy values necessary to induce moderate pain. In a separate experiment, cathodal stimulation significantly diminished mild pain sensation only when laser-stimulating the hand contralateral to the side of tDCS, while anodal stimulation modified warm sensation.

Conclusions. The possible underlying mechanisms of our findings in view of recent neuroimaging studies are discussed. To our knowledge this study is the first to demonstrate the mild antinociceptive effect of tDCS over the primary motor cortex in healthy volunteers.

Key Words. tDCS; Pain; Primary Motor Cortex; LEP

Introduction

The notion that the motor cortex is involved in modulating nociceptive processing was postulated more than 50 years ago by Penfield and colleagues [1]. He reoperated patients on whom he had previously resected a portion of the postcentral gyrus for epilepsy, and observed that stimulation of the corresponding primary motor cortex elicited sensory responses. Epidural electrical motor cortex stimulation (MCS) is a relatively safe clinical method for alleviating neuropathic pain [2]. In spite of its efficacy, MCS still remains an invasive procedure [3]. In recent years, several novel noninvasive techniques have

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been used in experimental and clinical neuroscience for manipulating brain function. In the field of pain research, repetitive transcranial magnetic stimulation (rTMS) of the motor cortex is the most promising method (for a review see: [4]).

Transcranial direct current stimulation (tDCS) has recently been reintroduced as a tool for inducing changes to cortical excitability in focal brain regions in a reversible, relatively selective, painless, and safe manner [5,6]. Motor cortex excitability is enhanced by anodal and decreased by cathodal stimulation when monitored with single-pulse TMS [7,8]. The primary effect of tDCS is a neuronal inhibition or excitation [9,10]: cathodal stimulation hyperpolarizes, while anodal stimulation depolarizes the resting membrane potential, whereby the induced after-effects depend on the polarity, the duration, and intensity of the stimulation. Even though in humans the effects of tDCS were first demonstrated on the motor system, it also influences visual, somatosensory, and cognitive functions [11,12]. In the field of pain perception, only two recently published tDCS studies have been performed. In a randomized, double-blind, parallel-group trial Fregni and colleagues demonstrated that a treatment of anodal tDCS over the primary motor cortex for five consecutive days significantly reduces the subjective pain rating scores of patients suffering from chronic, central, pharmacoresistant pain caused by spinal cord injury [13]. In a second study the same authors evaluated the effect of anodal stimulation vs sham stimulation in patient with fibromyalgia [14]. Despite the robust analgesic effect of anodal tDCS when compared with sham stimulation observed in both studies, the authors did not examine the efficacy of inhibitory, cathodal tDCS, which would have been very useful to understand the overall contribution of primary motor cortex to pain perception.

The aim of our study was to evaluate the effect of tDCS overt the motor cortex on laser evoked potentials (LEPs) and on acute pain and warm perception in healthy control subjects. LEPs are electrophysiological measures that represent the activation of distributed neural populations [15] and are quantitative neuronal correlates of pain processing [16–18]. We stimulated the dorsum of both hands of healthy subjects with a Tm : YAG laser (WaveLight Laser Technologie AG, Erlangen, Germany) [19] and recorded and analyzed LEPs prior to and following anodal, cathodal, and sham tDCS. Currently, early and late LEP components are considered to be differentially sensitive to the subjective variability of pain perception: the early N1 component is thought to be a preperceptual sensory response, whereas the late N2-P2 complex strongly correlates with perceived pain and can be modulated by either exogenous or endogenous factors [20,21]. Two separate experiments were conducted: an electrophysiological study evaluating changes of LEP amplitudes always induced by medium intensity pain and a psychophysical study where we focused on alterations in laser intensities in both sub-threshold (warm sensation) and supra-threshold (mild pain) perceptual categories.

Materials and Methods

Subjects

There were 10 (5 male and 5 female) volunteers who participated in the electrophysiological experiment and 16 (5 male and 11 female) subjects took part in the psychophysical experiment. All subjects were aged between 20 and 30 and none suffered from chronic pain syndrome or were taking medication regularly. They had no current or previous neurological or psychiatric diseases. Written informed consent was obtained from all participants. The study protocol conformed to the Declaration of Helsinki and was approved by the Ethics Committee of the University of Göttingen.

tDCS

tDCS was provided by a battery driven constant-current stimulator (NeuroConn, Ilmenau, Germany) using a pair of rubber electrodes placed in a 5×7 cm synthetic water-soaked sponge. One electrode was placed at position C3 (according to the international 10–20 system), while the other was situated above the right eyebrow. We chose this position as it is situated over motor areas [22,23], and recent studies found that the stimulation of this area is not only effective in reducing chronic pain [13,14], but also improved muscle endurance in patients with neuromuscular fatigue [24]. The electrodes were orientated approximately parallel to the central sulcus and the eyebrow. This montage had been already proven to be the most effective in modulating motor cortex excitability [7]. The type of stimulation (anodal or cathodal) refers to the polarity of the electrode above motor cortex. The current was applied for 10 minutes with an intensity of 1.0 mA, while for sham stimulation it was turned on only

for a few seconds to provide the slightly itchy sensation at the beginning of the stimulation. Subjects were not aware of the polarity and type of tDCS. We randomized the order of the sessions and separated them by at least 1 week in order to avoid the effect of interference.

Laser Stimulation and Psychophysical Evaluation

Pain was elicited using a Tm:YAG laser system. The thulium laser emits near-infrared radiation (wavelength 2,000 nm, pulse duration 1 ms, laser beam diameter 7 mm) with a penetration depth of 360 μm into the human skin [25]. It also allows the emitted heat energy to be precisely restricted to the termination area of primary nociceptive afferents without affecting the subcutaneous tissue [18]. The distal handpiece of the laser was positioned 30 cm from the radial part of the dorsal surface of the hand. The skin temperature of the stimulated area was checked prior to every switch of hands and corrected with a heating lamp when below 35°C. We stimulated slightly different spots in a square (5 × 5 cm) for each measurement to reduce receptor fatigue or sensitization through skin overheating [18]. In both experiments the right hand was stimulated first in half of the cases; in the other half we started with the left hand. This was done because increased response toward novel stimuli had already been described in evoked potential studies related to other sensory modalities [26]. Since in each case the left motor cortex was stimulated with tDCS, we anticipated that it would affect pain threshold dominantly on the contralateral right hand, which could have been masked by this initial orienting response.

We used a numeric analog score (NAS) to assess the subjective intensity of pain. We instructed the subjects to pay attention to the laser stimuli and to rate the perceived pain verbally with numbers (1 for warm, from 1.1 [for mildest] to 1.9 [most intensive] for painful sensation) about 2–3 seconds after each stimulation.

In order to obtain reliable pain rating scores, the subjects were trained to get accustomed to the NAS. Prior to the experimental stimulation sessions they were presented a series of laser stimuli from 200 mJ to 800 mJ and back during which they had to evaluate the intensity of pain.

In the electrophysiological experiment, at the beginning the pain threshold of both hands was determined by applying laser stimuli from 200 mJ in 50 mJ steps. We determined the pain threshold at a laser energy level, where subjects consistently perceived painful sensation between NAS scores 1.1–1.3. However, in order to get reliable LEP waveforms, we aimed to induce medium intensity pain by adapting the bioadaptive approach designed by Weiss and colleagues [27]. During the electroencephalographic (EEG) recording, we started with a laser intensity of 1.5–1.6 times of the threshold level and adjusted laser energy manually in order to keep the magnitude of pain between NAS scores 1.4–1.6. We delivered 40 laser pulses to each hand before and after tDCS, with an interstimulus interval between 8–15 seconds. The ears of the subjects were always plugged and white noise was presented during the measurements to avoid auditory artifacts due to laser stimulation.

In the psychophysical experiment we applied two series of stimuli for each hand before and after tDCS. We systematically increased laser intensity from 200 mJ (5.2 mJ/mm^2) in 50 mJ steps until subjects reported moderate pain. Then the laser energy was decreased from that intensity again in 50 mJ steps. This stimulation protocol was repeated twice. Hence we obtained four pain rating scores for each laser intensity prior to and following tDCS for each hand and simulation type.

Electrophysiological Recordings

The EEG was recorded using a five channel montage as described by Treede and colleagues [18]. This montage has been used in numerous experimental and clinical LEP studies as it enables the easy identification of both late LEP components. We placed three electrodes in the midline (Fz, Cz, and Pz) and two laterally above the temporal region (T3 and T4) in accordance with the international 10/20 system. The impedance was kept below 5 kOhm. We used the connected mastoids as reference (RLm) and the ground electrode was positioned on the forehead. Data were collected with a sampling rate of 1,000 Hz by the BrainAmp system (Brain Products GmbH, Munich, Germany) and were analyzed offline. A 0.5 Hz low cutoff and a 30 Hz high cutoff filter were used. After automatic artifact detection (200 μV amplitude criterion) all epochs were visually inspected as well, and those containing eye blinks or muscle movement artifacts were excluded. All recordings consisted of at least 35 artifact-free epochs. Baseline correction was performed on the basis of the 100 ms prestimulus interval. The amplitudes of N1 (referring to Fz) and N2-P2 (referring to RLm) components were measured offline.

Data Analysis

Concerning electrophysiological data, N1, N2, and P2 baseline amplitudes were entered into a repeated-measures ANOVA for both hands separately. For each LEP component, pre- and post-stimulation values (*time* factor), tDCS condition pairs (*type* factor) and electrodes (*electrode* factor) were entered into the statistical analyses. Here, we considered an amplitude change dependent on the tDCS condition only if the *time* × *type* interaction was significant. Furthermore we investigated whether this effect was dependent on the electrode positions by calculating the *time* × *type* × *electrode* interaction. Student's *t*-test was used to compare the baseline amplitudes between different conditions.

In the electrophysiological study, we did not get any significant difference between the different conditions with regard to the laser intensities necessary to induce moderate pain. Therefore in the psychophysical experiment, we divided the obtained pain rating scores into two perceptual categories: warm sensation (NAS = 1), mild (NAS between 1.1–1.3). We used this kind of classification because pain perception shows high inter-individual variability even among healthy subjects [28] and therefore subjective ratings do not necessarily correlate linearly with the laser energy applied.

Given that there were different numbers of laser stimuli belonging to the same perceptual category before and after tDCS, we applied a 2-way ANOVA for each hand and perceptual category in order to determine whether there was a significantly different change in laser energies (mJ) before and after cathodal vs sham, anodal vs sham, and cathodal vs anodal tDCS. Always two tDCS conditions were entered into ANOVA; hence, we could examine the efficacy of one stimulation type over another and avoid the masking effect of the third one. Since we were interested whether the change in laser energies was dependent on the tDCS conditions that had been used, we always examined the interaction of the *time* (before and after tDCS) and *type* (tDCS comparison pairs) factors.

Results

Electrophysiology

The laser stimulation induced a pricking pain in all subjects and a N1 and a biphasic N2-P2 component was clearly identified in all LEP measures (Figure 1).

In case of the N1 component the amplitudes recorded at T3 and T4 channels (referring to Fz) were analyzed separately for each hand. There was no significant main effect of *type* of stimulation on channel T3 (right hand: $F = 2.07$, $P = 0.12$; left hand: $F = 1.18$, $P = 0.3$) and on channel T4 (right hand: $F = 1.18$, $P = 0.21$; left hand: $F = 0.2$, $P = 0.8$). The *time* factor was also not significant on channel T3 (right hand: $F = 0.9$, $P = 0.4$; left hand: $F = 0.03$, $P = 0.9$) and on channel T4 (right hand: $F = 1.47$, $P = 0.3$; left hand: $F = 1.82$, $P = 0.2$). Similarly, there was no significant *time* × *type* interaction on T3 (right hand: $F = 0.21$, $P = 0.8$; left hand: $F = 0.21$, $P = 0.8$) and on channel T4 (right hand: $F = 0.7$, $P = 0.6$; left hand: $F = 0.5$, $P = 0.6$).

In the case of the N2 component, we found a significant *time* × *type* interaction only when the right hand was stimulated. When compared with sham and anodal tDCS, cathodal stimulation significantly diminished the N2 amplitude ($F = 6.02$, $P = 0.018$ for cathodal-sham and $F = 4.58$, $P = 0.038$ for cathodal-anodal comparison). The interaction with electrode position (*time* × *type* × *electrode*) was not significant though ($F = 0.39$, $P = 0.81$ and $F = 0.42$, $P = 0.79$). In contrast to cathodal stimulation, anodal stimulation, did not affect the N2 amplitude when compared with sham tDCS ($F = 0.08$, $P = 0.77$). The amplitude differences of the N2 component for all three tDCS conditions and both hands are shown in Figure 2a.

With regard to the P2 amplitude in comparison with the sham condition, we found a greater reduction after cathodal tDCS when the left hand was laser stimulated (from 18.31 μV to 15.62 μV and from 16.26 μV to 15.95 μV); however, the *time* × *type* interaction was not significant ($F = 0.01$, $P = 0.07$). In the case of the contralateral right hand, the repeated-measures ANOVA revealed a significant modulatory effect of tDCS. The interaction of *time* and *type* was significant for the cathodal-sham ($F = 9.86$, $P < 0.01$), marked but not significant for the anodal-sham ($F = 3.00$, $P = 0.09$) and not significant for the anodal-cathodal ($F = 0.78$, $P = 0.37$) comparison (Fig. 2). There was no significant interaction with electrode position ($F = 1.08$, $P = 0.37$; $F = 0.56$, $P = 0.69$ and $F = 0.06$, $P = 0.99$). The amplitude differences of the P2 component for all three tDCS conditions and both hands are shown in Figure 2b.

The means and standard deviations for both hands, LEP components and tDCS pairs are presented in Table 1.

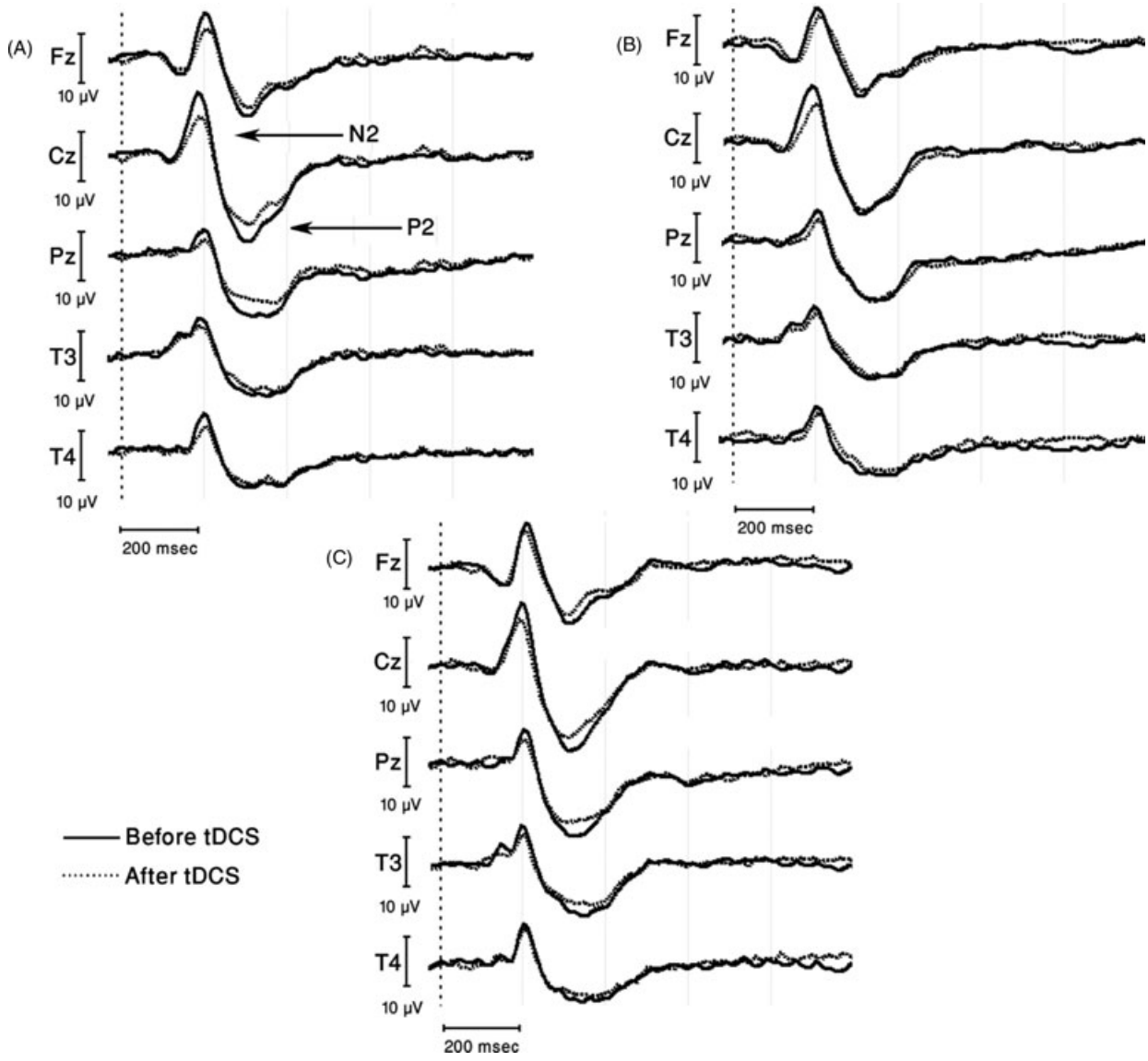

Figure 1 Grand averages of LEPs obtained by *right hand* laser stimulation for five scalp electrodes. The solid line shows LEPs before and the intermittent line after cathodal (A), sham (B) and anodal (C) tDCS. Please note that a greater amplitude reduction of the N2 and P2 components for cathodal tDCS is observed when compared with sham tDCS. LEP = laser evoked potentials; tDCS = transcranial direct current stimulation.

Psychophysics

In the electrophysiological experiment, moderate pain was always induced in order to get reliable LEP components. However, we did not get any significant difference among the different conditions with regard to the laser intensities necessary to induce moderate pain. For the left and right hand stimulations the two-way ANOVA revealed no significant change in laser intensities before and after tDCS in either pain categories or any of the tDCS-type comparisons ($F < 0.38$, $P > 0.37$ for all conditions, respectively). Therefore we implemented a new psychophysical experiment in order to see if tDCS has an effect for less intensive sensations.

In the case of the right hand, after anodal stimulation significantly lower laser energy values were necessary to induce warm sensation than before tDCS, when they were compared with sham tDCS ($F = 4.34$, $P = 0.038$; Figure 3a). On the contrary, cathodal stimulation significantly increased laser intensities that were needed to induce mild pain when compared with sham or anodal stimulation as shown in

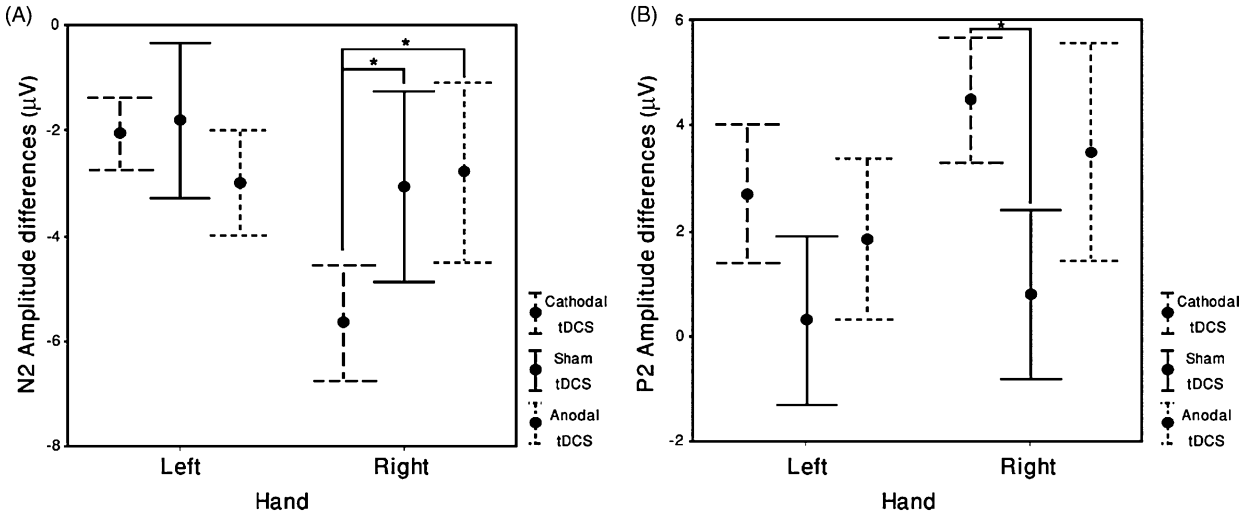

Figure 2 LEP component amplitude differences (before tDCS—after tDCS) in the three tDCS conditions for the N2 (A) and P2 (B) waveforms at the Cz electrode for both hands. The stars mark significant differences for the N2 component in the case of the right hand laser stimulation between cathodal-sham and cathodal-anodal tDCS conditions ($P < 0.03$). They also mark significant differences for the P2 component in the case of the contralateral hand between cathodal-sham tDCS conditions ($P < 0.01$). Please note, that in the case of the N2 wave a negative difference represents a decrease in the amplitude, while in the case of the P2 component the more positive the value is, the greater the amplitude reduction was caused by tDCS. LEP = laser evoked potentials; tDCS = transcranial direct current stimulation.

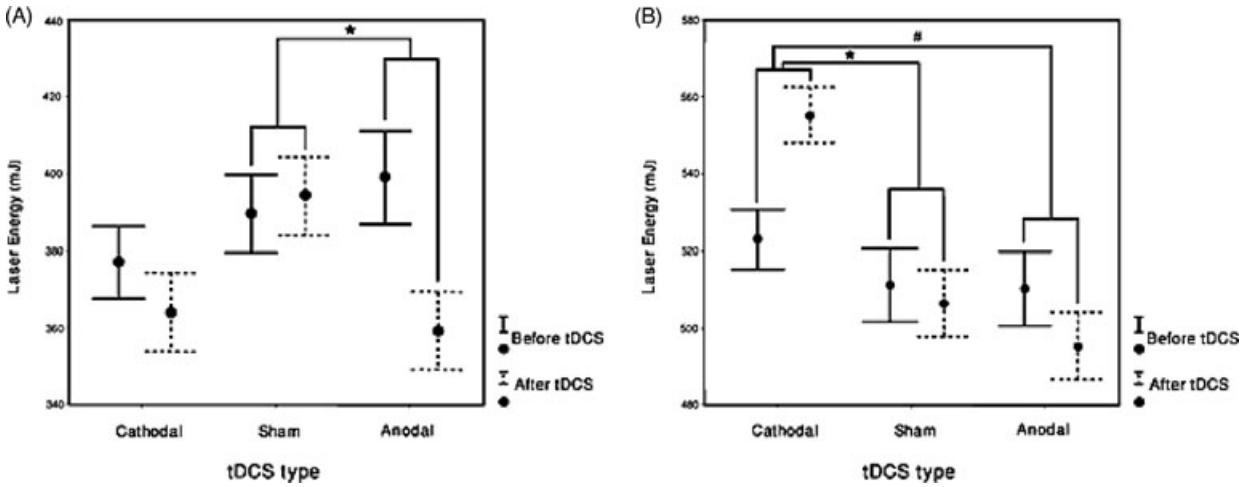

Figure 3 Laser energy changes in the perceptual categories (warm sensation [A], mild [B]) before and after cathodal, sham and anodal tDCS in the case of the *right hand* laser stimulation. The star on panel (A) marks significant difference between the changes of laser energy in the anodal and sham conditions necessary to induce warm sensation. The star on panel (B) represents significant difference between the changes of laser energy in the cathodal-sham, while the double cross shows significant difference between cathodal and anodal tDCS in the mild pain category. tDCS = transcranial direct current stimulation.

Table 1 Changes of LEP amplitudes at the Cz electrode

Hand	Laser evoked potentials component		Cathodal		Anodal		Sham	
			Before	After	Before	After	Before	After
Right Hand	N2 (μV)(Cz)	Mean ± SD	−13.9 ± 7.2	−8.2 ± 6.2	−13.3 ± 6.8	−10.6 ± 6.4	−12.24 ± 8.7	−9.1 ± 6.2
	P2 (μV)(Cz)	Mean ± SD	20.04 ± 9.25	15.56 ± 7.55	19.38 ± 8.11	15.87 ± 6.82	16.03 ± 6.44	15.23 ± 5.80
Left Hand	N2 (μV)(Cz)	Mean ± SD	−11.06 ± 6.77	−8.99 ± 5.89	−13.16 ± 8.00	−10.16 ± 6.40	−10.93 ± 7.86	−9.13 ± 7.06
	P2 (μV)(Cz)	Mean ± SD	18.31 ± 9.96	15.62 ± 6.85	18.01 ± 10.17	16.17 ± 7.62	16.26 ± 5.11	15.95 ± 5.27

Table 2 Statistical results of the psychophysical experiment

Hand	tDCS comparison	Warm	Mild
Right hand	Cathodal–Sham	F = 0.75, $P = 0.38$	F = 4.83, $P = 0.02$*
	Anodal–Sham	F = 4.34, $P = 0.038$*	F = 0.30, $P = 0.58$
	Cathodal–Anodal	F = 1.65, $P = 0.20$	F = 7.63, $P < 0.01$*
Left hand	Cathodal–Sham	F = 1.12, $P = 0.29$	F = 1.60, $P = 0.20$
	Anodal–Sham	F = 0.30, $P = 0.58$	F = 3.63, $P = 0.057$
	Cathodal–Anodal	F = 0.22, $P = 0.63$	F = 0.41, $P = 0.51$

* Significant changes between the tDCS conditions.
tDCS = transcranial direct current stimulation.

Figure 3b ($F = 4.83$, $P = 0.028$ for cathodal-anodal and $F = 7.63$, $P < 0.01$ for cathodal-sham comparison).

The F and *P*-values for all three tDCS comparison pairs, both hands and both perceptual categories are presented in Table 2.

Discussion

Several animal and human studies have shown that tDCS modifies the excitability of the stimulated cortical area in a polarity dependent way and as a result, causes perceptual changes (for a recent review see Antal et al. 2006 [11]). In our study, we explored the effects of this noninvasive technique on LEPs and acute pain perception. Cathodal stimulation significantly reduced the N2 and P2 components of LEPs when we induced pain in the contralateral hand. There was no effect on the N1 component observed. In the psychophysical experiment, cathodal stimulation of the motor cortex significantly diminished mild pain, whereas anodal stimulation facilitated warm sensation. Both effects were only present when the hand contralateral to the side of tDCS was stimulated with Tm:YAG laser.

The Effect of tDCS on LEPs and Pain Perception

Perhaps the most important finding of this study was the significantly decreased N2 and P2 amplitudes after cathodal tDCS on the contralateral hand. According to intracranial EEG and source localizing studies, the N2 component is generated mainly in the primary somatosensory cortex (SI) and in the operculoinsular region, while the P2 component mainly arises from the anterior cingulated cortex ([29–31], for a review see: [15]). Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies have also shown that the hemodynamic responses of these brain regions correlate with pain intensity [32–34]. It is likely that in our study, the observed diminution of both LEP components after cathodal tDCS reflects a modulation of the activation of at least some of these areas. Indeed, in a recent PET study, Lang and colleagues examined changes of regional cerebral blood flow (rCBF) in several brain regions while they used exactly the same stimulation protocol for modifying motor cortex excitability of 16 healthy subjects as we did in our study [35]. Concerning pain related regions, cathodal tDCS significantly changed rCBF in the right cingulate cortex and the right thalamus. As this area is situated on the medial surface of the brain, relatively far from the cortical convexity, it is not likely that tDCS could have directly modulated this structure. However, given that cingulate cortex is widely interconnected with primary and premotor areas [36], the secondary modification of its excitability could be a possible explanation of our results.

In the psychophysical experiment we found a significant reduction of mild pain perception after cathodal tDCS when the contralateral hand was stimulated with laser. In a recent study of Fregni and colleagues, anodal tDCS significantly reduced subjective pain perception in patients with intractable central pain [13], and this finding seems to contradict our results. One possible explanation for this discrepancy is that we examined experimentally induced acute pain in healthy subjects, while Fregni and colleagues have stimulated motor cortex in a chronic pain syndrome. Such chronic pathological states are characterized by both functional (i.e., reorganization of synaptic transmission [37–39] and structural (i.e., grey matter atrophy: see [40]) changes in cortical and subcortical areas, probably also leading to excitability changes [41,42].

Another important issue is that Fregni and colleagues [13] observed long lasting antinociceptive effects of 20-minute daily sessions with 2 mA strong anodal tDCS; that is a stimulation condition twice as long and strong as the one applied in our study. Interestingly enough, even by using this protocol they did not observe significant changes immediately after tDCS, but only the following day; this indicates that the effect of tDCS developed much slower than one would expect it in any other modality among healthy subjects. Although with our stimulation protocol, the possibility of

having induced subtle and slow-evolving effects of anodal tDCS on pain perception is less likely, taking into account the different subject population, it cannot be ruled out.

The Effect of tDCS on Warm Sensation

The effect of anodal stimulation on subjective warm assessment was to some extent opposite to cathodal tDCS as it facilitated warm sensation without influencing pain sensation or LEP amplitudes. Regarding the differential effect of anodal tDCS on warm and pain sensation there is some evidence that certain brain regions are differentially involved into processing of warm and painful stimuli. In a PET study, increased rCBF was found for mild painful stimulation but not for warm perception in the contralateral insular cortex, bilateral prefrontal cortex, bilateral inferior parietal cortex, and the ipsilateral premotor area [43]. Additionally, different blood oxygen level-dependent signal change in the contralateral operculoinsular region was reported for painful and warm stimulation as revealed by fMRI [44]. Thus, we might speculate that the modulation of all or some of these regions—namely the insular, motor/premotor cortex or the right frontopolar area (where the reference electrode was placed)—could contribute to the observed shift of pain threshold and manifest in thermal hyperaesthesia.

The discrepancy between the effect of anodal tDCS on warm and pain perception could also be explained by the different peripherial receptors involved in the two processes. Warm perception is mediated by C fiber nociceptors [45], while painful thermal stimuli trigger both A-delta and C-afferents [18]. The N2 and P2 late LEP components reflect A-delta activation, and albeit they remain unchanged after anodal tDCS, this might not be true for the so-called ultralate LEPs that reflect C-fiber firing. However, the analysis of ultralate LEP components requires special techniques. Moreover, warm sensation following laser skin stimulation is still controversial, as there is evidence that A-delta fiber activation suppresses C-fiber activation and consequent warm sensation [46,47].

Further Considerations

Since many imaging studies have reported that either MCS [17,48], TMS [49], or tDCS [35] over motor cortical areas are associated with altered activity in the thalamus and subthalamic nucleus, we cannot exclude the possibility of having modulated the involvement of these subcortical structures when interpreting our data. Indeed, in the case of MCS, Garcia-Larrea and colleagues proposed that the activation of the thalamus as a key structure would be the primary event that could trigger a cascade of synaptic events and reduce pain perception mainly by modulating activity of anterior cingulated cortex, orbitofrontal cortex, and the brainstem periaqueductal grey area [48].

tDCS is a relatively novel tool that causes focal and long lasting modulation of cortical excitability. It does not actively "stimulate" the cortex in the classic sense of the term, but rather it "modulates" cortical excitability. With regard to the increased use of tDCS in healthy subjects, the extent of cortical stimulation and the spatial distribution of the current density within the volume of the human brain for a given electrode montage is recently intensively investigated (for a recent article see: [50]). It was observed that approximately half of the current injected during tDCS is shunted through the scalp, depending on electrode dimension and position [51]. Using stimulating currents of 2.0 mA, the magnitude of the current density in relevant regions of the brain is of the order of 0.1 A/m^2, corresponding to an electric field of 0.22 V/m. Concerning the spatial distribution of the stimulation, Lang et al. [35] observed that tDCS is an effective means of provoking sustained and widespread changes in regional neuronal activity.

With regard to neuronal mechanisms, tDCS is likely to induce intracellular protein-synthesis and alterations of cAMP and calcium-levels [52–54]. NMDA receptors also seem to play a pivotal role in its cellular mechanism, as dextromethorphan—a selective NMDA receptor antagonist—abolishes any after-effects of stimulation [55]. Recent studies suggest that tDCS applied to motor and nonmotor areas according to the present tDCS safety guidelines is associated with relatively minor adverse effects in healthy humans and in patients as well [56–58]. The efficacy of this method has already been proven in several sensory modalities, recently in chronic pain [13,14]. To our knowledge, its efficacy was revealed for the first time in experimentally induced acute pain in healthy volunteers by our study. In the future, the pharmacological prolongation of the excitability diminishing after-effects [59] combined with the antinociceptive effect of cathodal stimulation would render the method of tDCS applicable to different patient populations with chronic pain.

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Electrophysiological correlates of reduced pain perception after theta-burst stimulation

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In an earlier study, we reported the antinociceptive effects of a special repetitive transcranial magnetic stimulation paradigm: continuous theta-burst stimulation (cTBS), when applied to human motor cortex. Here, we investigated whether the reduced subjective pain perception of 10 healthy individuals could be measured by changes in laser-evoked potentials, a reflection of pain related activations in the operculoinsular and midcingulate cortex. To minimize the effect of habituation during repeated laser stimulation, a bioadaptive design was used. However, both pain ratings and laser-evoked potential amplitudes were reduced after real and sham cTBS. When compared with sham stimulation, cTBS resulted in a significantly greater diminution of pain ratings and N2–P2 amplitudes on the hand contralateral to the site of motor cortex stimulation. ***NeuroReport***

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Introduction

Invasive stimulation of the primary motor cortex (M1) for the treatment of certain forms of pain has attracted much interest in recent years. Intracortical M1 stimulation alleviated chronic intractable pain [1–3] and non-invasive low-frequency and high-frequency repetitive transcranial magnetic stimulations (rTMS) over the M1 were reported to reduce both experimentally induced acute and chronic pains [4–9]. However, negative findings were also reported [10,11], which might be secondary to nonoptimized parameters of stimulation. This may change basically through a new TMS paradigm: recently, Huang and colleagues [12] showed that the application of a continuous theta-burst stimulation (cTBS) paradigm produces long-lasting (up to an hour) reversible inhibition of the M1. In this stimulation protocol, bursts consisting of three low-intensity pulses presented at 50 Hz are repeated in the theta-frequency range (5 Hz) for 40 s.

Two earlier studies investigated the effect of cTBS on pain perception. In the first study, laser-evoked pain perception was measured subjectively with the verbal analogue scale before, immediately after and 30 min poststimulation of M1 using cTBS [13]. Verum but not the sham stimulation resulted in a significant decrease in pain perception on both hands, accentuated on the contralateral hand with regard to laser stimulation. The main finding of the second study was that cTBS over the primary somatosensory cortex (SI) diminished the amplitude of the N2 laser-evoked potential (LEP) without any significant analgesic effects, when pain was induced contralaterally to the site of cTBS [14]. This indicates that LEPs are modified not only by low-frequency rTMS [11], but also by cTBS. The so-called late latency LEPs consist of the N2 and P2 waveforms, both with maximum amplitudes at the vertex [15]. Although the heat pulse from the laser activates both A-δ and C-fiber nociceptors, both the N2 and P2 components are considered to be mediated by A-δ fibers [16]. The N2 component (peaking around 160–220 ms) is generated partly in the operculoinsular region and the midcingulate cortex, whereas the P2 component (peaking around 300–360 ms) arises mostly from the midcingulate cortex and correlates with attentional and cognitive aspects of pain perception [17–19]. In earlier studies, positive correlations between the magnitude of subjective pain and the N2–P2 amplitudes were reported [11,20], rendering the N2–P2 complex an ideal tool to objectively monitor psychophysical findings.

In this study, we aimed to (i) reproduce our earlier finding with regard to the analgesic effects of cTBS applied over the M1 [13] using a bioadaptive method that minimizes the effect of habituation [21], and to (ii) investigate the effect of stimulation on LEP components. We hypothesized that in parallel with the

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change of subjective pain perception, cTBS would also reduce LEP amplitudes primarily contralateral to the side of laser stimulation.

Methods

Participants

Thirteen healthy volunteers aged between 20 and 30 years signed an informed consent. Three of them chose not to continue the experiment after the first trial, and therefore 10 volunteers (five male, five female) were included into the study. All volunteers were different from those who participated in our earlier pain-related studies [13,14]. We conformed to the Declaration of Helsinki, and the experimental protocol was approved by the Ethics Committee of the University of Göttingen. None of the individuals suffered from chronic pain syndromes, neurological or psychiatric illnesses, nor took any medications regularly.

Continuous theta-burst stimulation

cTBS was applied over the hand area of the left M1 using a standard, slightly bent figure-of-eight-coil with a posterior–anterior current flow and an outer radius of one half-coil of 9 cm (Dantec S.A., Skovlunde, Denmark). Motor-evoked potentials of the right abductor digiti minimi muscle were recorded by Ag–AgCl electrodes in a belly-tendon-montage. Active motor threshold was defined as the minimum intensity eliciting a motor-evoked potential of a superior size compared with spontaneous moderate muscular activity in at least three out of six trials. cTBS was delivered using a Magstim Super Rapid stimulator (Mastim Company Ltd., Whitland, Wales, UK). TMS pulse intensity was 80% of active motor threshold. A burst consisted of three pulses applied at 50 Hz, which was repeated every 200 ms for 40 s. In accordance with earlier studies [12,13], we stimulated above the position of electrode C3, which is situated above the primary M1.

In separate experimental sessions, sham stimulation was applied with the same parameters, but using a sham coil (Dantec S.A.) with output reduced to 15% of real stimulation. The experimental sessions were separated from each other by at least 5 days. The individuals were blinded concerning the type of the magnetic stimulation. The order of the sessions was randomized across individuals.

Laser stimulation

A Tm:YAG laser system (WaveLight Laser Technologie AG, Erlangen, Germany) was used for noxious stimulation (wavelength: 2010 nm, pulse duration: 1 ms, laser beam diameter: 7 mm). The distal hand piece of the laser was positioned 30 cm from the dorsal surface of the hand. Before LEP recording, we determined the pain threshold on both hands by applying laser stimuli from 200 to 1000 mJ in 50 mJ steps. During LEP recording, each pulse was delivered with an intensity of 1.4–1.6 times the threshold intensity to a slightly different spot in a square (5×5 cm) to reduce receptor fatigue or sensitization by skin overheating [15]. To get reliable LEP waveforms and to avoid habituation, we adopted the bioadaptive design described by Weiss and colleagues [21]: laser intensity was continuously adjusted to always evoke moderate pain sensation. In addition, both in the real and sham cTBS conditions, the right hand was stimulated first in half of the cases and the left hand was stimulated first in the other half of the cases. Every run consisted of 40 laser stimuli on each hand. The interstimulus interval ranged from 8 to 15 s.

Psychophysical evaluation

We used the Numeric Rating Scale to assess subjective pain intensity. The individuals were instructed to pay attention to the laser stimuli and to rate the perceived pain verbally (0: warm, 1: smallest pain, 10: most intense pain) about 2–3 s after each stimulation. Individuals wore earplugs, and white noise was presented during the measurements to avoid auditory artifacts because of laser stimulation.

Electrophysiological recordings

The electroencephalogram was recorded using a 64-channel montage with ring electrodes (inner diameter: 6 mm, outer diameter: 12 mm; EasyCap; Falk and Minow Gmbh, Munich, Germany). The electrodes were placed in accordance with the extended international 10/20 system. The impedance was kept less than 5 kΩ. Fz was used as reference, the ground was placed 2 cm anterior to the tragus of the right ear. Data were collected with a sampling rate of 1000 Hz using the BrainAmp system (Brain Products GmbH, Munich, Germany) and were analysed off-line. The electroencephalogram was re-referenced to connected mastoids, and a 0.5 Hz low-cutoff and a 30 Hz high-cutoff filters were used. After automatic artifact detection (200 μV amplitude criterion), all epochs were visually inspected, and those containing eye blinks or muscle movement artifacts were excluded. Baseline correction was performed on the basis of the 100-ms prestimulus interval.

Although we recorded data on 64 channels, we assessed LEPs according to the scalp distribution of the analysed peaks. The N2–P2 components are larger over the lateral temporal and frontocentral areas, and therefore LEP analysis was performed with regard to the three scalp regions: central (FCz, Cz, Pz), left (FC3, FC5, C3, C5, CP3, CP5) and right (FC4, FC6, C4, C6, CP4, CP6) instead of separate electrodes.

Data analysis

As pain thresholds differed across individuals, normalization of the data was necessary. We divided the pain

rating scores (1–10) by the actual laser energy (J). These values were compared for both hands separately with repeated-measures analysis of variance (ANOVA), where rTMS stimulation types (real and sham cTBS) and time (before and after cTBS) served as within-subject factors.

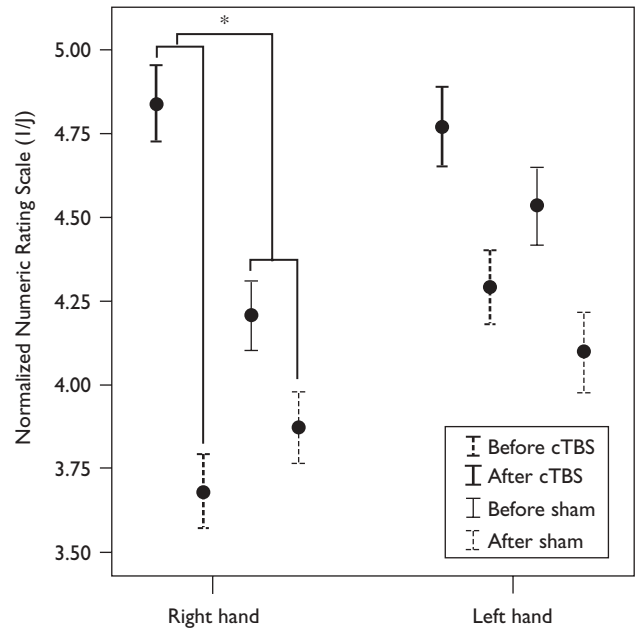
N2 and P2 baseline amplitudes and the N2–P2 peak-to-peak amplitude were entered into a repeated-measures ANOVA for both hands separately. Condition (real cTBS and sham cTBS) and time (before cTBS and after cTBS) were within-subject factors, whereas scalp regions were between-subject factors.

Results

Psychophysics

A repeated-measures ANOVA revealed a significant decrease in normalized pain rating scores in both hands ($F = 97.94$, $P < 0.001$ for the right hand and $F = 29.90$, $P < 0.001$ for the left hand) (Fig. 1 and Table 1). This indicates a robust habituation to laser stimuli, despite the bioadaptive design used. However, the interaction with stimulation type was significant only in the right-hand laser stimulation condition ($F = 25.14$, $P < 0.001$ for the right hand and $F = 0.91$, $P = 0.34$ for the left hand), for real cTBS causing almost four times larger decrease in pain sensation compared with sham stimulation (Fig. 1 and Table 1).

Fig. 1



Normalized Numeric Rating Scale scores (1/J) for both continuous theta-burst stimulation (cTBS) conditions and hands (mean ± SEM). The asterisk marks significant ($P < 0.001$) difference for right (contralateral)-hand stimulation between real cTBS and sham cTBS.

Electrophysiology

The laser stimulation induced a pricking pain in all individuals and biphasic N2–P2 components were clearly identified in all LEP measurements (Fig. 2a and b).

The N2 amplitude decreased in both cTBS conditions for both hands after stimulation ($F = 191.14$, $P < 0.001$ for the right side and $F = 76.78$, $P < 0.001$ for the left side; Table 1). The interaction with the condition (real vs. sham cTBS) was also significant for both hands ($F = 12.52$, $P < 0.001$ for the right hand and $F = 4.00$, $P = 0.04$ for the left hand), indicating that after real cTBS the N2 amplitude reduction was larger than after sham stimulation. Separate analysis of the N2 component over the three regions revealed that in cases of left-hand stimulation, the change was independent of the scalp distribution ($F = 0.712$, $P = 0.40$ for the central region, $F = 1.280$, $P = 0.25$ for the right region and $F = 1.749$, $P = 0.18$ for the left region), whereas for right-hand stimulation, a significant effect of condition (real sham) over the central ($F = 3.984$, $P = 0.05$) and left hemisphere ($F = 5.227$, $P = 0.02$), but not for the right hemisphere ($F = 0.104$, $P = 0.74$) was found.

With regard to the P2 amplitude, there was also a significant modulation for both hands and cTBS conditions ($F = 225.38$, $P < 0.001$ for the right hand and $F = 239.65$, $P < 0.001$ for the left hand), but there was a significant interaction with condition only for left-hand laser stimulation ($F = 0.69$, $P = 0.40$ for the right hand and $F = 22.50$, $P < 0.001$ for the left hand). Interestingly, for the left hand, the magnitude of amplitude change was significantly less when real cTBS was used (Table 1), showing that in this case real cTBS caused dishabituation. Analysis of scalp distribution showed a significant effect for condition over the left and right hemispheres ($F = 6.95$, $P < 0.01$ for the left region, $F = 18.448$, $P < 0.001$ for the right region and $F = 2.085$, $P = 0.15$ for the central region).

As for the separate LEP components, the N2–P2 peak-to-peak amplitudes also decreased significantly regardless of cTBS condition or hand ($F = 224.21$, $P < 0.001$, for the right hand and $F = 241.24$, $P < 0.001$ for the left hand). The interaction with cTBS condition was significant only for right-hand laser stimulation ($F = 4.53$, $P = 0.03$ for the right hand and $F = 1.64$, $P = 0.20$ for the left hand), regardless of the scalp region (Fig. 2a and b).

Discussion

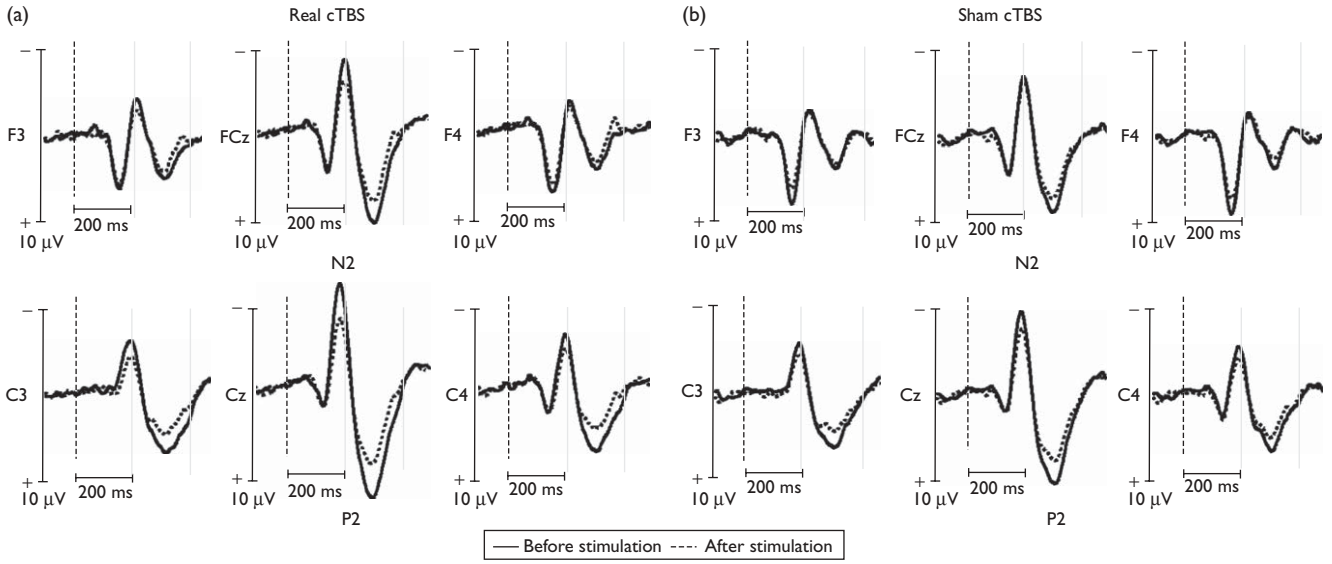
In our study, we could confirm that the previously reported analgesic effect of cTBS applied over the M1 [13] was because of excitability changes of neural assemblies responsible for the generation of late LEP components. Despite the bioadaptive design, habituation

Table 1 Characteristics of psychophysical and electrophysiological data for both hands, time periods and cTBS conditions

		Right hand		Left hand	
		Real cTBS	Sham cTBS	Real cTBS	Sham cTBS
Rating/energy (1/J)	Before	4.81 ± 0.10	4.21 ± 0.10	4.76 ± 0.10	4.53 ± 0.11
	After	3.67 ± 0.08	3.87 ± 0.10	4.32 ± 0.09	4.10 ± 0.12
	Before–after	1.14 ± 0.09	0.32 ± 0.12	0.44 ± 0.13	0.43 ± 0.14
	Real cTBS–sham cTBS	0.83 ± 0.17		0.02 ± 0.22	
N2–P2 amplitudes (μV)	Before	17.87 ± 0.65	17.09 ± 0.73	16.27 ± 0.74	14.79 ± 0.72
	After	13.44 ± 0.50	13.49 ± 0.66	13.06 ± 0.61	10.78 ± 0.55
	Before–after	4.42 ± 0.42	3.59 ± 0.26	3.21 ± 0.34	4.01 ± 0.32
	Real cTBS–sham cTBS	0.83 ± 2.81		−0.80 ± 3.69	
Absolute N2 amplitudes (μV)	Before	7.31 ± 0.26	6.88 ± 0.32	6.51 ± 0.32	5.14 ± 0.31
	After	5.31 ± 0.20	5.45 ± 0.28	5.06 ± 0.27	4.20 ± 0.22
	Before–after	2.00 ± 0.18	1.40 ± 0.14	1.45 ± 0.22	0.91 ± 0.17
	Real cTBS–sham cTBS	0.60 ± 2.51		0.55 ± 3.59	
P2 amplitudes (μV)	Before	8.59 ± 0.31	8.34 ± 0.31	7.99 ± 0.32	8.15 ± 0.31
	After	6.71 ± 0.26	6.60 ± 0.28	6.62 ± 0.25	5.70 ± 0.26
	Before–after	1.88 ± 0.20	1.74 ± 0.12	1.37 ± 0.14	2.45 ± 0.17
	Real cTBS–sham cTBS	0.14 ± 2.84		−1.08 ± 2.58	

cTBS, continuous theta-burst stimulation.

Fig. 2



Grand averages of laser-evoked potentials at six scalp electrodes obtained for right-hand laser stimulation in the (a) real continuous theta-burst stimulation (cTBS) and (b) sham cTBS conditions. Note that there is a greater N2–P2 reduction for real cTBS (most prominent at electrode Cz), which is mainly because of smaller N2 amplitudes after motor cortex stimulation.

of both pain rating scores and LEP amplitudes occurred (Figs 1 and 2). This effect was independent from the type of stimulation and is most likely because of central habituation effects [22]. However, after real cTBS, significant higher energy was needed to induce acute pain at the contralateral side than after sham stimulation. In accordance with our psychophysical results, the N2–P2 amplitude reduction was observed only after contralateral-hand laser stimulation, which was mainly because of changes in N2 amplitude.

We previously reported N2 reduction after real cTBS of the primary somatosensory cortex, although pain rating scores did not deviate from that of sham stimulation. Here, the same stimulation protocol slightly anterior to the somatosensory cortex resulted in a similar N2 diminution, which was in this case accompanied by an analgesic effect as well. During invasive electrical stimulation, increased regional cerebral blood flow in the ipsilateral thalamus, cingulate gyrus, orbitofrontal cortex and brainstem was reported, but regional cerebral blood flow in the M1 or SI remained unchanged [2]. This indicates that the analgesic effect is not likely to be caused by changes in M1 activity *per se*, but rather by secondary activation of the cingulate/orbitofrontal cortex that influences the affective/emotional component of

chronic pain and subsequently leads to the descending inhibition of pain impulses by activation of the brainstem [2]. This is further supported by the fact that the M1 is intensively interconnected with anterior cingulate cortex [23].

With regard to the ipsilateral side, the decreased habituation of the P2 amplitude after real cTBS is a rather puzzling finding of the study. A similar phenomenon was reported in the visual modality as well: local inhibition of the visual cortex by applying 900 pulses of 1 Hz rTMS significantly reduced the habituation to pattern reversal visual-evoked potential amplitudes in healthy volunteers, an effect which was prolonged to several weeks after five daily rTMS sessions in another study [24]. The reduced P2 habituation in our study could be a consequence of a generalized dishabituation that evolves on the grounds of local inhibition of the ipsilateral M1. Whatever the exact underlying mechanisms are, changes of the P2 component did not affect the N2–P2 amplitudes, the magnitude of which is considered to be an electrophysiological index of subjective pain experience. In addition, this ipsilateral effect did not influence subjective pain rating scores during left-hand stimulation.

In earlier studies, TBS produced a controllable, consistent, long-lasting and powerful effect on M1 excitability and behaviour after an application period of only 20–190 s [12,25]. The effect on cortical excitability depends on the stimulation protocol: intermittent stimulation produces facilitation, whereas continuous TBS induces inhibition of the underlying cortical area [12]. The reasons underlying these effects are still speculative at this stage, but probably involve long-term potentiation-like and long-term depression-like mechanisms. Our results further strengthen the application of cTBS in pain research, which may contribute to a more efficient manipulation of the brain plasticity for therapeutic purposes.

Conclusion

Our study showed that 40 s of cTBS applied over the M1 alleviated laser-induced pain perception on the contralateral hand. This analgesic effect was accompanied by reductions in the N2–P2 LEP amplitudes, which indicates that such stimulation does not exhibit only local effects, but might also modulate the excitability of other pain-related brain areas.

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Nem-invazív agyi ingerlési módszerek alkalmazása akut és krónikus fájdalom csillapítására

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Rövid cím: rTMS és tDCS akut és krónikus fájdalomban

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Non-invasive brain stimulation for relieving acute and chronic pain

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Absztrakt

A fájdalomcsillapítás mindig is az orvostudomány legnagyobb kihívásai közé tartozott, és a gyógyszeripari erőfeszítések ellenére sok kórképben a mai napig nem megoldott a tartós fájdalommentes állapot elérése. Az elmúlt 40 év során számos olyan műtéti beavatkozás terjedt el, amely a központi idegrendszer működésének modulálásával eredményesen csökkentette a krónikus, farmakorezisztens fájdalmat. Az ilyen beavatkozások azonban sokszor igen komoly kockázattal és számos mellékhatással jártnak. Ezzel párhuzamosan, a kilencvenes években megjelent több olyan nem-invazív agyi ingerlési módszer, amelyek lehetővé tették viszonylag körülírt kérgi agyterületek működésének átmeneti serkentését vagy gátlását. Ezen eljárások az agy elektromos ingerlésén alapulnak, és az alkalmazás helyétől valamint az ingerlési paraméterektől függően mindezidáig eredményesen alkalmazták őket a perceptuális, kognitív és motoros folyamatok befolyásolására mind egészséges személyekben, mind különféle kórképekben. Jelen összefoglalásban két ilyen módszer, a repetitív transcranialis mágneses ingerlés és a transcranialis egyenáram-ingerlés alkalmazásával kapcsolatos eredményeket ismertetjük kísérletesen kiváltott, akut fájdalomészlelés és krónikus fájdalom szindrómák esetében.

Kulcsszavak: fájdalomcsillapítás, transcranialis mágneses ingerlés, transcranialis egyenáram-ingerlés, lézer által kiváltott agyi potenciál

Abstract

Controlling pain has always been one of the biggest challenges of medical science. Despite pharmacological developments, still many patients suffer from long-lasting pain. During the last 40 years several surgical interventions have been used to modulate the activity of the central nervous system in order to control chronic, pharmacoresistant pain. Because such interventions may involve very serious adverse events, safer and at least equally efficient methods are still required. In the 90's new techniques of non-invasive brain stimulation have been introduced that enable the facilitation or inhibition of distinct cortical areas. These methods are based on the electrical stimulation of brain structures and to date they have been successfully used to modulate perceptual, cognitive and motor functions in healthy subjects and various diseases as well. In this review we describe such techniques of non-invasive brain stimulation, namely repeated transcranial magnetic stimulation and transcranial direct current stimulation and review the current literature about their efficacy in controlling acute and chronic pain.

Key words: pain relief, transcranial magnetic stimulation, transcranial direct current stimulation, laser-evoked potential

A fájdalom gyógyszeres csillapítása mellett az egyik legelterjedtebb eljárás a perifériás és központi idegrendszeri elektromos inger általi modulálása. Az elektromos impulzusok ilyen jellegű alkalmazása nem új: már az ókori Egyiptomban és Görögországban alkalmazták az elektromos rájákat végtag- és fejfájás csillapítására. A római Scribonius Largus Kr.u. 47-48-ban írt „De Compositionibus Medicamentrum" című munkájában javasolja, hogy fájdalomcsillapítás céljából helyezzük a ráját az érintett területre. A mai modern eljárások azonban nyilvánvalóan sokkal pontosabb tervezést igényelnek, amelyekhez elengedhetetlen a fájdalomészlelés idegrendszeri hátterének alapos ismerete.

A fájdalomészlelés elektrofiziológia és hemodinamikai korrelátumai

A 90-es években az agyi képalkotó eljárások által kínált jó térbeli és időbeli felbontás lehetővé tette a fájdalom által kiváltott agyi aktivitásváltozások feltérképezését mind egészséges személyeknél, mind krónikus fájdalom szindrómákban szenvedő betegeknél. Bár az állatkísérletek valamint a humán hemodinamikai és elektrofiziológiai vizsgálatok eredményei a mai napig néhol ellentmondásosak, lényegében mégis sikerült azonosítani azon agyi struktúrákat, amelyek különböző aspektusból járulnak hozzá a fájdalomészleléshez. Mint számos neurológiai kórképben, így a krónikus fájdalom szindrómák esetén is kimutathatóak olyan plasztikus idegrendszeri változások, amelyek során megváltozik egyes agyterületek makro- és mikroszkópos struktúrája, neurokémiai profilja és neuronjainak válaszkészsége. Mivel krónikus fájdalom esetén az ilyen jellegű eltérések nagymértékben befolyásolják az alkalmazott nem-invazív ingerlés paramétereit, lényegesnek tartjuk a krónikus fájdalomban szenvedő betegeknél tapasztalható strukturális és funkcionális eltéréseket külön tárgyalni.

Az akut fájdalomészlelés idegrendszeri háttere egészséges személyekben

A fájdalom érzékeléséért szabad idegvégződések felelősek, amelyek viszonylag gyorsan vezető, Aδ-rostokon és lassabban vezető, velőshüvely nélküli C-rostokon lépnek be a központi idegrendszerbe. A felszálló fájdalomérzékelő pályák innen hagyományosan két nagy rendszerre, a medialis és a lateralis rendszerre oszthatók. Ezek mind a gerincvelői hátulsó szarvi átkapcsolódás, mind a gerincvelői fehérállományi elhelyezkedés, mind pedig az érintett thalamus magok szempontjából különböznek egymástól. Mindezek ismertetése messze meghaladja jelen tanulmány kereteit, annyit azonban fontosnak tartunk hangsúlyozni, hogy a lateralis rendszer elsősorban a fájdalmat kiváltó inger fizikai tulajdonságainak (behatás helye, intenzitása, modalitása, időtartama, stb.) feldolgozásáért felelős, míg a medialis rendszer főként a fájdalommal kapcsolatos érzelmi-kognitív folyamatokat szabályozza.

A klinikai tudomány fejlődése folyamán nagyon sokáig kétséges volt, hogy létezik-e egyáltalán a fájdalomészlelés szempontjából specifikus kérgi terület. Head és Holmes 1911-es

megfigyelései alapján a kérgi sérülések nem jártak fokozott vagy csökkent fájdalomészleléssel, és később Penfield és Boldrey közvetlen kérgi ingerlésessel sem talált egyértelműen fájdalomérzékeny agyi területet[1]. Később a modern agyi képalkotó vizsgálatoknak köszönhetően sikerült azonosítani négy kérgi terület, nevezetesen az elsődleges és a másodlagos somatosenoros cortexet (SI és SII), az insulát és az anterior cingularis cortexet (ACC), amelyek az agyi fájdalomészlelési hálózat részeit képezik **(1. ábra)**. A fájdalom feldolgozását e négy terület mellett több struktúra (dorsolateralis praefrontalis cortex (DLPFC), amygdala, hippocampus) is befolyásolja, amelyek elsősorban a fájdalomhoz kapcsolt figyelmi, tanulási és kontroll folyamatokban játszanak szerepet[1]. Témánk szempontjából e területek közül transcranialis megközelíthetősége miatt a DLPFC különösen fontos.

Az SI és az SII a lateralis fájdalomrendszerhez tartozik. Mindkettő közvetlen afferentációt kap a thalamusból (eltérő magokból)[1], és a fájdalom által kiváltott aktiválódásuk időben átfedi egymást[2]. Ebből következik, hogy egyéb somatosensoros alrendszerektől eltérően, a fájdalom esetében nem szekvenciális, hanem párhuzamos kérgi feldolgozás valósul meg. Az SI a Gyrus postcentralisban helyezkedik el, és pozitron emissziós tomográfiás (PET) valamint funkcionális mágneses rezonanciás képalkotó (fMRI) vizsgálatok alapján az SI aktivitás a fájdalom lokalizációjával contralateralisan jelentkezik, többek között a kiváltó inger térbeli elhelyezkedésének kódolásában játszik szerepet[3], és a mért hemodinamikai jel nagysága az inger intenzitásával (de nem a fájdalom kellemetlenségével) arányos[4].

Az SII a Sylvius árok felett, a parietalis operculumban helyezkedik el. Az insulához való közelsége miatt a két terület képalkotó eljárással való különválasztása igen nehéz, így azokat közös néven supra- vagy parasylvianus areaként vagy operculoinsularis területként is illetik. E terület fájdalmat kiváltó inger általi aktivációja bilateralis[5], és humán léziós vizsgálatok tanulsága szerint az SII sérülése magasabb fájdalomküszöböt eredményez[6]. Az insula sérülése kevésbé befolyásolja a fájdalomküszöböt[6], ellenben csökkenti a fájdalmas ingerekre adott affektív reakciókat valamint somaticus és vegetatív válaszokat.

Régóta ismert, hogy az ACC és az alatta lévő fehérállomány szerepet játszik a fájdalom észlelésében. A terület műtéti sértése csökkenti a fájdalom által okozott kellemetlenséget[7]. Fájdalominger hatására mindkét oldali ACC véráramlása megnő[1], és az aktiváció mértéke arányos a fájdalom affektív komponensével[8]. Emellett az ACC aktivitás szerepet játszhat a fájdalom által kiváltott figyelmi orientáció és motoros reakciók szabályozásában[9].

Egyre több adat mutat rá a DLPFC meghatározó szerepére a fájdalomészlelésben. Egy PET vizsgálatban a bilateralis DLPFC fokozott véráramlása negatívan korrelált a kísérletesen kiváltott fájdalom intenzitásával[10], míg egy fMRI vizsgálatban a DLPFC aktivitása a placebo által kiváltott analgesia mértékével párhuzamosan emelkedett, míg csökkent a klasszikus „fájdalomspecifikus" területeken (thalamus, insula, ACC) mért hemodinamikus válasz[11]. Mindezek arra utalnak, hogy a DLPFC jelentős szerepet játszik a fájdalomészlelés „top-down" szabályozásában, amit a régió felett alkalmazott viszonylag kisszámú számú nem-invazív ingerléses vizsgálat is megerősíteni látszik (lásd később).

A kísérletesen kiváltott, akut fájdalom mértékét nem csupán a vizsgálati személyek szubjektív értékelése illetve az agyi véráramlás-változás alapján lehet megítélni, hanem elektro- és magnetoenkefalográfiás (EEG és MEG) vizsgálattal is. Laboratóriumi körülmények között több eljárás is alkalmas kontrollált fájdalomindukcióra (extrém hideg és meleg, közvetlen elektromos ingerlés, átmeneti ischaemia, intradermalis capsaicin injekció), azonban csupán az 1970-es évek óta elterjedt lézeres ingerlési eljárásokkal vált lehetővé a fájdalom feldolgozásának pontos időbeli követése. Az alkalmazott CO_2, Tm-YAG vagy argon lézerek rendkívül rövid (1-100 milliszekundumos), a fájdalomküszöböt 1,5-2-szer meghaladó intenzitású impulzusai szelektíven ingerlik az A-δ és C-rostokat, és az epidermis ritkán jelentkező, gyorsan gyógyuló égését leszámítva nem rendelkeznek mellékhatással[12]. Az ingerlés ideje alatt a skalpról EEG/MEG segítségével ú.n. lézer által kiváltott agyi potenciálok (laser-evoked potential, LEP-ek) regisztrálhatók, amelyek a fájdalom által kiváltott idegi jel perifériás és centrális továbbításáról és az inger központi idegrendszeri feldolgozásáról szolgálnak információval. A LEP komponensek latenciájuk alapján korai (N1 és P1), kései (N2 és P2) illetve ultra-kései komponensekre oszthatók. Az egyes hullámok megjelenési ideje függ az ingerelt terület agytól való távolságától, az ingerelt axonok vezetési sebességétől és az ingerlés idejétől, míg amplitúdójuk a keletkezésükért felelős agyterületek aktivitását tükrözi. A korai N1/P1 potenciálok (latencia 160 msec körül) maximuma a temporalis elvezetések felett jelentkezik, és feltehetően a suprasylvianus area (elsősorban az SII) aktivitását tükrözi[13]. Az N2/P2 potenciálok (latencia kézfej ingerlése esetén 210/330 msec, talp ingerlése esetén 250/380 msec) maximuma a vertexen (Cz elvezetés) jelentkezik **(2.a ábra)**, és mind a suprasylvianus, mind az ACC aktivitásának szerepe van kialakulásukban[13]. A fájdalom intenzitása és kellemetlensége az N2/P2 komponens amplitúdójával korrelál, de kognitív folyamatok, mint pl. a fájdalomra irányuló figyelem is befolyásolhatja[13]. Az ultra-kései potenciálok (latencia 700-1300 msec körül, az ingerlés helyétől és módjától függően) olyan speciális ingerlési technikával jelentkeznek, amely során csupán a lassan vezető C-rostokat

ingerlik. Amint azt később ismertetjük, a LEP amplitúdók elemzésével sikeresen követhető a nem-invazív agyi ingerlés fájdalomészlelést befolyásoló hatása.

A fájdalomészlelés eltérései krónikus fájdalomban szenvedő betegeknél

A centrális eredetű fájdalom központi idegrendszeri (gyakran corticalis, thalamicus, agytörzsi vagy gerincvelői) laesio által közvetlenül, vagy perifériás idegsérülés (fantom végtagfájdalom, derékfájdalom, fibromyalgia) következtében kialakuló, a centrális fájdalomfeldolgozó pályák túlérzékenységével jellemezhető tartós fájdalom. Egy nemrég közölt összefoglaló tanulmány rámutat arra, hogy különféle krónikus fájdalom szindrómában szenvedő betegeknél hasonló morfológiai eltérések azonosíthatóak, vagyis feltételezhető egy krónikus fájdalomra jellemző "közös agyi mintázat"[14].

Krónikus hátfájdalomban leírták a bilateralis DLPFC szürkeállományi és a thalamus degenerációját[15]. Fantom végtagfájdalomban régóta ismert a végtaggal ellenoldali somatosensoros és motor cortex reprezentációinak átrendeződése[16]. Krónikus tenziós fejfájásban a betegség fennállásának idejével arányos szürkeállományi csökkenést észleltek a cingularis, insularis, orbitofrontalis, parahippocampalis és cerebellaris területeken[17]. Migrénben szintúgy egyértelműnek tűnik a cingularis cortex, az insula és a temporalis lebeny szürkeállományának elvékonyodása[18]. Fibromyalgiában parahippocampalis, insularis, cingularis és medialis praefrontalis szürkeállományi elvékonyodást észleltek[19].

A fenti strukturális eltérésekkel némileg egybecseng, hogy krónikus fájdalom szindrómában szenvedőknél eltér a kísérletesen kiváltott akut fájdalom okozta agyi aktivitásváltozás. Egy meta-elemzés szerint ilyen betegeknél erőteljesebb praefrontalis kérgi véráramlás-fokozódás tapasztalható, valamint csökken a klasszikus fájdalomészlelésben jelentős területeken (SI, SII, Thalamus, ACC) mért hemodinamikai jel[20].

Összességében megállapítható, hogy bár a krónikus fájdalom „közös agyi mintázatát” még korántsem sikerült egyértelműen azonosítani, a fenti kórképekben morfológiai leépülés-átrendeződés tapasztalható a fájdalomészlelés szempontjából kulcsfontosságú struktúrákban, mint a cingularis, insularis és praefrontalis területeken[14]. A fenti eltérések közül kiemelendő a fájdalomra irányuló figyelmi és anticipációs folyamatokban, valamint a placebo-analgesia során aktiválódó ACC és DLPFC[21] eltérése. Mindkét területnek szerepe van a PAG-ból kiinduló leszálló fájdalomcsillapító pályák szabályozásában, így az ACC és DLPFC eltérései módosíthatják a fájdalom affektív-kognitív értékelését, és így hosszú távon megváltoztathatják a fájdalom szubjektív megélését, annak krónikussá válását eredményezve. Mindezek alapján joggal feltételezhető, hogy fájdalomcsillapítás céljából alkalmazott agyi

ingerlés során a medialis-lateralis praefrontalis cortex működésének közvetlen vagy közvetett modulálása lehet az egyik fő célpont.

Invazív elektromos fájdalomcsillapítás

Az orvostudomány fejlődésével megjelentek azon invazív eljárások, amelyek a központi idegrendszeri struktúrák elektromos ingerlésével hatásosnak bizonyultak krónikus, gyógyszeres terápiára refrakter fájdalom szindrómákban. A thalamus szenzoros magjait illetve az agytörzsi periaqueductalis szürkeállományt (preiaqueductal gray matter; PAG) célzó mély agyi ingerlés már 30 éve ismert eljárás, és a mai napig alkalmazzák többek között amputáció illetve stroke utáni fájdalom csillapítására[22].

A kilencvenes évek elején Tsubokawa és munkatársai macskákon végzett kísérletei rámutattak arra, hogy a motor cortex ingerlése csökkenti a thalamicus relé neuronok spinothalamicus deafferentációt követően kialakuló hiperaktivitását[23]. Ebből kiindulva a szerzőknek hatékony fájdalomcsillapítást sikerült elérniük a Gyrus praecentralis epiduralis elektródákkal történő krónikus ingerlésével thalamicus fájdalom szindrómában szenvedő betegeknél[23]. Az eljárás motor cortex stimuláció (MCS) néven vált ismertté, és a mai napig javasolt arcfájdalom és stroke utáni neuropathiás fájdalom csillapítására[22]. Az MCS pontos hatásmechanizmusa nem ismert: PET vizsgálatok sem az alkalmazás helyén, sem a primer somatosensoros cortexben nem találtak jelentős agyi regionális véráramlás-változást[24]. Ezzel ellentétben, jelentős véráramlás-növekedést tapasztaltak számos, a fájdalomészlelés szempontjából releváns agyi struktúrában, mint a thalamusban, az ACC-ben, az orbitofrontalis cortexben, az anterior insularis cortexben és a felső agytörzsi régiókban. A cingularis és orbitofrontalis véráramlás-fokozódás szignifikánsan erőteljesebb volt azon betegeknél, akiknél a beavatkozás jelentősebb fájdalomcsillapítást eredményezett. Ez egybecseng a krónikus fájdalom szindrómákban szenvedő betegeknél tapasztalható ACC elvékonyodással és hypoaktivitással, amely MCS általi helyreállítása kedvezően befolyásolhatja a fájdalom megélését. A beavatkozás hatásmechanizmusának jelenleg legelfogadottabb modellje szerint a motor cortexből induló corticothalamicus rostok a lateralis, majd a medialis thalamus magok aktiválásával egyrészt a fájdalom affektív komponensét csökkentik a cingularis/orbitofrontalis cortex befolyásolása által, másrészt pedig a PAG-ból a gerincvelői hátulsó szarvhoz leszálló pályák a nociceptív ingerek belépését és felszállását gátolják, feltehetően ópiáterg mechanizmus által[25]. A motor cortex fájdalomcsillapításban betöltött szerepe a nem-invazív módszerek szempontjából különösen fontos, hiszen ezen eljárások fájdalomcsillapító hatásának tekintetében az elsődleges motor cortex (M1) ingerlésével kapcsolatban áll rendelkezésünkre a legtöbb pozitív eredmény. A továbbiakban ismertetjük a két

leggyakrabban alkalmazott nem-invazív agyi ingerlést, valamint azok alkalmazhatóságát a fájdalomcsillapítás terén.

A transcranialis mágneses ingerlés

A transcranialis mágneses ingerlést (transcranial magnetic stimulation; TMS) Barker és munkatársai vezették be a klinikai gyakorlatba 1985-ben[26]. A módszer alapja az elektromágneses indukció, amely szerint egy vezetőben létrehozott rövid idejű, nagy erősségű áram változó mágneses teret hoz létre. A TMS készülék esetén a feltekercselt vezeték egy általában kézben tartható fémházban található, amelyet az ingerelni kívánt agyterület fölé, a fejbőrre helyeznek **(3. ábra)**. A keletkező rendkívül erős (kb. 2 T) mágneses mező kb. 1 milliszekundum alatt lecseng, de a skalpon, koponyacsonton és liquortereken áthatolva elektromos térerő-változást indukál az agyban. Ennek hatására depolarizáció, majd akciós potenciál keletkezik a kérgi idegsejtek axonjaiban, amelyek könnyebben ingerelhetők, mint a neuronok szómája[27]. A manapság leggyakrabban használt nyolcas alakú tekercsben a két hurok találkozásánál alakul ki a maximális mágneses térerő-változás, ami lehetővé teszi az ingerlés helyének pontosabb meghatározását, és a viszonylag körülírt agyi ingerlést (ezt különböző neuronavigációs eljárásokkal lehet még pontosabbá tenni). A számítógépes modellek szerint a mágneses tér csak a felszíni szürkeállományig hatol, így a módszerrel egyelőre nem lehetséges mélyebb agyi struktúrák közvetlen ingerlése. Az axonokban keletkező akciós potenciál azonban az axon mentén mind ortodrómos, mind antidrómos irányban végigfut, és így nem csupán a tekercs alatti neuronokban okoz membránpotenciál-változást, hanem akár távoli corticalis és subcorticalis struktúrákban is. Ez a fájdalomcsillapítás szempontjából is rendkívül fontos, ugyanis a legtöbb, fájdalom feldolgozás szempontjából releváns idegrendszeri struktúra (pl. insula, ACC) a skalptól való távolsága miatt TMS által közvetlenül nem ingerlehetők.

Az egyszeri TMS még nem alkalmas az idegi aktivitás hosszabb ideig tartó megváltoztatására, ahhoz ismételt ingerlés (repetitív TMS, rTMS) szükséges. Számos rTMS ingerlési protokoll létezik, amelyeknek főbb paraméterei a létrehozott térerő-változás intenzitása, a tekercs orientációja, az ingerlés frekvenciája, az összesen alkalmazott impulzusok száma, és az egyes sorozatok ismétlődése, akár napi szinten. Míg az egyszeri ingerlés intenzitása küszöb feletti, azaz az ingerlés helyétől függően objektív módon mérhető változásokat idéz elő (pl. a motor cortex ingerlése akaratlan izomkontrakciókat, ú.n. motoros kiváltott potenciálokat, MEP-eket hoz létre a megfelelő izomcsoportokban), az rTMS impulzusok (elsősorban biztonsági okból) jóval gyengébbek, intenzitásuk küszöb alatti (a pontos értéket általában az ú.n. motoros küszöb százalékában szokták meghatározni). Az

elmúlt évtized tapasztalatai alapján az rTMS frekvenciafüggő módon, akár több óráig képes megváltoztatni az ingerelt agyterület idegsejtjeinek ingerlékenységét: az alacsony frekvenciájú (általában 1 Hz-es) ingerlés gátló hatású, míg az 5 Hz-nél magasabb (általában 10 vagy ritkábban 20 Hz-es) ingerlés serkentő hatású. Mindez a kérgi neurontípusok eltérő ingerlékenységével áll összefüggésben: a gátló GABAerg neuronok könnyebben ingerelhetőek, így az alacsony frekvenciájú rTMS elsősorban ezen sejtek excitabilitását változtatja meg átmenetileg, míg a serkentő piramissejteken érvényesülő hatás csak magasabb frekvenciáknál jön létre.

Az rTMS hosszan tartó hatásának molekuláris mechanizmusai még nem teljesen tisztázottak, de minden bizonnyal az ingerlési frekvenciától függően hosszú távú potenciációt (long-term potentiation, LTP) vagy depressziót (LTD) idéznek elő az ingerelt neuronokban. Ezt támasztják alá azon újabb vizsgálati eredmények, melyek szerint az rTMS utóhatásai kivédhetők a dextrometophran vagy a memantin N-metil-D-aszpartát (NMDA) glutamát receptor antagonisták bevitelével[28].

Az rTMS hatékonyságát legelőször a motor cortex esetében mutatták ki: nagy frekvenciájú ingerlést követően nagyobb amplitúdójú MEP-eket mértek a motor cortex ingerlésének hatására[29], míg az 1 Hz-es ingerlés csökkentette a MEP amplitúdókat az ingerlés befejezését követő 30 percen át[30]. A motoros rendszer mellett az rTMS segítségével sikeresen befolyásoltak számos perceptuális[31] és kognitív folyamatot[32].

A klasszikus magas és alacsony frekvenciájú rTMS protokollok mellett az elmúlt pár évben terjedtek el az ún. theta frekvenciájú sorozatingerlés (theta-burst stimulation, TBS) különböző változatai. A módszer azon a régi megfigyelésen alapul, hogy a 4 ingerből álló, gyors (100 Hz-es) sorozatingerek, amelyek theta (5 Hz) frekvenciával (azaz 200 milliszekundumonként) követik egymást, kifejezetten hatékonyan idéznek elő LTP-t a hippocampus CA1 régiójában[33]. Huang és munkatársai a TBS protokollt az rTMS-hez adaptálván kidolgozták a folyamatos (continuous TBS, cTBS), illetve az intermittáló (iTBS) ingerlést[34]. A primer motor cortex felett alkalmazva az iTBS szignifikáns mértékben megnövelte a MEP amplitúdót, míg a cTBS erőteljesen csökkentette azt. Az iTBS az ingerlést követően kb. 20 percig fejtette ki hatását, a cTBS pedig a korábbi rTMS eljárásoknál lényegesen hosszabb ideig, mintegy 60 percen át bizonyult gátló hatásúnak.

Az TMS alkalmazására vonatkozó biztonságossági előírásokat már több mint 10 éve publikálták[35]. Az ingerlés viszonylag hangos kattanó hanggal jár, és a vizsgálati személyek az egyes TMS impulzusokat a fejbőrt ért enyhe ütésként észlelik, amit főként a skalpizomzat ingerlése okoz. A leggyakoribb mellékhatások az átmeneti, enyhe fejfájás, hányinger; magas

frekvenciájú ingerlés esetén epileptiform rohamok előidézéséről is beszámoltak. Ellenjavallott TMS alkalmazása intracranialis mágnesezhető fémimplantátumok és cardialis pacemaker esetén, míg számos egyéb állapot (pl. terhesség, görcsküszöböt csökkentő gyógyszerek szedése) relatív kontraindikációt képez.

Minden orvosi beavatkozásnál figyelembe kell venni a placebo hatást, ami fájdalomcsillapító módszereknél különösen kifejezett lehet. Emiatt fontos olyan kontroll eljárás alkalmazása, amelyet a vizsgálati személy nem tud megkülönböztetni a valós ingerléstől. A TMS esetén ez az ingerléssel járó somatosensoros jelenségek és hangok miatt különösen nehéz. Placebo ingerlés során (amit az angol irodalom gyakran „sham" (hamis) ingerlésnek nevez) általában vagy 90 fokkal elforgatják a tekercset úgy, hogy annak síkja a fejbőrre merőleges legyen, vagy a kereskedelmi forgalomban lévő „sham tekercsek" egyikét alkalmazzák. Ezek a valódi ingerléshez hasonló hangot adnak, ugyanakkor valamivel kisebb mértékű taktilis ingerekkel járnak, és sajnos csupán csökkentik, de nem szüntetik meg teljes mértékben a mágneses térerőt.

Az rTMS fájdalomészlelésre gyakorolt hatásai

Az rTMS-t már a kilencvenes évek közepe óta számos idegrendszeri kórképben, mint pl. depresszióban, Parkinson-kórban, dystoniában, stroke-ban vagy epilepsziában alkalmazták sikeresen a tünetek csökkentésére[27]. Mivel az MCS klinikai hatékonysága felvetette annak lehetőségét, hogy az M1 rTMS általi ingerlése is fájdalomcsillapító hatású lehet, már az első tanulmányban az M1 ingerlékenységének változtatásával próbálkoztak két, centrális eredetű fájdalomtól szenvedő betegnél[36]. A vizsgálatban azonban igen alacsony, 0,2 Hz-es ingerlést alkalmaztak a fájdalom oldalával contralateralisan, amely csupán az egyik beteg esetében bizonyult analgetikus hatásúnak. Egy későbbi vizsgálatban thalamus eredetű fájdalomban, agytörzsi vagy plexus brachialis sérülésben szenvedő betegeken hasonlították össze az alacsony (0,5 Hz-es) és magas (10 Hz-es) rTMS hatásait, és az eredmények alapján a 10 Hz-es ingerlés volt csupán hatásos[37]. Az M1 10 Hz-es ingerlésének fájdalomcsillapító hatását később számos vizsgálat megerősítette, sőt egyes tanulmányok szerint a 20 Hz-es ingerlés még hatékonyabbnak tűnik[38,39]. Az M1 ingerlése leggyakrabban nem jár azonnali fájdalomcsillapító hatással: egyszeri rTMS után a maximális hatást 2-4 nappal észlelték, amely még egy héttel az ingerlést követően is szignifikáns fájdalomcsillapítást okozott[40]. Az ingerlés ismétlése még erőteljesebb lehet: egy plexus brachialis laesioban szenvedő betegnél havi egyszeri, a sérüléssel ellenoldali M1 felett alkalmazott 10 Hz-es rTMS jelentős mértékű, 16 hónapig tartó fájdalomcsillapító hatást eredményezett[41]. Ennél még meggyőzőbb az a tanulmány, amely összesen 48, trigeminus neuralgiában vagy post stroke fájdalom

szindrómában szenvedő betegnél mutatta ki az egymás után 5 napon át, naponta ismételt 20 Hz-es, napi 2000 impulzusból álló rTMS szignifikáns fájdalomcsillapító hatását, amely a kúra befejezését követően két héten át kimutatható volt[38]. Az ingerlési frekvencia mellett tehát az ingerlés többszöri ismétlése és az egy alkalommal összesen alkalmazott TMS impulzusok száma is fontos szempont (lehetőleg minimum 1000 impulzus), továbbá lényeges, hogy az ingerlés fókuszált térerő-változást létrehozó, nyolcas alakú TMS tekerccsel történjen, lehetőleg postero-anterior orientációban tartva[22].

Számos tanulmány igazolta, hogy az M1 felett alkalmazott rTMS nem csupán a krónikus, hanem a kísérletesen kiváltott akut fájdalmat is csillapítja egészséges személyekben, bár az optimális ingerlési paraméterek kevésbé egyértelműek. Annak ellenére, hogy a 10 Hz-es rTMS egyértelműen hatékony különféle krónikus fájdalom szindrómákban, egészséges személyekben a mai napig ellentmondásos ezen rTMS protokoll fájdalomcsillapító hatása[42,43,44]. Az új rTMS protokollok közül az M1 felett alkalmazott cTBS azonban szignifikáns mértékben csökkentette a lézer által kiváltott fájdalmat és N2/P2 LEP amplitúdókat **(2. ábra)**[45,46]. A tónusos, C-rostok által közvetített, capsaicin okozta fájdalmat ezzel szemben az 1 Hz-es rTMS mérsékli[47].

Az eddig rendelkezésre álló kísérletes eredmények alapján a motor cortex rTMS általi ingerlése nem magyarázható kizárólag a motor cortexen belül bekövetkező változásokkal. Mivel a TMS az ingerelt neuronok axonjaiban akciós potenciálokat kelt, így egy corticalis area ingerlése távoli hatásokat is előidéz. Amint azt az MCS feltételezett hatásmechanizmusai kapcsán részleteztük, az M1 ingerlés megnöveli a thalamus, ACC, anterior insula és a PAG véráramlását[24]. E struktúrák közül gyakorlatilag mindegyiknél kimutattak már strukturális és/vagy funkcionális elváltozásokat krónikus fájdalom szindrómákban, így joggal feltételezhető, hogy a naponta ismételt rTMS kúrák jótékony plasztikus változásokat idéznek elő bennük, ami klinikailag jelentős fájdalomcsillapításban nyilvánulhat meg.

A DLPFC ingerlése is fájdalomcsillapító hatású lehet bizonyos ingerlési protokollok esetén: egy vizsgálatban a jobb DLPFC 1 Hz-es ingerlése[48], egy másik tanulmányban pedig a bal DLPFC 10 Hz-es ingerlése volt fájdalomcsillapító hatású egészséges személyekben[49]. Krónikus fájdalomban is kimutatták, hogy a jobb és a bal DLPFC eltérő frekvenciájú ingerlése fejt ki analgéziás hatást: a jobb DLPFC 1 Hz-es ingerlése fibromyalgiában[50], míg a bal DLPFC 10 Hz-es ingerlése arcfájdalom[51], izom-ízületi fájdalom[52], krónikus migrén[53] és műtét utáni fájdalom esetén[54] volt hatásos. A gyors, serkentő és a lassú, gátló hatású rTMS DLPFC-re gyakorolt elérő lateralizációjú hatása kísértetiesen hasonlít a depresszióban tapasztalt tünetenyhítő protokollokhoz[27]. Jól ismert tény, hogy a krónikus fájdalom gyakran

szövődik depresszióval, valamint régóta dokumentált a subgenualis anterior cingularis cortex pathológiás elváltozása major depresszióban[55]. Elképzelhető tehát, hogy az rTMS DLPFC-re gyakorolt hatása hasonló változásokat okoz az affektív rendszert szabályozó praefrontalis és/vagy limbikus struktúrákban, amely mind fájdalomcsillapító, mind hangulatjavító hatású lehet.

A fentiektől jóval kevesebb adat áll rendelkezésünkre az SI és az SII ingerlés fájdalomcsillapító hatásáról, és az eddigi eredmények igen ellentmondásosak. Egy korai vizsgálatban a Gyrus postcentralis 20 Hz-es ingerlése rTMS-sel csökkentette a kar átmeneti leszorítása által okozott fájdalmat[56]. Az analgéziás hatás naloxon adásával kivédhető volt, ami az ópiáterg rendszer szerepére utal. Egy másik tanulmányban az SI különböző TBS protokollokkal való ingerlése erőteljesen mérsékelte az N2 LEP komponens amplitúdóját, ami az N2 keletkezéséért felelős területek (suprasylvianus cortex, ACC) aktivitásának befolyásolására utal[57]. Végül, az SII 1 Hz-es ingerlése hatékonyan csillapította a pancreatitis által okozott, visceralis eredetű fájdalmat[58].

A transcranialis egyenáram-ingerlés

Mivel az egyszeri, küszöb feletti TMS impulzus közvetlenül is képes akciós potenciálokat kelteni az axonokban, míg a küszöbalatti intenzitású rTMS hosszú távon befolyásolja a sejtek ingerlékenységét, a TMS-t neurostimulációs és neuromodulációs eljárásnak is tekintik. Ezzel ellentétben, a most ismertetendő transcranialis egyenáram-ingerlés (transcranial direct current stimulation, tDCS) kizárólag a membránpotenciál viszonylag hosszú távú (percekig tartó) modulálása révén fejti ki hatását, vagyis tisztán neuromodulációs eljárás.

Először a 60-as években sikerült állatkísérletek során az agykéreg gyenge egyenáramú ingerlésével tartósan megváltoztatni a corticalis neuronok nyugalmi membránpotenciálját és ezáltal befolyásolni a sejtek ingerlékenységét[59]. Az ezredforduló környékén két kutatócsoportnak humán vizsgálatokban sikerült igazolnia, hogy a motor cortex fölé, a fejbőrre helyezett elektródákkal történő egyenáramú ingerlés módosítja a TMS-sel kiváltott MEP amplitúdókat[60,61]. Az eljárás során két, 20-35 cm^2 felszínű, vízzel átitatott szivaccsal körülvett elektródát (egy anódot és egy katódot) helyeznek a fejre, amelyek között egy elemmel működtetett ingerlő segítségével általában 1 vagy 2 mA-es egyenáram áramlik az anód felől a katód felé legfeljebb 20 percen keresztül **(4. ábra)**. Bár az áram egy része a skalpon keresztül áramlik, jelentős része belép az agyba, és az elektródák elhelyezkedésétől, az áram iránytól, intenzitásától és az ingerlés idejétől függően viszonylag hosszú, akár 1 órás változásokat idéznek elő az ingerelt kéregrész neuronjainak ingerlékenységében[61]. Amint ezt

egyes fájdalomszindrómák esetében kimutatták, a hatás akár több héten keresztül is észlelhető akkor, ha az ingerlést kúraszerűen, naponta megismétlik (lásd később). Az anodális ingerlés depolarizálja a neuronokat, míg a katodális hiperpolarizálja azokat, ezáltal modulálván az idegsejtek akciós potenciáljainak frekvenciáját. Mindkét típusú ingerlés hosszú távú hatása kivédhető NMDA glutamátreceptort blokkoló molekulákkal, emellett az anodális tDCS rövid távú hatását a Na^+-csatorna blokkoló karbamazepin és a Ca^{2+}-csatorna blokkoló flunarizin is csökkenti[62]. Mindezek rendkívül komplex hatásmechanizmusra utalnak, amelyben az ioncsatornák megfelelő működése elengedhetetlen, ugyanakkor a hosszú távú hatás feltehetően LTP- illetve LTD-szerű neuroplasztikus változások következtében alakul ki.

A tDCS segítségével sikeresen befolyásolható a motoros, visualis és a somatosensoros cortex ingerlékenysége, továbbá a praefrontalis cortex ingerlése hatással volt az implicit tanulásra és a munkamemóriára[63]. A TMS-hez viszonyítva a tDCS hatása kevésbé lokális, ami egyrészt az elektródák méretéből adódik, másrészt pedig a hatást befolyásolja az elektródák helyzete és egymástól való távolsága, így azon idegi struktúrák funkciója, amelyeken az egyenáram keresztüláramlik.

A tDCS biztonságos, ismert tartós mellékhatások nélküli eljárás. A beavatkozás leggyakoribb velejárója az elektródák alatt érezhető enyhe bizsergő-viszkető érzés, amely azonban pár percen belül elmúlik. Erősebb vagy hosszabb ideig tartó ingerlés az elektródák alatti bőr enyhe égését okozhatja, valamint beszámoltak már az ingerlést követő enyhe gyengeségérzésről, fejfájásról, émelygésről és alvászavarról is[64].

A placebo ingerlés a tDCS esetében sokkal könnyebb, mivel az ingerlés nem produkál hallható vagy erősebb somatosensoros ingereket. Az ú.n. "sham" ingerlés során az elektródákat a személy fejéhez erősítik, majd pár másodpercre bekapcsolják az ingerlést a bizsergés kiváltására, majd azután kikapcsolják azt.

A tDCS fájdalomészlelésre gyakorolt hatásai

Az rTMS-hez viszonyítva lényegesen kevesebb vizsgálat foglalkozott eddig a tDCS fájdalomcsillapító hatásával annak ellenére, hogy krónikus fájdalom szindrómák esetében az első vizsgálatok igen bíztatóak. Ennél a módszernél is a motor cortex ingerlésével kapcsolatosan áll rendelkezésünkre messze a legtöbb adat, de a tDCS kevésbé lokalizált hatásai miatt korántsem beszélhetünk kizárólagos M1 ingerlésről, mint az rTMS esetében. A motor cortex ingerléséhez az egyik elektródát általában az EEG mérésekhez használt nemzetközi 10/20 rendszer pozíciói (oldaltól függően C3 vagy C4) alapján szokták elhelyezni, de lehetséges az M1 helyének TMS-sel történő pontos meghatározása is[65]. A másik elektródát általában az ellenoldali supraorbitalis területre helyezik. Krónikus fájdalom

csillapításához a motor cortex fölé helyezett anodális ingerlés bizonyult hatásának, amely (a 10-20 Hz-es rTMS-hez hasonlóan) fokozza a terület neuronjainak excitabilitását[61]. Az első vizsgálatban spinalis laesioban szenvedő betegek részesültek 5 napig tartó, naponta 20 perces, 2 mA erősségű anodális vagy placebo ingerlésben[66]. Az ingerlés a fájdalmas oldaltól contralateralisan vagy szimmetrikus fájdalom esetén a domináns hemispherium felett történt. A vizsgálat eredményei alapján az anodális tDCS csökkentette a betegek fájdalmát, ami azonban csupán az 5 napos kúra ideéig eredményezett szignifikáns fájdalomcsökkenést. A szubjektív fájdalomérzet a 2. kezeléstől tért el szignifikáns mértékben a placebo ingerléstől, és bár az egyes aktív kezelések után közvetlenül nem változott a fájdalomérzet, a kezelés előrehaladtával kumulatív módon, egyre erőteljesebb fájdalomcsillapító hatást eredményezett. Egy másik vizsgálatban fibromyalgiában szenvedő betegeket randomizáltak anodális motor cortex, anodális bal oldali DLPFC vagy placebo ingerléses csoportokba[67]. Csupán a motor cortex ingerlése bizonyult analgéziás hatásúnak, ez azonban a kúra végét követően 3 héten keresztül fennmaradt. A kezelés hatása ebben a betegcsoportban is additívnak bizonyult. Egy későbbi esettanulmányban a domináns oldali motor cortex anodális ingerlése négy órán át teljesen megszűntette a hasnyálmirigyrák okozta fájdalmat[68]. Azóta több vizsgálat is megerősítette a kúraszerűen alkalmazott tDCS hatékonyságát: az ingerlés analgéziás hatású volt krónikus pelvicalis fájdalom[69] és sclerosis multiplexhez kapcsolt fájdalom[70] esetén, továbbá egy post stroke fájdalomban, trigeminus neuralgiában, hátfájdalomban és fibromyalgiában szenvedő kevert betegcsoportban[71]. Az ilyen jellegű, naponta ismételt kezelés hatása minden esetben legalább 1, maximum 3 héttel meghaladta a kezelés idejét.

Kevés tanulmányt szenteltek a tDCS akut fájdalomérzetre gyakorolt hatásának vizsgálatának egészséges személyekben. Az egyik első vizsgálatban a fájdalommal contralateralis SI katodális ingerlése (1 mA, 15 perc) a placebóhoz képest szignifikáns mértékben csökkentette a kézfejen a lézer okozta fájdalmat és az N2 LEP amplitúdót, míg az anodális ingerlés eredményei nem tértek el a placebótól[72]. Egy másik tanulmányban csak az anodális ingerlést vetették össze a placebóval, hiszen krónikus fájdalomban szenvedőknél is ez a stimuláció volt hatásos[73]. Három corticalis területet (M1, DLPFC, V1) ingereltek külön kísérleti ülésekben, és eredményeik szerint az M1 és a DLPFC ingerlése emelte meg a fájdalomküszöböt, emellett az M1 feletti tDCS a fájdalmatlan ingerek detekciós küszöbét is módosította. Ez arra utal, hogy míg az M1 ingerlése a fájdalom szenzoros-diszkriminatív és affektív komponenseit is befolyásolja (azaz a medialis és a lateralis fájdalomészlelő rendszerre is hat), addig a praefrontalis területek ingerlékenységének befolyásolása csak az utóbbit modulálja.

Korábban bemutattuk, hogy egészséges személyeknél a különböző rTMS protokollok hatékonysága korántsem olyan egységes, mint krónikus fájdalomtól szenvedő betegeknél, és ez sajnos a tDCS esetében is így van. A motor cortex felett alkalmazva ugyanis a katodális tDCS is hatékony fájdalomcsillapításhoz vezethet, legalábbis a kézfej lézeres ingerlése esetén, ha a lézerimpulzusok csupán enyhe fájdalomérzetet okoznak[74]. Erősebb fájdalom esetén a fájdalomküszöb nem változik katodális tDCS-t követően, de a placebóhoz képest lecsökkennek az N2/P2 LEP amplitúdók, ami a távoli, suprasylvianus és cingularis cortexre gyakorolt hatás mellett szól. Egy további vizsgálatban a D2-receptor agonista pergolid 40 percről 2 órára tolta ki az M1 felett alkalmazott katodális tDCS fájdalomcsillapító hatását, és 2 óra helyett 24 órás N2 amplitúdócsökkenést eredményezett[65].

Az ismertetett vizsgálatok tanulsága szerint krónikus fájdalom esetén az M1 felett alkalmazott anodális tDCS, akut fájdalom esetén pedig a DLPFC feletti anodális, az SI feletti katodális, valamint az M1 felett mind az anodális, mind a katodális tDCS fájdalomcsillapító hatású lehet. Krónikus fájdalom esetén fontos a naponta ismételt ingerlés, ami kumulatív módon felerősítheti és meghosszabbíthatja a fájdalomcsillapító hatást. Emellett úgy tűnik, hogy a legfeljebb 2 mA-es, 20 perces ingerlést a betegek viszonylag jól tolerálják.

A kezelés hatásmechanizmusát illetően mindezidáig a motor cortex feletti tDCS hatásával kapcsolatban áll rendelkezésünkre a legtöbb információ. Egy PET vizsgálatban a primer motor cortex 10 perces, 1 mA intenzitású anodális és katodális ingerlése nem csupán az ingerlés helyén okozott szignifikáns mértékű regionális véráramlás-változásokat, hanem számos, a fájdalomészlelés szempontjából kulcsfontosságú corticalis (pl. cingularis cortex, praefrontalis cortex) és subcorticalis (pl. thalamus) régióban is[75]. Ezek alapján a tDCS az MCS-hez és az rTMS-hez hasonlóan, képes megváltoztatni távoli területek működését, és így befolyásolni a fájdalmas ingerek feldolgozását, amit az N2/P2 LEP amplitúdókra gyakorolt hatás is támogat, hiszen e potenciálok korántsem a motor cortex aktivitását tükrözik.

Összefoglalás

Az Európai Neurológiai Társaságok Szövetségének (EFNS) felmérése szerint a krónikus neuropathiás fájdalomban szenvedő betegek csupán 30-40%-ánál érhető el legalább 50%-os fájdalomcsillapítás gyógyszerek segítségével[76]. Ebből adódóan világszerte számos laboratóriumban próbálkoznak új fájdalomcsillapító módszerek kidolgozásával, amelyek közül az általunk ismertetett rTMS és tDCS biztonságosság és alkalmazhatóság tekintetében kiemelkedő, és az eddigi kísérletes eredmények is bíztatóak. Ugyanakkor fontos hangsúlyozni, hogy egyik módszerrel sem érhető el jelenleg tartós terápiás siker. Egy nemrég közölt összefoglaló elemzés szerint az M1 magas frekvenciájú rTMS ingerlése kb. 20-45%-os

fájdalomcsökkenést eredményez, és a betegek 35-60%-a esetében sikerült legalább 30%-os fájdalomcsillapítást elérni a fájdalom típusától függően[77]. tDCS esetében sokkal kevesebb tanulmányt közöltek, de a motor cortex feletti anodális ingerlés 58%-os fájdalomcsökkenést okozott spinalis laesio okozta hátfájdalom esetében, ami magasabb az rTMS vizsgálatokban tapasztalt eredménynél. Egy másik meta-elemzés szerint rTMS esetében a betegek 36,8%-a (95%-os konfidencia intervallum 30,5-43,0), míg tDCS esetében 71,4%-a (95%-os konfidencia intervallum 52,1-90,7) reagált a kezelésre[78]. Kiemelendő továbbá, hogy kúraszerű alkalmazás esetén a tDCS átlagosan hosszabb ideig volt hatásos, mint az rTMS. Ezen eredmények azonban még mindig messze alulmúlják az MCS mutatóit, ahol a válaszadók aránya 72,6% (95%-os konfidencia intervallum 67,7-77,4), és 4 éves kezelést követően a legalább 50%-os fájdalomcsökkenést tapasztaló betegek aránya még mindig 22,6-47% közötti[78,79]. Az MCS metodikáját tekintve azonban egy invazív ingerlés.

Bár jelenleg még nem rutinszerű az rTMS és a tDCS klinikai alkalmazása fájdalomcsillapítás céljából, az EFNS ajánlása szerint a megfelelően alkalmazott rTMS B szintű evidenciával rendelkezik post stroke fájdalom és számos egyéb neuropathiás fájdalom esetében, továbbá ajánlott alkalmazása MCS-re váró betegeknél az MCS későbbi hatékonyságának megjóslására[22]. Az eddig elvégzett viszonylag kisszámú vizsgálat miatt a tDCS ugyan nem rendelkezik hasonló ajánlással, de olcsóbb volta, könnyebb alkalmazhatósága és hordozhatósága miatt a tDCS akár otthoni használatra is alkalmas, ami lehetővé teszi az egyszerű és hosszú távú alkalmazást. Egy tanulmány nemrég mutatta ki, hogy költséghatékonyság tekintetében egy éves kezelés esetén a tDCS olcsóbb az rTMS-nél és az MCS-nél, azonban 5 éves kezelés esetén továbbra is az MCS bizonyul a három módszer közül a legkifizetődőbbnek[79].

Az rTMS és a tDCS közötti hasonlóságokat és különbségeket az **1. táblázat** ismerteti. Ezek alapján úgy tűnhet, hogy a tDCS számos előnnyel bír az rTMS-sel szemben, de nem szabad megfeledkeznünk arról, hogy mindeddig nagyságrendekkel több vizsgálat számolt be az rTMS fájdalomcsillapító hatásáról. Az eddigi eredmények arra utalnak, hogy az rTMS és a tDCS centrális és perifériás fájdalomban is hatékony, ami egy alapbetegségtől viszonylag független hatásmechanizmus meglétére utal. Mindkét módszernél egyértelműen a motor cortex serkentő ingerlése bizonyult a legeredményesebbnek, ami elsőre meglepő lehet, hiszen e terület nem a fájdalomészlelésben betöltött szerepéről ismert. Ugyanakkor az M1 közvetlen kapcsolatban áll a lateralis fájdalomrendszerhez tartozó thalamus magokkal, ami következtében az M1 ingerlése elsősorban a fájdalom szenzoros-diszkriminatív feldolgozását befolyásolja. Ezzel szemben a DLPFC ingerlése inkább a medialis fájdalomrendszeren

keresztül a fájdalommal kapcsolatos érzelmeket, szorongást, motivációs folyamatokat módosítja[79]. Ennek megfelelően, a motor cortex rTMS és tDCS általi ingerlése a fájdalomküszöb eltolása mellett az ártalmatlan ingerek észlelését is befolyásolja, míg a praefrontalis cortex ingerlése nincs ilyen hatással[42,73].

Mindkét általunk ismertetett neuromodulációs eljárás feltehetőleg hosszan tartó változásokat idéz elő az agyi mikorarchitektúrában. Az rTMS számos neuromodulátor, mint a noradrenalin, szerotonin és dopamin felszabadulását fokozza, valamint elősegíti az agyi eredetű neurotrophicus faktor (BDNF) expresszióját[80], amely anyagok az ingerlés idejét meghaladván módosíthatják a neurotranszmissziót. A neurális plaszticitás függ az idegsejtek aktuális állapotától, és így a beavatkozás eredménye akár farmakológiailag is befolyásolható, mint ahogy azt a pergolid esetében bemutattuk[65]. Ezek igen bíztató eredmények, amelyek további protokollok kidolgozását serkentik a hosszú távú, nem-invazív fájdalomcsillapítás elérése céljából.

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1. táblázat. Az rTMS és a tDCS összevetése.

	rTMS	**tDCS**
Készülék ára	20 000 – 100 000 USD	400 – 10 000 USD
Placebo ingerlés	Nehezen kivitelezhető	Könnyen kivitelezhető
Biztonságosság	Jó, convulsio előfordulhat	Jó, enyhe bőrégés az ingerlés helyén előfordulhat
Abszolút ellenjavallat	Mágnesezhető fémből készült implantátum, epilepszia	Mágnesezhető fémből készült implantátum, epilepszia
Eddigi hatékonyság krónikus fájdalom esetén	Trigeminus neuralgia[38,39,40] Fibromyalgia[50] Post stroke fájdalom[36,37,38,39,40,] Gerincvelő laesio[39] Plexus brachialis laesio[37,39,41] Foghúzás utáni arcfájdalom[51] Izom-ízületi fájdalom[52] Migrén[53] Postoperatív fájdalom[54] Krónikus pancreatitis[58]	Trigeminus neuralgia[71] Fibromyalgia[67,71] Post stroke fájdalom[71] Gerincvelő laesio[66] Pelvicalis fájdalom[69] Sclerosis multiplex[70] Hasnyálmirigyrák[68] Hátfájdalom[71]
Javasolt ingerlési protokoll	Tekercs típusa: nyolcas alakú Tekercs orientációja: postero-anterior Frekvencia: 10-20 Hz (M1, bal DLPFC) vagy 1 Hz (jobb DLPFC, jobb SII) Intenzitás: motoros küszöb 80-.90%-a Impulzusszám: min. 1000 Ismétlés: naponta, legalább 5 napig	Elektródák mérete: 20-35 cm^2 Intenzitás: 1-2 mA Időtartam: 10-20 perc Ismétlés: naponta, legalább 5 napig
Ingerlési hely	Fájdalommal ellenoldali vagy domináns oldali M1, bal vagy jobb DLPFC, visceralis fájdalom esetén esetleg jobb oldali SII	Anód: fájdalommal ellenoldali vagy domináns oldali motor cortex Katód: anóddal ellenoldali

		supraorbitalis régió
Egyéb előny	Körülírt ingerlés, célterület jó lokalizálhatósága	Otthoni alkalmazhatóság

Ábramagyarázatok:

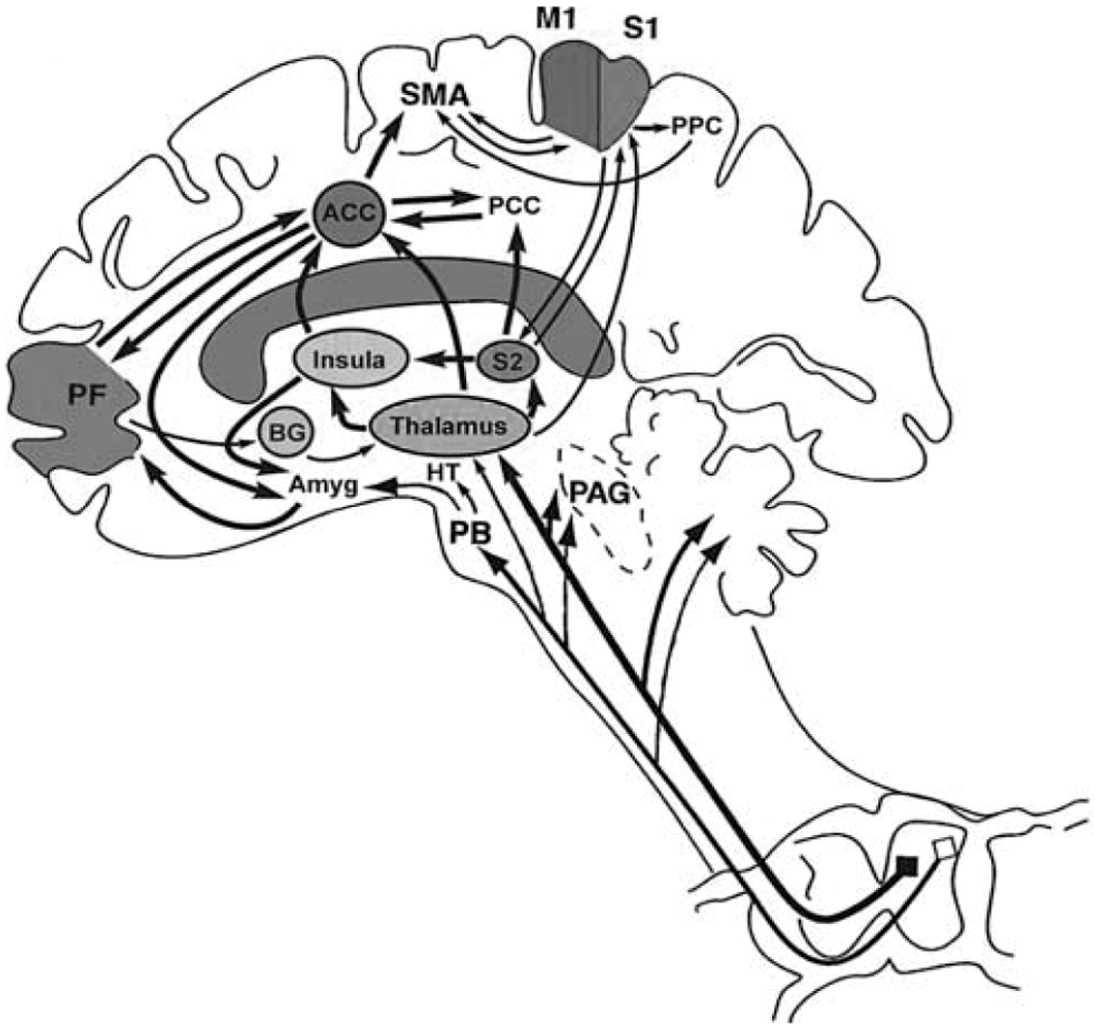
1. ábra. A fájdalomészlelés szempontjából fontos központi idegrendszeri struktúrák és azok összeköttetései. Rövidítések: ACC: anterior cingularis cortex, AMYG: amygdala, BG: basalis ganglionok, HT: hypothalamus, M1: primer motor cortex, PAG: periaqueductalis szürkeállomány, PB: nuclei parabrachiales, PCC: posterior cingularis cortex, PF: praefrontalis cortex, PPC: posterior parietalis cortex, S1: primer somatosensoros cortex, S2: secunder somatosensoros cortex, SMA: supplementer motor area. (Engedéllyel átvéve a 20. hivatkozásból.)

2. ábra. A bal motor cortex feletti cTBS hatása az N2/P2 LEP amplitúdókra (a) és a szubjektív fájdalomérzetre (b) a contralateralis (jobb) kéz lézeres ingerlése esetén. A csillagok szignifikáns ($p<0,01$) csökkenést jeleznek a placebo ingerléshez viszonyítva (a LEP amplitúdók változását csak valós ingerlés esetén mutatjuk be). (Engedéllyel átvéve és módosítva a 46. hivatkozásból.)

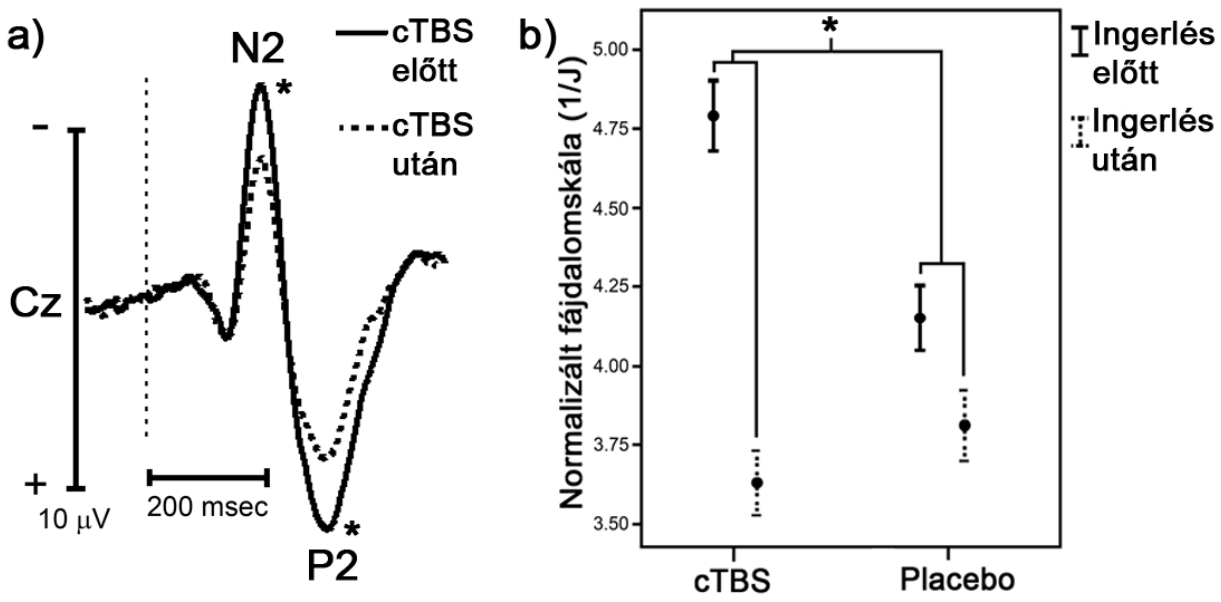
3. ábra. rTMS alkalmazása a bal motor cortex felett, a tekercs postero-anterior orientációban.

4. ábra. Anodális tDCS alkalmazása a bal motor cortex felett. A katód a contralateralis supraorbitalis régió felett helyezkedik el.

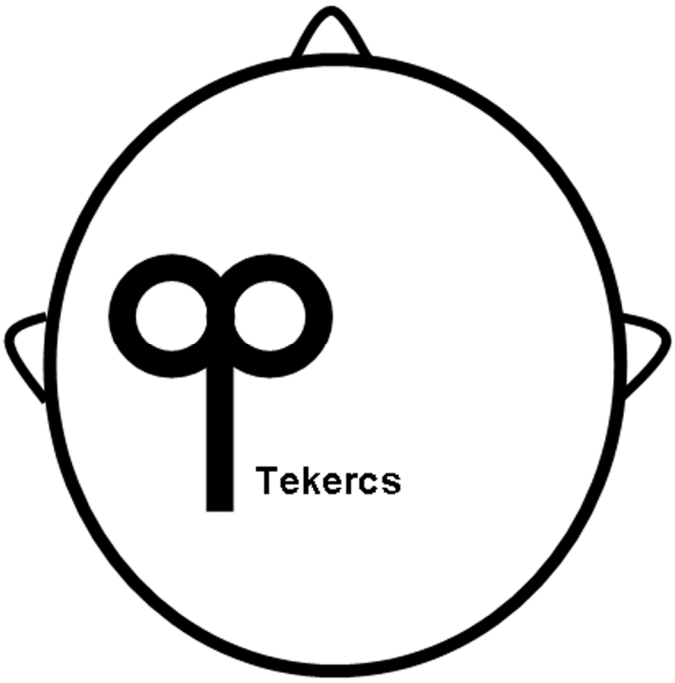
1. ábra



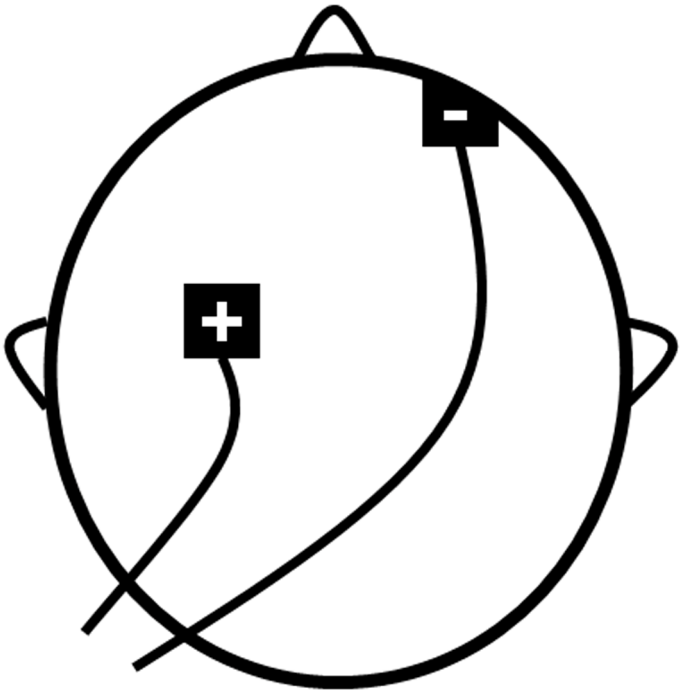
2. ábra


a)
N2
cTBS előtt
cTBS után
-
Cz
+
10 μV
200 msec
P2
b)
Normalizált fájdalomskála (1/J)
5.00
4.75
4.50
4.25
4.00
3.75
3.50
*
cTBS
Placebo
Ingerlés előtt
Ingerlés után

3. ábra



4. ábra



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I G A Z O L Á S

Igazoljuk, hogy **Csifcsák Gábor, Antal Andrea „Nem-invazív agyi ingerlési módszerek alkalmazása akut és krónikus fájdalom csillapítására" c. közleménye a *Clinical Neuroscience/Ideggyógyászati Szemle* Szerkesztőbizottságánál közlésre, elfogadásra került.**

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Tisztelettel:

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