

## Review article

# A systematic review of the proposed mechanisms underpinning pain relief by primary motor cortex stimulation in animals



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## ABSTRACT

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Experimental treatments for treating neuropathic pain include transcranial magnetic stimulation (TMS) and invasive electric motor cortex stimulation (iMCS) of the primary motor cortex (M1). Mechanisms of action of both methods, however, remain largely elusive. Within this paper, we focus on animal-based experiments in order to investigate the biological mechanisms that are involved in alleviating pain by use of TMS and/or iMCS.

Therefore, this paper systematically reviewed the animal-based evidence on these mechanisms. Multiple online databases were systematically searched and retrieved articles were assessed using predefined inclusion and exclusion criteria.

Twenty-three suitable articles were included; six on TMS and seventeen on iMCS. In general, iMCS and TMS were found to impact the primary motor cortex structure and function in animals. Furthermore, structural and functional changes within the thalamus, striatum, periaqueductal grey, rostral ventromedial medulla and dorsal horn were reported to occur.

Although widespread, all areas in which structural and functional changes occurred after TMS and iMCS have been found to be interconnected anatomically. This could provide a rationale for future investigations of treating neuropathic pain by use of neuromodulation.

## 1. Introduction

Chronic neuropathic pain remains a poorly understood neurological disorder with a broad variety of affected brain regions and -networks involved [40]. As a consequence, a corresponding wide range of pharmaceutical andventional treatments have been employed to treat these patients [6]. In addition, neuromodulation treatments have been developed to treat chronic neuropathic pain. In the 1990s, Tsukamoto et al. reported that placing an active electrode on the primary motor cortex (i.e., invasive motor cortex stimulation; iMCS) induced analgesic effects in pain patients [41]. The same group reported that stimulation of the primary motor cortex was capable to decrease thalamic hyperactivity induced by spinothalamic deafferentation, whereas

stimulation of the primary somatosensory cortex increased the thalamic burst firing pattern, indicating increased thalamic hyperactivity [42].

Non-invasive stimulation of the primary motor cortex (i.e., transcranial magnetic stimulation; TMS) related to pain research was originally used as a predictive test to preoperatively distinguish responders from non-responders in iMCS treatment. In TMS, a high current pulse generator produces a strong, ultra-brief electric current in a coil with a time-varying magnetic field [18]. This magnetic field will create a secondary current in nerve tissue that, depending on stimulation frequency, can increase or decrease excitability of neural tissue directly beneath the coil [11,23]. The therapeutic use of TMS for neuropathic pain has so far been limited due to its relatively short-lived effect, which is thought to last for approximately one week [22].

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Despite the documented analgesic effects, the exact mechanisms of action of TMS and iMCS in pain management remain largely elusive. The modulating mechanisms of TMS and iMCS are considered to have a substantial amount of overlap [27], as it is believed that both techniques induce changes in cortical excitability, which in turn affects the excitability of neurons in subcortical structures and -tracts involved in the processing of nociceptive input. Based on the finding that unilateral TMS can induce bilateral analgesic effects, it thought that the regions modulated by TMS/iMCS are found in diencephalon and/or are part of the descending pain modulation system (including the periaqueductal grey and the rostral ventromedial medulla) [28].

In order to investigate the involved neural pathways and other mechanisms of action, animal models have been studied with TMS and iMCS. Animal models allow for more experimental designs, including the evaluation of brain structure and function by post-mortem, histological assessment. Vis-à-vis comparison between the mechanistic findings in animals and humans is dubious at best considering that the level of analysis that could be done in animals is much higher than in humans. This paper therefore provides an overview of these animal-based experiments which investigate the underpinnings of analgesia achieved by TMS and/or iMCS.

## 2. Materials and methods

Five investigators (E.G., M.v.d.H., M.K., S.L. and D.H.) independently and systematically searched online databases (i.e., PubMed, Embase, MEDLINE, Google Scholar, and the Cochrane Library), with assistance of an independent librarian. Keywords included: “Transcranial Magnetic Stimulation”, “Motor Cortex Stimulation”, “Mechanisms”, “Physiology” and “Analgesic effects”. In order to enrich the results, Medical Subject Headings (MeSH-) terms with major subheadings were added to the search strategy. Literature was searched for until August 2018. Inclusion criteria were: 1) Animal studies; 2) Neuromodulation (TMS or iMCS) carried out to alleviate pain (either chronic or experimental); 3) Primary outcome of the paper was the mechanisms of action via which aforementioned forms of neuromodulation provided pain relief. Systematic reviews and articles written in other languages than English were excluded as well. Observational studies, case reports and randomized-controlled trials were excluded when they did not report on the mechanisms of action of neuromodulation in ameliorating pain. In total, 1241 original articles were retrieved after conducting the searches. Each paper was randomly assigned and reviewed on relevance by title, abstract and full-text by two investigators independently. Incongruently assessed articles were evaluated by a third, independent investigator. A total of 1126 papers were excluded, resulting in 115 articles remaining. For the final assessment, papers were only included when they discussed the mechanisms involved in the analgesic effects of TMS and/or iMCS. After in-depth, full-text analysis, a total of 23 papers were included based on this last inclusion criterion (Fig. 1).

## 3. Results

Twenty-three animal-based studies were included in this review; six publications discussed the proposed mechanisms of TMS and seventeen papers discussed the actions underpinning iMCS.

### 3.1. TMS in animals

Five of the included studies used rat-models, whereas one paper used a macaque monkey as model in a neuroimaging study. The measurements used in the included rat studies can be divided into two categories: 1) effect of TMS on cortical excitability; and 2) effect of TMS on biochemical composition of the nervous system. See Tables 1 and 2 for a detailed overview of the included TMS studies. Regarding the applied stimulation protocol in the TMS studies can be found in Table 2.

It can be appreciated that various TMS protocols are used.

Muller et al. showed that repetitive TMS (rTMS) reduced cortical excitability in healthy rats. rTMS induced decrease in cortical excitability which was found to be NMDA receptor dependent as rTMS was not capable of changing motor cortex excitability after administering a competitive and non-competitive NMDA receptor antagonist [30]. Hsieh et al. carried out a comparable study and investigated the cortical excitability depending on the GABA $\alpha$  receptor in healthy rats. Administering a GABA $\alpha$  receptor antagonist and agonist respectively reduced and enhanced TMS-induced cortical inhibition, suggesting TMS-induced cortical excitability is GABA $\alpha$  receptor dependent [12].

Regarding the biochemical composition of the central nervous system, Löffler et al. documented increased serotonin levels in the nucleus accumbens shell after rTMS in rats. They furthermore found that increased outflow serotonin levels in the nucleus accumbens shell region after active rTMS, whereas sham TMS did not induce such changes [24]. Another animal based study that the lipid profiles in the rat changed in the prefrontal cortex and striatum after rTMS [21]. More specific, there were significant alterations in plasmalogen phosphatidylethanolamines, phosphatidylcholines, and increases in sulfated galactosylceramides or sulfatides. Plasmalogen species with long chain polyunsaturated fatty acids showed decrease in abundance together with corresponding increase in lysophospholipid species. These changes suggest endogenous release of long chain fatty acids in the central nervous system. The hippocampus, another studied region, showed no significant changes, whilst changes in the striatum were often opposite to that of the prefrontal cortex [21]. Another animal based study investigated the expression of neuroglia in the spinal cord. For this, they induced spinal cord injury in rats [16]. They found decreased expression of the proteins Iba1 and GFAP after rTMS, suggesting pain modulation by rTMS-induced attenuation of microglia and astrocyte activity in the dorsal horn of L4 and L5 segments.

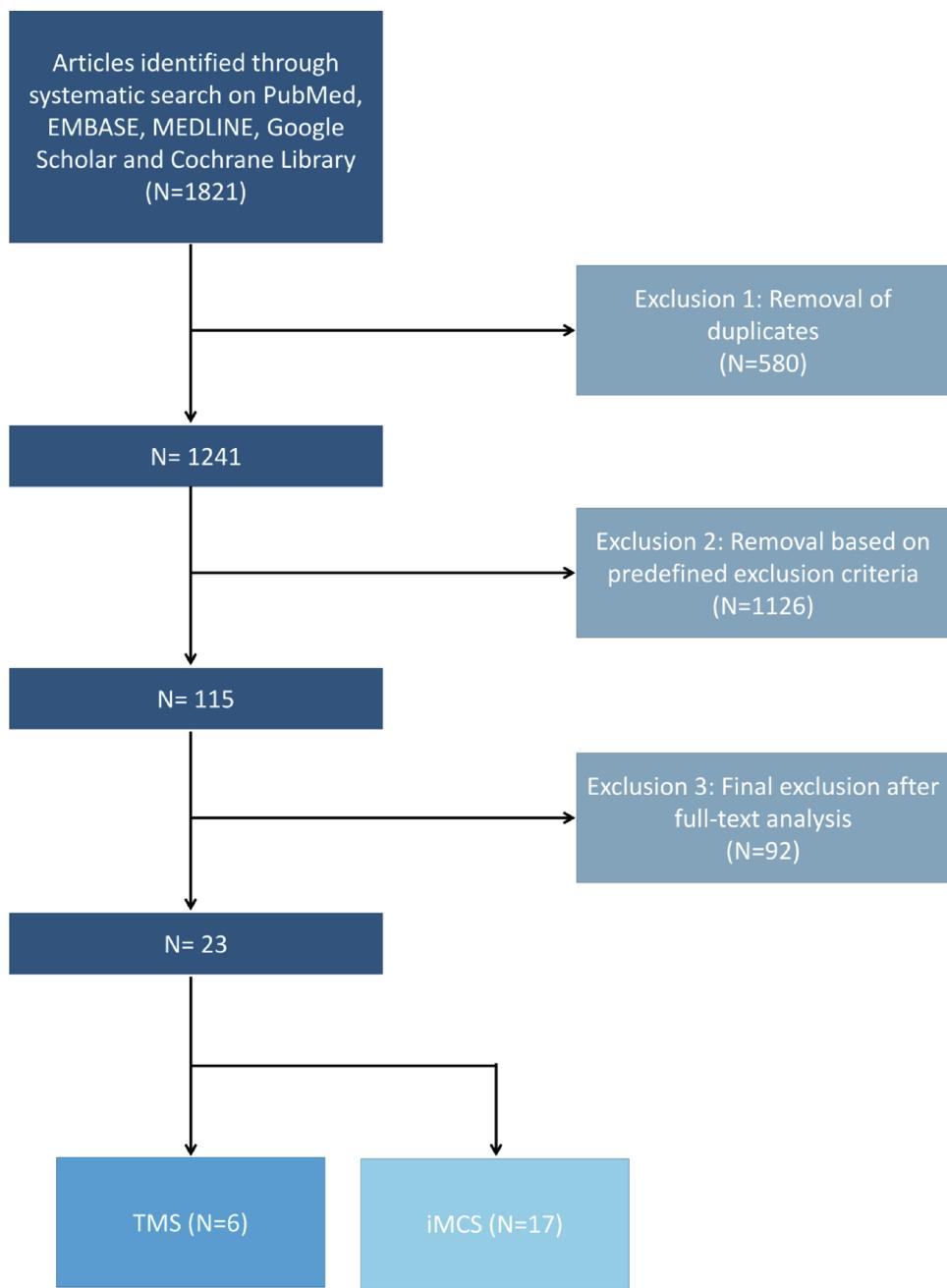
Ohnishi et al. investigated the biochemical effects of rTMS by using [ $^{11}$ C]raclopride positron emission tomography in macaque monkeys. After rTMS, it was found that after unilateral rTMS the extracellular dopamine concentration increased in the ventral striatum, including the nucleus accumbens, in both hemispheres. In addition, rTMS over the right M1 decreased the extracellular dopamine concentration in the ipsilateral putamen, as compared to sham stimulation [31].

### 3.2. iMCS in animals

Fifteen of the included studies used rat-models, whereas one paper used a rabbit-model and another one used a cat-model. Animal-based studies have extensively investigated the mechanisms underlying the analgesic effect of iMCS. In general, four types of research have been conducted: 1) neuroimaging studies to investigate changes in blood flow and/or activity induced by iMCS *in vivo*; 2) immunohistochemical studies investigating patterns of increased activity induced by iMCS *ex vivo*; 3) agonist/antagonist studies to diminish/enhance the results of iMCS; and 4) studies investigating structural changes within the neural system induced by iMCS. See Table 3 for an overview of the included iMCS studies.

Using [ $^{18}$ F]-FDG PET to measure brain activity in rats after iMCS, it was observed that alterations in activity occurred in the striatum, thalamic areas and cerebellum [15]. The same study showed that neuronal activity in the thalamus (ventral posterolateral nucleus) was effectively suppressed by iMCS [15]. Another neuro-imaging study showed significant lower blood oxygen level dependent (BOLD) responses in the primary somatosensory cortex (S1) and prefrontal cortex following noxious stimulation after iMCS [13], indicating that the areas involved in processing nociceptive input were impacted by iMCS.

This was corroborated by an immunohistochemical study, in which an increased cFos- and Egr-1 immunoreactivity in the PAG and decreased cFos- and Egr-1 immunoreactivity in the dorsal horn of the spinal cord was observed in rats after iMCS. These findings indicate an



**Fig. 1.** Flow chart describing the study selection process.

inhibited transmission of noxious stimuli from the dorsal horn of the spinal cord [9]. Such an iMCS induced inhibited transmission of noxious stimuli from the dorsal horn, as visualized by use of immunohistochemistry, was in agreement with findings of other groups [15,32]. With regard to the midbrain structures, immunohistochemistry studied found decreased Egr-1, GABA and glutamic acid decarboxylase expression in the ipsilateral PAG [33], which conflicts with the findings of França et al, who reported increased Egr-1 immunoreactivity in the PAG [9]. Other studies which investigated cell activation with cFos immunoreactivity found that iMCS suppresses neuronal activity in the ventral posterolateral nucleus [15], anterior cingulate cortex (ACC), left principal sensory nucleus of the trigeminal [20] and central and basolateral amygdaloid nuclei [20,32]. No Egr-1 immunoreactivity changes were observed in the ACC and central amygdaloid nucleus [32].

In 2012 and 2013, Chiou et al. observed two phenomena in rats

with iMCS. First, they observed suppressed short-latency somatosensory evoked potentials (SSEPs) and second, decreased neuronal responses of the primary somatosensory cortex were observed after noxious stimuli. These effects could be reversed by administering an opioid antagonist and a nonspecific dopamine receptor antagonist within the periaqueductal gray (PAG) [4,5]. Therefore, they concluded that iMCS possibly induces the release of endogenous opioids in the PAG [4]. As the PAG is considered a site at which endogenous opioids are released, other animal-based studies focussed on the effect of opioid antagonists on the analgesia provided by iMCS. Infusion of an opioid antagonist or a GABA agonist in the zona inserta (ZI) showed a complete block of iMCS-induced effects [26]. Cha et al. studied neuron activity within the posterior thalamus by use of electrophysiological recordings during iMCS using and the effects of administering a GABA agonist. They showed that iMCS enhanced activity of the activity of the neurons in the zona incerta en reduced the activity of the neurons in the posterior thalamus.

**Table 1**  
Overview of studies that investigate the possible mechanisms involved in the anti-nociceptive effects of TMS.

Author (year) ref	Study design ref	Animal	N	Males (%)	Condition	Intervention	Measurements	Methods	Findings	Conclusion	
Ohnishi et al. (2004) [30]	Experimental study	Macaque monkey	8	100	Healthy subjects	Sham or rTMS	Endogenous dopamine release	$[^{11}\text{C}]$ -labeled PET	1) rTMS over the right M1 increased the extracellular dopamine concentration in the bilateral ventral striatum, including the nucleus accumbens, as compared to sham stimulation 2) rTMS over the right M1 decreased the extracellular dopamine concentration in the ipsilateral putamen, as compared to sham stimulation	rTMS modulated dopamine release in the (bilateral) ventral striatum and (ipsilateral) putamen	
Hsieh et al. (2012) [12]	Experimental study	Rat	34	100	Healthy subjects	TMS	Cortical excitability	EMG Agonizing and antagonizing the GABA $\alpha$ receptor	1) Antagonizing the GABA $\alpha$ receptor reduced TMS-induced cortical inhibition 2) Agonizing the GABA $\alpha$ receptor enhanced TMS-induced cortical inhibition	TMS decreased cortical excitability by use of GABA $\alpha$	
Lee et al. (2011) [20]	Experimental study	Rat	8	100	Healthy subjects	rTMS	Lipid profiles in the PFC, hippocampus and striatum	HPLC Mass spectrometry	1) No changes in lipid profile are detected in the hippocampus after rTMS 2) rTMS-induced changes in lipid profile in the left and right striatum are opposite to changes in respectively the left and right PFC 1) rTMS increased dopamine levels of basal outflow in the nucleus accumbens shell 2) No significant change in basal outflow of dopamine metabolites for rTMS or sham stimulation	rTMS induced changes in brain lipid profiles which can underlie the clinical effects of rTMS	
4	Löffler et al. (2012) [23]	Experimental study	Rat	X	100	Healthy subjects	Sham or rTMS	Monoamine outflow in the nucleus accumbens shell	Microdialysis HPLC with electrochemical detection	1) rTMS increased dopamine levels in the nucleus accumbens shell region, as compared to sham stimulation 3) rTMS increases serotonin levels in the nucleus accumbens shell region, as compared to sham stimulation	rTMS increased dopamine and serotonin levels in the nucleus accumbens shell region, as compared to sham stimulation
Kim et al. (2013) [15]	Experimental study	Rat	8	0	Induced spinal cord injury	TMS	Neuroglial expression in the spinal cord	Immuno-histochemical staining for microglial and astrocytic markers and light microscopy	1) Iba1 positive areas in the dorsal horn of lumbar segments 4–5 decreased after rTMS, as compared to sham stimulation 2) GFAP expression in the dorsal horn of L4–5 segments decreased after rTMS, as compared to sham stimulation	Attenuation of the nociceptive activations of microglia and astrocytes	
Muller et al. (2014) [29]	Experimental study	Rat	48	100	Healthy subjects	TMS	Cortical excitability	EMG Antagonizing the NMDA receptor	1) rTMS reduced motor cortex excitability, as compared to sham stimulation 2) Administering a non-competitive and a competitive antagonist of the NMDA receptor did not change motor cortex excitability after rTMS	rTMS reduced cortical excitability which was found to be NMDA receptor dependent	

EMG: electromyography; GABA: y-aminobutyric acid; GFAP: glial fibrillary acidic protein; HPLC: high performance liquid chromatography; Iba1: ionized calcium-binding adapter molecule 1; N: number of participants; NMDA: N-methyl-D-aspartate; rTMS: repetitive transcranial magnetic stimulation;  $[^{11}\text{C}]$ -labeled PET:  $[^{11}\text{C}]$ -raclopride positron emission tomography.

**Table 2**  
Overview of the parameters used in the applied TMS-protocols (list of parameters is derived from Klein et al. [18]).

Author (year)	Coil design		Coil placement		Stimulation parameters						
	Shape	Size (mm)	Orien-tation	Target	Localization-method	Pulse intensity (%) of RMT)	Pulse frequency	Total length	Train duration	Trains (N)	Intertrain interval
Ohnishi et al.(2004) [30]	Double figure-of-eight coil	62	X	M1	At the optimum scalp position to elicit EMG responses in the contralateral MF. Localization by use of T1-weighted MRI	35%	5 Hz	X	20s	20	40s
Hsieh et al. (2012) [12]	Figure-of-eight coil	66	ML	M1	Fixation in stereotactic frame	X	X	X	X	X	7s
Lee et al. (2011) [20]	Figure-of-eight coil	70	ML	M1	Stereotactic frame and fixation of the coil with dental resin	30/100%	15 Hz	X	30%: 50s 100%: 200s	4	X
Löffler et al. (2012) [23]	Figure-of-eight coil	75	X	M1	Stereotactic frame and fixation of the coil with dental resin	10,20,30 and 40%	20Hz	X		6	60s
Kim et al. (2013) [15]	Round prototype coil	70	X	M1		100%	25 Hz	20min	3s	200	3s
Muller et al. (2014) [29]	Figure-of-eight coil	70	ML	M1	Stereotactic frame and fixation of the coil	55-95% (in steps of 5%)	0.25 Hz; 0.5 Hz; and 1.0Hz	10.5min	X	5-10	7s
Session parameters											Sham conditions
Ref	Pulses per session	Number of sessions	Between session intervals (days)	Maintenance session parameters	Strategy of allocation	Extent of blinding	Control of sensations	Identify sham vs. real	Rate sensations of sham		
Ohnishi et al.(2004) [30]	2000	2	14	Yes	No allocation	No blinding	Audiographic feedback	N/A	N/A		
Hsieh et al. (2012) [12]	X	X	X	X	No allocation	No blinding	X	N/A	N/A		
Lee et al. (2011) [20]	200 or 800	5	0	X	No allocation	No blinding	X	N/A	N/A		
Löffler et al. (2012) [23]	300	4	2	X	No allocation	No blinding	X	N/A	N/A		
Kim et al. (2013) [15]	200		5 consecutive days of stimulation	X	No allocation	No blinding	X	N/A	N/A		
Muller et al. (2014) [29]	300	1	N/A	X	No allocation	No blinding	X	N/A	N/A		

EMG = Electromyography; MRI = Magnetic resonance imaging; MF = Fascicular muscle; X = Missing.

N/A = Not applicable; X = Missing.

**Table 3**  
Overview of studies that investigate the possible mechanisms involved in the anti-nociceptive effects of iMCS.

Author (year)	Study design	Animal	N	Males (%)	Condition	Intervention	Measurements	Methods	Findings	Conclusion
Simpson et al. (1991) [37]	Experimental study	Rabbit	27	Healthy subjects	iMCS of M1	Concentrations of aspartate, GABA, glutamate, glycine and taurine in the ECS of the lumbar spinal cord	Microdialysis HPLC	1) Trend towards increased concentrations of excitatory amino acids in the segmental ECS in response to cortical stimulation	Glycine and taurine increase significantly after iMCS	iMCS of M1 activates LC neurons. However LC or its descending noradrenergic pathways may not have a major role in the spinal antinociceptive effect
Viisanen et al. (2010) [44]	Experimental study	Rat	X	Induced spinal nerve ligation	iMCS and chemical stimulation of M1	Activity of LC neurons	Electro-physiological recording	1) iMCS produced a significant increase in the discharge rate of LC neurons, independent of heat stimulation 2) Administration of an opioid antagonist failed to attenuate iMCS induced spinal antinociception 3) Blockage of spinal alpha2-adrenoreceptors failed to produce an attenuation of iMCS induced spinal antinociception	1) IMCS of M1 activates LC neurons. However LC or its descending noradrenergic pathways may not have a major role in the spinal antinociceptive effect	IMCS possibly reduces neuropathic pain by interrupting the transmission of noxious information from the spinal cord level through the activation of descending pain suppressor systems
Pagano et al. (2011) [32]	Experimental study	Rat	X	Induced sciatic nerve neuropathy	iMCS of M1	Pattern of neuronal activation	Immuno-histochemistry of the spinal cord and brain	1) Decrease of cFos immunoreactivity in the DHSC, VPL and medial nuclei of the thalamus 2) Increase of cFos immunoreactivity in the PAG, ACC and central and basolateral amygdaloid nuclei 3) Egr-1 results were similar to those for cFos, although no changes were observed in the ACC and central amygdaloid nucleus after iMCS	The RVM and the descending serotonergic pathway acting on the spinal 5-HT <sub>1A</sub> receptor contribute to spinal antinociception induced by M1 stimulation in neuropathic animals	IMCS reduces hyperalgesia by increasing activity in the ZI and suggest that the ZI may play an integral role in mediating the reduction in hyperalgesia observed after iMCS by affecting nociceptive transmission within the thalamus through corticothalamic interactions
Viisanen et al. (2010) [45]	Experimental study	Rat	X	Induced spinal nerve ligation	iMCS of M1	Spinal nociception	5-HT <sub>1A</sub> receptor antagonist GABA receptor antagonist	1) Blockade of the RVM by a 5-HT <sub>1A</sub> receptor antagonist or GABA receptor antagonist attenuated spinal antinociception induced by electric stimulation of M1 1) Opioid antagonist infusion in the ZI completely blocked the effects of iMCS 2) GABA agonist blocked iMCS effects	Activation and morphological changes of astrocytes may contribute to the mechanisms underlying pain relief or functional recovery from stroke or movement disorders	IMCS may induce neuroplasticity through the activation of astrocytes
Lucas et al. (2011) [26]	Experimental study	Rat	15	Induced spinal nerve ligation	iMCS of M1	Changes in mechanical withdrawal	Opioid antagonist GABA agonist Withdrawal reflex	3) Saline infusion had no effect on the iMCS induced reduction in hyperalgesia 1) Astrocytes were enlarged 2) The number of astrocytes increased in the cortex and the thalamus of the stimulated hemisphere	IMCS blocked the transmission of somatosensory information to the SI, and this interference was mediated by the endogenous opioid system.	IMCS suppressed SSEPs on the SI ipsilateral to iMCS
Morishita et al. (2011) [29]	Experimental study	Rat	12	Healthy	iMCS of sensorimotor cortex	Morphological changes following long-term chronic IMCS	Immuno-chemical staining	1) Electrical stimulation on the motor cortex suppressed SSEPs on the SI evoked by stimulation of the contralateral forepaw 3) Inhibition of SSEPs induced by	IMCS decreased neuronal responses of the SI evoked by stimulation of the contralateral forepaw	1) Electrical stimulation on the motor cortex suppressed SSEPs on the SI ipsilateral to iMCS
Chiou et al. (2012) [5]	Experimental study	Rat	88	Healthy	iMCS of M1	Effect of iMCS on cortical SSEPs	Electro-physiological recording Opioid antagonist	1) The number of astrocytes increased in the cortex and the thalamus of the stimulated hemisphere	(continued on next page)	2) Responses of the SI evoked by stimulation of the contralateral forepaw

**Table 3 (continued)**

Author (year)	Study design	Animal	N	Males (%)	Condition	Intervention	Measurements	Methods	Findings	Conclusion
Viisanen et al. (2012) [43]	Experimental study	Rat	X	Induced spinal nerve ligation	iMCS of M1	Noxious heat-evoked responses in spinal dorsal horn wide-dynamic range and nociceptive-specific neurons	Electro-physiological recording of dorsal horn	Opioid antagonist D2R antagonist	iMCS was blocked by an opioid antagonist.	Descending pain control induced by stimulation of the M1 cortex in neuropathic animals involves supraspinal (presumably striatal) and, through A11, spinal D2Rs.
Pagano et al. (2012) [33]	Experimental study	Rats	X	Healthy	iMCS of M1	Neuronal activation patterns in the thalamic nuclei and midbrain PAG	Immuno-histochemistry Electro-physiological recording of thalamic complex and the PAG		1) iMCS produced a suppression of the heat response in WDR neurons 2) Striatal administration of a D2R antagonist enhanced the heat-evoked baseline responses of WDR but not nociceptive-specific neurons and reversed the M1 stimulation-induced suppression of the heat response in WDR neurons 3) Spinal administration of a D2R antagonist enhanced the heat response of nociceptive-specific neurons and enabled iMCS to suppress the heat response of WDR neurons 4) Blocking A11 with an opioid antagonist, M1 stimulation failed to suppress the noxious heat-evoked withdrawal reflex	Inhibition of thalamic sensory neurons and disinhibition of the neurons in PAG are at least in part responsible for the iMCS-induced antinociception
Cha et al. (2013) [3]	Experimental study	Rat	48	Induced spinal cord injury	iMCS of M1	Spontaneous activity from well-isolated single neurons in ZI and Po	Extracellular electrophysiological recording GABA <sub>A</sub> receptor-agonist		1) iMCS enhanced spontaneous activity in 35% of the ZI neurons and suppressed spontaneous activity in 58% of Po neurons 2) Inactivation of the ZI using a GABA receptor agonist blocked the effects of iMCS in 73% of Po neurons	iMCS activates the incertothalamic pathway by increasing inhibition within the thalamus, which will hinder nociceptive information transduction to cortical areas (such as the somatosensory cortex, AC and the insula) involved in nociceptive processing
Chiou et al. (2013) [4]	Experimental study	Rat	X	Healthy	iMCS of M1	Responses of the SI to electrical stimuli	Electro-physiological recording Opioid antagonist injection		1) SSEPs of the ipsilateral hemisphere decreased after iMCS 2) Saline decreased SSEPs ipsilateral to iMCS, but contralateral it had no effect 3) Administration of an opioid antagonist in the PAG blocked the effect of iMCS on SSEPs 4) Application of a nonspecific dopamine receptor antagonist to the PAG blocked the inhibition of SSEPs after iMCS 5) Local application of a D <sub>1</sub> antagonist in the PAG inhibited SSEPs after iMCS but a D <sub>2</sub> antagonist had no effect	iMCS possibly induces the release of endogenous opioids in the PAG and this is involved in the inhibition of SSEPs induced by iMCS

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**Table 3 (continued)**

Author (year)	Study design	Animal	N	Males (%)	Condition	Intervention	Measurements	Methods	Findings	Conclusion
França et al. (2013) [9]	Experimental study	Rat	X	Healthy		iMCS of M1	Density of nuclei in the DHSC and rostral portion of the midbrain PAG	Immuno-histochemistry	1) iMCS decreased cFos immunoreactivity in the superficial layers of the DHSC. 2) Increased cFos immunoreactivity in the PAG 3) Egr-1 results were similar to those obtained for cFos	The motor cortex is involved in neuronal circuits of endogenous pain control and when applying iMCS, activation of the endogenous analgesic pathway through the PAG, which consequently inhibits the transmission of noxious stimuli in the spinal cord, is accomplished After iMCS, evoked BOLD signals are significantly attenuated in the S1 and PFC
Jiang et al. (2014) [14]	Experimental study	Rat	10		Induced spinal cord lesion	iMCS of M1	Changes in evoked cortical BOLD signals	fMRI	1) Animals with spinal cord lesion exhibited significant bilateral reduction in mechanical withdrawal thresholds 2) After iMCS, a significant reduction in BOLD signals in response to noxious hindpaw stimulation was observed bilaterally in S1, S2 and PFC	Pain relief due to iMCS appears to be caused by activation of the cingulate gyrus, amygdala and medial lemniscus system and it is not dependent on the descending enkephalinergic and serotonergic analgesic mechanism of the brainstem
Kudo et al. (2014) [20]	Experimental study	Cat	19		Induced destruction of trigeminal ganglion	iMCS of M1	cFos-immuno-reactivity	Immunohistochemistry	1) iMCS increases the percentage of cFos-positive cells in the anterior CG and left main sensory nucleus of the trigeminus 2) iMCS increases the percentage of cFos-positive cells in the basolateral nucleus and central amygdala	'Through the cannabinoid system, iMCS inhibits spinal astrocyte and microglia activity and therefore neuroinflammation'
Silva et al. (2015) [36]	Experimental study	Rat	X		Induced spinal nerve ligation	iMCS of M1	Changes in glial cells, cytolines, cannabinoid type 2, $\mu$ -opioid and purinergic P2 $\times$ 4 receptors in the DHSC	Immuno-histochemistry Cannabinoid receptor antagonist	1) iMCS reversed mechanical hyperalgesia 2) iMCS inhibited astrocyte and microglial activity 3) iMCS decreased proinflammatory cytokine staining 4) iMCS enhanced Ch2 staining 5) iMCS downregulated P2 $\times$ 4 receptors in the DHSC ipsilateral to sciatic injury 6) Pre-treatment with a cannabinoid receptor antagonist blocked the effects of iMCS	'Through the opioid system, iMCS suppresses spinal nociceptive neuron excitability and therefore transmission of persistent pain'
Kim et al. (2016) [15]	Experimental study	Rat	47		Induced sciatic nerve transection as compared to healthy controls with MCS	iMCS of M1	Changes in cFos and serotonin expression Changes of glucose uptake Brain activity in the VPL	Immuno-histochemistry $[^{18}\text{F}]FDG$ -mPET Electro-physiology	1) cFos labelling in lamina I-III of the left dorsal horn was increased by the onset of neuropathic pain, it decreased slightly with electrical stimulation 2) iMCS increased serotonin labelling 3) With $[^{18}\text{F}]FDG$ -mPET altered brain activity was observed in the striatum, thalamic area and cerebellum 4) Neuronal activity in the VPL was effectively suppressed by iMCS	Descending pathway: iMCS suppresses pain by stimulating the striatum and PAG and regulating the levels of opioids or GABA in the descending part of the thalamic area Ascending pathway: suppression of the VPL

(continued on next page)

Table 3 (continued)

Author (year)	Study design	Animal	N	Males (%)	Condition	Intervention	Measurements	Methods	Findings	Conclusion
Kobaïter-Maarrawi et al. (2017) [19]	Experimental study	Cat	20	Healthy		iMCS of M1 VPL	Changes in single-unit activities of the thalamic VPL	Electro-physiology	1) iMCS induced a significant depression of WDR cells firing rate, concomitant with activity enhancement of non-nociceptive units 2) iMCS exhibited activity increase in non-nociceptive cells	iMCS could limit the priming of thalamocortical loops to nociceptive information, by reducing burst occurrence through modulation of the WDR cell excitability

A11: dopaminergic cell group/nucleus within the hypothalamus; ACC: anterior cingulate cortex; BOLD: blood oxygen-level dependent; CB2: cannabinoid type 2; CBF: cerebral blood flow; CG: cingulate gyrus; D2R: dopamine D2 receptor; DHSC: dorsal horn of the spinal cord; ECS: extracellular space; Egr-1: early growth response protein 1; MRI: functional magnetic resonance imaging; GABA:  $\gamma$ -Aminobutyric acid;  $H_2^{15}O$ -PET:  $^{15}O$ -labeled water positron emission tomography; iMCS: invasive motor cortex stimulation; LC: locus coeruleus; M1: primary motor cortex; MCC: midcingulate cortex; MOR: mu opioid receptor; N: number of participants; N/A: not applicable; PAG: periaqueductal gray; pgACC: pregenual anterior cingulate cortex; PFC: prefrontal cortex; PMBS: post-movement beta synchronization; Po: posterior thalamus; P2  $\times$  4: purinoreceptor involved in neuropathic pain; RVM: rostroventromedial medulla; SD: standard deviation; SSEP: somatosensory evoked potential; SI: primary somatosensory cortex; S2: secondary somatosensory cortex; VPI: ventral posterolateral nucleus; WDR: wide-dynamic range; ZI: zona incerta; 5-HT<sub>1A</sub>: serotonin 1A; [<sup>11</sup>C]-PET: [<sup>11</sup>C]diprenorphine positron emission tomography; [<sup>18</sup>F]FDG- mPET: 2-deoxy-[<sup>18</sup>F]fluoro-D-glucose micro-petron emission tomography.

Inactivation of the neurons in the zona incerta by administering a GABA agonist reversed the effects of iMCS on the posterior thalamus neurons. The authors concluded that iMCS activated the incertothalamic pathway by increasing inhibition within the thalamus, which was considered to be a GABA-mediated process [3]. A third rat study administered an opioid antagonist blocking the cell-region A11, which completely reversed the analgesic effects of iMCS. Blocking dopamine 2 receptors with an antagonist had the same effect [43]. The studies of Cha et al. and Viisanen et al. support the hypothesis that iMCS inhibits thalamic processing of noxious inputs, which seems highly dependent on the opioidergic system [3,43]. Viisanen et al. showed activation of locus coeruleus (LC) neurons by electro as a result of iMCS. However, an opioid antagonist and blockage of spinal alpha2-receptors both failed to attenuate iMCS effects. Therefore it was concluded that the LC or its descending noradrenergic pathways may not have a major role in the spinal antinociceptive effect [44]. Another animal-based study in rats found pre-treatment with cannabinoid antagonist (AM251) blocked the effects of iMCS, which led the authors to suggest that iMCS decreases proinflammatory processes by activating the cannabinoid system and suppresses pain by activation of the opioid system [36]. An increase in concentrations of glycine and taurine after iMCS was found in rabbits [37]. Others administered a 5-HT<sub>1A</sub> receptor antagonist and a GABA receptor agonist and concluded that the rostral ventromedial medulla (RVM) and the descending serotonergic pathway acted on the spinal 5-HT<sub>1A</sub> receptor, which contributed to spinal antinociception induced by iMCS [45]. The study of Kim et al. showed that iMS also increased serotonin labelling in rats with induced neuropathic pain [15]. The studies of Viisanen et al. and Kobaïter-Maarrawi et al. measured firing rates of neurons in the rat and cat, respectively. After administrating a dopaminergic antagonist into the striatum, baseline responses were enhanced and iMCS effects were reversed in firing rates. In nociceptive specific neurons, it had no effect on baseline responses [19,43]. Spinal administration of the same antagonist enhanced the heat response of nociceptive neurons and disabled iMCS to suppress the heat response of neurons. Therefore, the investigators concluded that the supraspinal- and spinal dopaminergic systems play an important role in the descending system [43].

One study investigated morphological changes and found that astrocytes were enlarged and their number increased in the thalamus and cortex of the ipsilateral hemisphere after chronic iMCS. The hypothesized mechanism involved induced neuroplasticity by iMCS through the activation of thalamic astrocytes [29].

#### 4. Discussion

##### 4.1. Connections between the different regions impacted by primary motor cortex neuromodulation in animals

In general, this review shows that animal models provide unique insights in our understanding of primary cortex neuromodulation as a pain treatment. The analgesics effects of (r)TMS and iMCS seem to be based on direct modulation of the primary motor cortex and indirect modulation of three subcortical structures. Predominantly functional changes have been described in the reviewed literature, which has been reported to be regulated on neurotransmitter level. These functional changes have been investigated by each study individually and together form a cascade of neural structures and their interplay, which can possibly explain the analgesic effect of iMCS.

Functional changes within the primary motor cortex have been described after iMCS and TMS [12,30]. Both the functional and structural changes could be explained by the involvement of the GABA $\alpha$ -receptor. The GABA $\alpha$ -receptor is the most widespread inhibitory receptor in the central nervous system and is mainly found on post-synaptic membranes [46]. In neuropathic pain models, upregulation of the neuronal expression of the GABA $\alpha$ -receptor has been described [25], although this alone is generally not regarded as sufficient to cause

grey matter volume changes [34]. With regard to the subcortical changes, modifications of thalamic function and structure have been described to occur after iMCS and TMS [3,26,29,32,33]. Second, functional changes within the striatum have been documented as well [21,24,31,43]. Third, the brainstem centers (i.e., PAG, LC and RVM) [9,15,20,33,44] and the spinal cord structures [15,16,19,36,37,45] that are thought to modulate pain were also found to be impacted by invasive and non-invasive primary motor cortex stimulation. Anatomical evidence that all these regions are interconnected in rats was provided by Kita and Kita in 2012 [17]. In this study, an anterograde tracing method was applied to examine the cortical layer of origin, the sizes of parent axons and the specific projections to other brain regions of corticofugal tracts in rats. The study revealed that from the motor cortex various collaterals innervate multiple brain sites including the striatum, associative thalamic nuclei, superior colliculus, ZI, pontine nucleus, multiple other brainstem areas, and the spinal cord. It is furthermore known that the ventral posteromedial nucleus- and the ventral posterolateral nucleus of the thalamus receive feedback from the sixth layer of the motor cortex, suggesting the existence of an extensive modulation circuitry [35]. The nociceptive system is generally regarded to consist of an ascending pain processing system and a descending pain modulating system [39]. The ascending pain processing system consists out of a medial- and lateral pain pathway. The medial pain pathway encodes the motivational and affective components of pain [1]. It is activated by C-fibers which synapse within the fifth and other deep layers of the dorsal spinal horn. These fibers connect to the posterior part of the ventromedial nucleus, the ventrocaudal part of the medial dorsal nucleus, the parafascicular nucleus and the centrolateral nucleus. From the thalamic nuclei, connections course to the anterior cingulate gyrus and the insula [7]. The lateral pain pathway serves the discriminatory and sensory dimensions of pain [1] and is activated by C-, A $\delta$ - and/or A $\beta$ -fibers that synapse at the level of the first and fifth laminae of the dorsal horn. It connects to the ventral posterolateral nucleus, the ventral posteromedial nucleus and the ventral posterior inferior nucleus. From the thalamic nuclei, fibers reach the somatosensory cortices and the parietal area [2]. Bearing the anatomical evidence of structural and functional connections in mind, the widespread impact of neuromodulation of the primary motor cortex stimulation to treat chronic pain can be explained.

#### 4.2. Strengths and limitations

One of the strengths of this review comprises the extensive literature review of animal-based studies on iMCS/TMS in alleviating pain. The reviewing of both iMCS and TMS of the primary motor cortex provides more extensive insights from different studies, although both techniques are not the same. The systematical approach methods used in this review strengthens our conclusion. Limitations of this review can be found in the heterogeneity of the applied stimulation protocols. Especially in TMS-related research, these protocols are of crucial importance in order to create an understanding of the results. These protocols might explain contradictory findings as some protocols are regarded as excitatory (e.g., continuous theta-burst stimulation) or inhibitory (e.g., intermittent theta-burst stimulation). Nevertheless, this dichotomy is rather relative as prolonging a TMS protocol can also reverse the neural effects [10]. In addition, the exact reasoning on which this dichotomy is based remains topic of debate among scientists [8,38]. Therefore, the comparison of different TMS studies might be hindered by the different TMS protocols applied in these studies.

#### 5. Conclusion

To conclude, this systematic review illustrates that the proposed analgesic mechanisms elicited by TMS or iMCS have a considerable amount of overlap in animals. Further progress can be made by a more systematic study of the various mechanisms involved and a consensus

about the optimal stimulation parameters to be used. This could provide new leads for developing novel ways to treat chronic neuropathic pain or to optimize future neuromodulation therapy.

#### Author contributions

*D.J.H.A. Henssen* contributed to the systematic literature search and reviewed the retrieved papers. He synthesized the found evidence and wrote the body of the text and constructed the tables.

*E. Giesen* contributed to the systematic literature search and reviewed the retrieved papers. She contributed to writing the first version of the manuscript. She also provided feedback on the first and second version of the manuscript.

*M.L.E. van der Heiden* contributed to the systematic literature search and reviewed the retrieved papers. She contributed to writing the first version of the manuscript. She also provided feedback on the first and second version of the manuscript.

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*S.A. Lange* contributed to the systematic literature search and reviewed the retrieved papers. She contributed to writing the first version of the manuscript. Lange also created the figures of the manuscript. She also provided feedback on the first and second version of the manuscript.

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*E. Kurt* provided feedback on the first version of the manuscript.

*R. van Dongen* provided feedback on the first version of the manuscript.

*D.J.L.G. Schutter* provided feedback on the different versions of the manuscript and helped in re-writing the body of the text, especially those sections discussing TMS.

*K. Vissers* provided feedback on the different versions of the manuscript and helped in re-writing the body of the text.

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