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Translational Neuromodulation: Approximating Human Transcranial Magnetic Stimulation Protocols in Rats

Andrew M. Vahabzadeh-Hagh, MD*†, Paul A. Muller, BS*, Roman Gersner, PhD‡, Abraham Zangen, PhD‡, Alexander Rotenberg, MD, PhD*†

Objective: Transcranial magnetic stimulation (TMS) is a well-established clinical protocol with numerous potential therapeutic and diagnostic applications. Yet, much work remains in the elucidation of TMS mechanisms, optimization of protocols, and in development of novel therapeutic applications. As with many technologies, the key to these issues lies in the proper experimentation and translation of TMS methods to animal models, among which rat models have proven popular. A significant increase in the number of rat TMS publications has necessitated analysis of their relevance to human work. We therefore review the essential principles for the approximation of human TMS protocols in rats as well as specific methods that addressed these issues in published studies.

Materials and Methods: We performed an English language literature search combined with our own experience and data. We address issues that we see as important in the translation of human TMS methods to rat models and provide a summary of key accomplishments in these areas.

Results: An extensive literature review illustrated the growth of rodent TMS studies in recent years. Current advances in the translation of single, paired-pulse, and repetitive stimulation paradigms to rodent models are presented. The importance of TMS in the generation of data for preclinical trials is also highlighted.

Conclusions: Rat TMS has several limitations when considering parallels between animal and human stimulation. However, it has proven to be a useful tool in the field of translational brain stimulation and will likely continue to aid in the design and implementation of stimulation protocols for therapeutic and diagnostic applications.

Keywords: Motor evoked potential, rat, repetitive transcranial magnetic stimulation, translation of human magnetic stimulation protocols

Conflict of Interest: Andrew M. Vahabzadeh-Hagh reports no biomedical financial interest or potential conflicts of interest. Paul A. Muller reports no biomedical financial interest or potential conflicts of interest. Roman Gersner reports no biomedical financial interest or potential conflicts of interest. Abraham Zangen is a consultant for and has financial interest in Brainsway Inc., a company that develops transcranial magnetic stimulation (TMS) coils designed for stimulation of deeper brain areas. Alexander Rotenberg does not currently serve on any advisory board, but does hold intellectual property for TMS technology and the combination of TMS with electroencephalogram.

INTRODUCTION

Transcranial magnetic stimulation (TMS) is a well-established method for noninvasive focal cortical stimulation in humans that provides a means to investigate or modulate cortico-cortical and corticospinal circuits (1–5). In its most common embodiment, TMS is coupled with surface electromyography (EMG) and applied to stimulate the motor cortex of humans to elicit motor evoked potentials (MEPs) in targeted contralateral muscle groups. Quantitative attributes of these signals, such as stimulus threshold to activation, MEP amplitude, and latency, are then used as measures of cortical and corticospinal excitability. Such measures have been shown to change in correlation with a range of physiologic and disease states such as epilepsy, stroke, and head trauma, which are characterized

Address correspondence to: Alexander Rotenberg, MD, PhD, Department of Neurology, Children's Hospital, 300 Longwood Avenue, Boston, MA 02115, USA. Email: alexander.rotenberg@childrens.harvard.edu

* Department of Neurology, Children's Hospital, Harvard Medical School, Boston, MA, USA;
† Berenson-Allen Center for Noninvasive Brain Stimulation, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA; and
‡ Department of Neurobiology, The Weizmann Institute of Science, Rehovot, Israel

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by pathologic alterations in cortical excitability (1,3,6,7). The potential for future application in clinical diagnostics is quite clear.

TMS is also applied to cortical regions in repetitive trains; namely as repetitive TMS (rTMS). rTMS enables use-dependent modulation of cortical excitability likely via mechanisms analogous to long-term potentiation and long-term depression (LTD) (3,8–13). The capacity of rTMS to induce changes in cortical excitability which outlast stimulation protocols has led to its experimental and clinical application in neuropsychiatric disease where the pathophysiology involves over- or under-activation of a cortical region (14–16).

Relevant to this discussion, TMS has recently been translated to use in rodent models (17–24) where more mechanistic insights into TMS-derived measures of cortical excitability and rTMS-mediated changes in cortical function can be obtained. The benefits of this translation are only now being realized as rodent, particularly rat, TMS methods are undergoing optimization, their underlying physiologic effect is being teased out at the synaptic and molecular levels, and are ultimately applied in controlled disease models. Here we discuss the growing body of translational TMS research and address the hurdles which still need to be overcome in the translation of TMS techniques from human to rat.

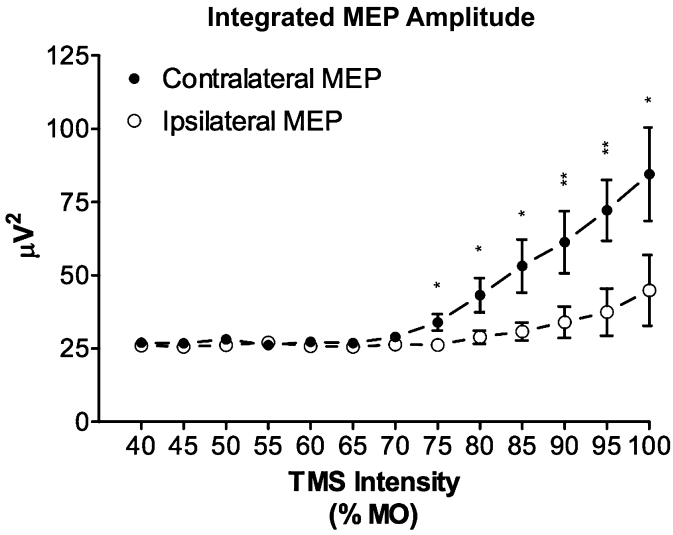

Figure 1. Input–output curves for rat transcranial magnetic stimulation (TMS) motor evoked potentials (MEPs). Data averaged across 13 male adult rats demonstrating MEP metric discrepancy between the contralateral and ipsilateral brachioradialis muscle as a function of TMS intensity. Demonstrates the lower MT of the contralateral target muscle group as well as the ability to activate the contralateral limb without ipsilateral activation. Error bars represent the standard error of the mean; * $p < 0.05$, ** $p < 0.01$. Adapted from Rotenberg et al. (22). MO, machine output.

ADAPTING TMS TO RATS

TMS Coils and Stimulation Focality

Focalization of stimulation in TMS is of immense importance in experiments aimed to mimic human stimulation protocols. While steps toward focal rat TMS are not without their caveats, its introduction and evolution are vital to the field of noninvasive brain stimulation, especially in its capacity to measure cortical-specific excitability. The first and most formidable impediment is the discrepancy in the ratio of TMS coil size to head size. In human studies where focal stimulation is desired, TMS is often applied unilaterally, usually with a figure-of-eight coil to activate or alter the function of discrete brain regions. Most human TMS coils are capable of safe cortical stimulation at depths up to approximately 1.5 cm from the coil and provoke direct stimulation of volumes as focal as half a cubic centimeter (25). Yet this volume of stimulated brain would not produce a focal effect in a rat. Thus, special adaptation to mimic focal human TMS in the rat is necessary.

A variety of different coil shapes have been used in rat TMS studies. Among them are round, Helmholtz, teardrop, and figure-of-eight (Supporting Information Tables S1, S2, and S3). More focal stimulation results from the commonly used figure-of-eight coil (26). Interestingly, both documented (27) and empiric evidence from our laboratory demonstrates that the average adult rat brain size is on the order of 1.5–1.6 cm^3, depending on age. Although TMS-induced electrical fields in rats are expected to be smaller than in humans due to the relatively poor electromagnetic coupling of the small rat head with the large magnetic field (28), nevertheless one can expect the relatively diffuse stimulation of the rat brain to impair translation of human focal TMS techniques to rodent models, at least when neuroanatomical considerations affect the outcome related of the research question.

To improve the focality of TMS, smaller TMS coils may, in principle, be used in rats. However, their development has been limited by a requirement for smaller electrical current loads to limit intracoil repulsive Lorentz forces (29), while also requiring a greater electrical intracoil current to produce an effective field due to greater cancellation induced in smaller coils (25,30). In addition to smaller coil size, although untested *in vivo*, is the development of an attachment or shield to use concomitantly with a human coil to more accurately direct the magnetic field (31).

Recent studies from our lab demonstrate that orienting the TMS coil eccentrically to targeted regions in the rat brain allows for at least uni-hemispheric stimulation (22). In this work, we obtained input–output curves from 13 adult male rats anesthetized with intraperitoneal (i.p.) pentobarbital. The pentobarbital dose was optimized to allow for the comfortable placement of the rats in a stereotactic frame while preserving their withdrawal response to foot pinch. We utilized a relatively small figure-of-eight coil (20 mm outer lobe diameter) positioned lateral to the midline of the dorsal scalp. We then gradually increased the machine output while recording MEPs from the brachioradialis muscle bilaterally with EMG needle electrodes. The input–output curves demonstrated that motor threshold (MT), defined as the stimulus intensity required to achieve MEPs with a peak-to-peak voltage $\geq$15 μV in at least five of ten consecutive trials, was reached in all rats for the target muscle contralateral to stimulation. As the stimulus intensity was increased, only 15% of the animals reached the MT of their ipsilateral limb. Figure 1 further illustrates the ipsilateral–contralateral MEP discrepancy as a function of TMS intensity.

These data prove the feasibility of using conventional TMS equipment, with a relatively small coil, in rats to obtain focal brain stimulation at the resolution of a single hemisphere.

Anesthesia in Rat TMS Experiments

Another difficulty in translating TMS techniques is the requirement for anesthesia in order to provide humane, reproducible focal stimulation in rats. In contrast to human studies, experimenters are unable to control volitional motor activity in rats. Also, rather than the frameless stereotactic system used in humans, accuracy in rats is greatly increased by the use of a fixed stereotactic frame. Therefore,

to suppress spontaneous motor activity and to eliminate pain and distress associated with placement in the stereotactic frame, anesthesia becomes a necessity. Early studies suggested a strict dependence of the success of TMS to elicit MEPs on the choice and dose of anesthesia (19). To date, a majority of rat studies use either urethane or propofol anesthesia, as both have been shown to preserve spinal reflexes (32,33) (Supporting Information Tables S1, S2, and S3). More recent studies in our lab demonstrate the capacity to achieve TMS evoked MEPs under i.p. pentobarbital, ketamine-atropine-xylazine (KAX) (22,24), or inhaled isoflurane (Vahabzadeh-Hagh, Lo, Pascual-Leone, and Rotenberg, unpublished) anesthesia with equal success. The broad range of anesthetics successfully used in our work, as well as current and previous studies, allows for experimental optimization and avoidance of anesthetic confounding of data relevant to testing contributions of specific neurotransmitter receptor families.

While anesthesia provides a vital tool to allow for focal stimulation, it is important to keep in mind that the effect of TMS is reliant upon the underlying activity of the brain at the time of stimulation; e.g., the brain state (34). In human studies the effect of both sleep and anesthesia on state-dependent neural activity and the resulting changes in TMS wave propagation have been well documented (34,35). In rats, a recent study from our group (36) compared the lasting neurochemical effects of rTMS in awake and anesthetized animals. We found that the effects of stimulation on brain-derived neurotrophic factor (BDNF) and 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid (AMPA) receptor GluR1 subunit levels were opposite for the awake vs. anesthetized states. These data emphasize the importance of anesthesia selection in translational rTMS experimental design.

In some experiments, such as those designed to simulate clinical rTMS applications, mechanical restraint, rather than anesthesia, has been used (37). Also, to facilitate translational studies in awake rats, our group has also been looking at unanesthetized TMS with a new alternative method to EMG, mechanomyography (38). However, what is lost in the conscious awake state is the ability to target a single hemisphere or more focal anatomical structure. In many cases, this loss of focality is not significant, as whole volume stimulation may indiscriminately activate all circuits, thus allowing for the desired metric to possibly be isolated post-acquisition. However, where neuromodulatory stimulation paradigms are utilized, the need to target specific sites becomes more imperative to exclude the contribution of multiple areas to the observed effect. The present review highlights this continued hurdle to the application of focal TMS in rats. Thirty-seven of 100 studies analyzed in this review employed anesthesia. This comprises only 30% of the non-focal (holocranial) TMS studies, but is seen in 92% of focal TMS reports.

Also relevant to the issue of TMS focality in translational studies, the development of a very small, potentially implantable coil might forgo the use of anesthesia altogether. However, development of such a coil is not being actively pursued and presents a multitude of engineering problems (25,30).

TMS PROTOCOLS IN RATS

Paired-Pulse TMS in Rats

Among the advances in translation of human diagnostic TMS techniques to rodents is the adaptation of paired-pulse stimulation (ppTMS) to rats. ppTMS applied with variable interstimulus intervals (ISIs) is a tool capable of noninvasive interrogation of both excitatory and inhibitory cortico-cortical circuits (2,39–41). In ppTMS protocols, a conditioning stimulus (CS) precedes each successive test stimulus (TS). The provocation of inhibitory or excitatory cortical circuits has been shown to largely depend on CS intensity and ISI. Short (1–6 msec) ISIs with subthreshold CS and suprathreshold TS lead to suppression of the MEP produced by the TS; i.e., short-interval intracortical inhibition. Longer (8–30 msec) ISIs with a subthreshold CS and suprathreshold TS result in facilitation of the MEP; i.e., intracortical facilitation (2,40–45). Still longer (50–300+ msec) ISIs with suprathreshold CS and TS result in MEP inhibition; i.e., long-interval intracortical inhibition (LICI) (39). Last, discrete ISIs (1.1–1.5 msec, 2.3–3.0 msec, and 4.1–5.0 msec) with a supra- or at MT CS followed by a subthreshold TS provoke MEP facilitation; short-interval intracortical facilitation, also known as facilitatory I-wave interaction (44–46). Thus, with a pair of stimuli applied to the same cortical location there are four main circuits one can activate in humans; namely, short-interval intracortical inhibition, intracortical facilitation, LICI, and short-interval intracortical facilitation.

In a recent study, we sought to translate human LICI protocol to anesthetized rats. LICI is a commonly used and robust ppTMS technique with previously demonstrated potential clinical applications. Notably, LICI is suppressed in diseases of excess cortical excitability such as epilepsy (42,47–50). In this study we sought to demonstrate the ability to obtain LICI-like measures in rats, the dependence of these measures on two commonly used injectable anesthetics, and the capacity of these measures to detect acute changes in cortical inhibition as provoked by a chemoconvulsant, pentylenetetrazol (PTZ). Here we used a figure-of-eight double 40 mm coil (Magstim Company, Wales, UK). We applied stimulation using the same eccentric positioning described in Rotenberg et al. (22). One subset of rats was anesthetized with i.p. pentobarbital while the other with i.p. KAX. We then provided both single and ppTMS (ISIs of 50, 100, 200, 300, 400, and 500 msec) at 120% MT presented in a randomized order with an intertrial interval of 8 sec. In a separate subset of rats, anesthetized with pentobarbital, we then assessed the capacity of these LICI-like measures to detect acute changes in cortical excitability as provoked by PTZ. Here we obtained baseline long-interval ppTMS (LI-ppTMS) measures, injected PTZ (70 mg/kg i.p.) or the equivalent volume of 0.9% saline (control), and then repeated LI-ppTMS measures at 5, 30, and 45 min post injection while recording the electroencephalogram to confirm electrographic seizures.

Figure 2 demonstrates the paired-pulse inhibition profile as a function of ISI for pentobarbital and KAX anesthesia. We found that both single-pulse TMS evoked MEPs and human-like measures of LICI were preserved under either anesthetic. In addition, the rat LI-ppTMS inhibitory profile, as in Figure 2, strongly resembles the human LICI profile, suggesting the interrogation of perhaps similar intracortical processes. PTZ administration was shown to reduce LI-ppTMS inhibition by as much as 53% when compared with saline-injected control subjects (24). This study was the first successful translation of a ppTMS protocol to rat models as well as the first to evaluate the sensitivity of LI-ppTMS measures to changes in cortical excitability during acute chemoconvulsant-induced seizures. This work also underscores the importance of animal TMS studies in helping us to better understand human TMS mechanisms.

LICI has generally been accepted to be mediated by the activation of gamma-aminobutyric acid B receptors ($GABA_BR$) based upon limited data for the enhancement of LICI via the administration of baclofen, a $GABA_BR$ agonist. However, such dependence has proven difficult to replicate, suggesting that LICI is a more multifaceted process (51,52). The data from our study, demonstrating the reduction of LI-ppTMS, an LICI-like measure, in the presence of a $GABA_AR$ antagonist (e.g., PTZ), corroborate the notion that LICI may not be an exclusive $GABA_BR$-mediated process. Additionally, in more recent

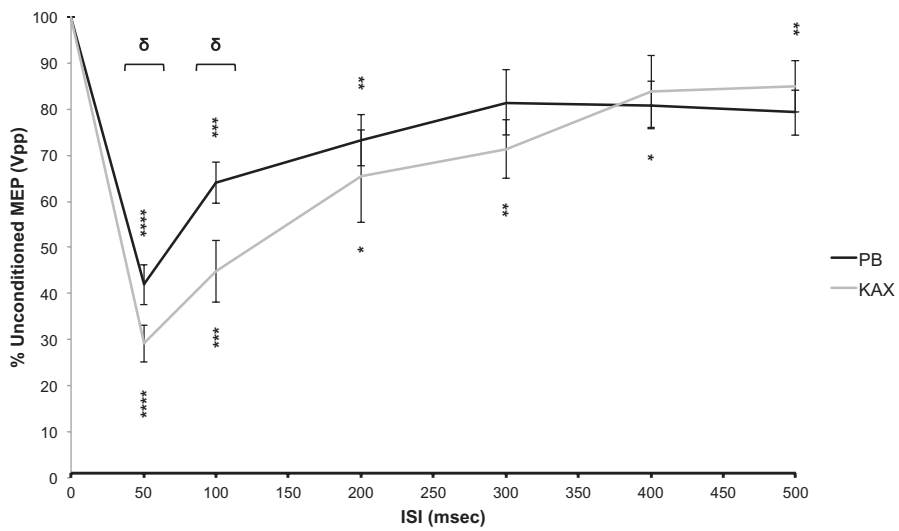

Figure 2. Long-interval paired-pulse transcranial magnetic stimulation (LI-ppTMS) inhibitory profile in anesthetized rats**.** Graph depicts the conditioned motor evoked potential (MEP) size as a function of both anesthetic and interstimulus interval (ISI). Values were derived as the conditioned MEP peak-to-peak amplitude normalized to that of the unconditioned MEP. MEP inhibition is represented by values <100%. Significant inhibition relative to baseline unconditioned MEP values is noted by asterisks (* $p < 0.05$, ** $p < 0.02$, *** $p < 0.002$, **** $p \leq 0.0002$). ISIs demarcated with brackets indicate significant intergroup (e.g., interanesthetic) differences ($\delta < 0.05$). Values indicate the mean ± standard error. Adapted from Vahabzadeh-Hagh et al. (24). KAX, ketamine-atropine-xylazine; PB, pentobarbital; Vpp, peak-to-peak voltage.

years, studies have demonstrated an intricate cross-talk that exists between $GABA_A$ and $GABA_B$ receptor subclasses. For example, the interaction of $GABA_BR1$ with $GABA_A$ receptor gamma2S has been shown to regulate surface expression and internalization of $GABA_BR1$ under different circumstances (53,54). As such, while $GABA_BR$ activity may still be the final mediator of LICI, we cannot exclude that $GABA_AR$ activity may be at least indirectly involved. The establishment of this ppTMS protocol in rats opens the door to additional pharmacologic studies that will help us to understand these neurophysiologic processes even further.

rTMS in Rats

Translational rTMS has been applied in rodents largely for one of two objectives: 1) to study rTMS effects on biochemical or electrophysiologic markers of cell injury or synaptic plasticity, and 2) to test the capacity of rTMS to intervene in models of neuropsychiatric disease. *Ex vivo* changes in molecular markers of altered neuronal excitability and alterations in hippocampal electrophysiology suggest the modulation of neuronal activity following rTMS (13,36,55–57). Rat data also demonstrate the potential for rTMS-induced measures to serve as markers of neuronal plasticity. For example, Luft and colleagues demonstrated that simulated motor learning (by somatosensory afferent stimulation) leads to an enhanced cortical motor excitability in anesthetized rats (18).

rTMS may be customized by frequency and the pattern (continuous or intermittent) of stimulation. When applied in a low frequency manner (i.e., 1 Hz) rTMS suppresses cortical excitability, while high frequency (i.e., 5–20 Hz) enhances cortical excitability. In the majority of rat rTMS publications, stimulation has been applied globally without confirmation of lateralized stimulation by limb EMG (17,21,37,58,59). More recently, we adapted lateralized TMS protocols in anesthetized rats to an rTMS experiment. We applied low frequency rTMS to induce an LTD-like suppression of the MEP and further utilized the rodent preparation to demonstrate the dependence of this process on the N-methyl-D-aspartate (NMDA)-type glutamate receptor (60). Demonstration of a reliable, focal increase or decrease in excitability by rTMS holds implications for studying disease states affecting discrete brain regions or overall cortical activity and helps to broaden the potential applications of this technique.

Utilization of animals to study the neurophysiologic effects of rTMS is beneficial even without the anatomical localization that can be achieved in humans. For example, experiments where rTMS was delivered non-focally showed how rTMS parameters can differentially affect neuronal excitability (55) and neurochemical markers of neuroplasticity such as immediate early genes (IEGs), BDNF or AMPA (36) and NMDA (56) receptors, depending upon stimulation parameters and animal conditions. Some studies in animal models evaluated the acute effects of either high-frequency (61) or low-frequency (57) rTMS on plasticity markers or clinically relevant neurochemical alterations such as the release of dopamine, glutamate, or acetylcholine (62,63). An early landmark study found that specific stimulation patterns of rTMS can trigger the expression of IEGs, surrogate measures of induced neuronal activity (17). IEGs are rapidly expressed following synaptic stimulation or behavioral experience and typically assume either a regulatory transcription factor or an effector phenotype. C-fos and Zif268, two regulatory transcription factors, have been implicated in the processes of synaptic plasticity and memory consolidation (64). From the work of Aydin-Abidin and colleagues (17) it was shown that c-fos protein expression was enhanced by either low- or high-frequency rTMS, but only partially by intermittent theta-burst stimulation. On the other hand, Zif268 expression was increased by intermittent theta-burst stimulation, partially by high- but not by low-frequency stimulation. Thus, c-fos appears to have a more nonspecific pattern of activation induced by rTMS. By measuring the expression of these genes in different brain regions, Aydin-Abidin et al. (17) deciphered that different brain regions appear to exhibit different susceptibilities to entrainment by rTMS. Interestingly, they also found a reflexive increase in c-fos expression in the limbic or arousal system in response to sham rTMS, indicating a general level of stress on the

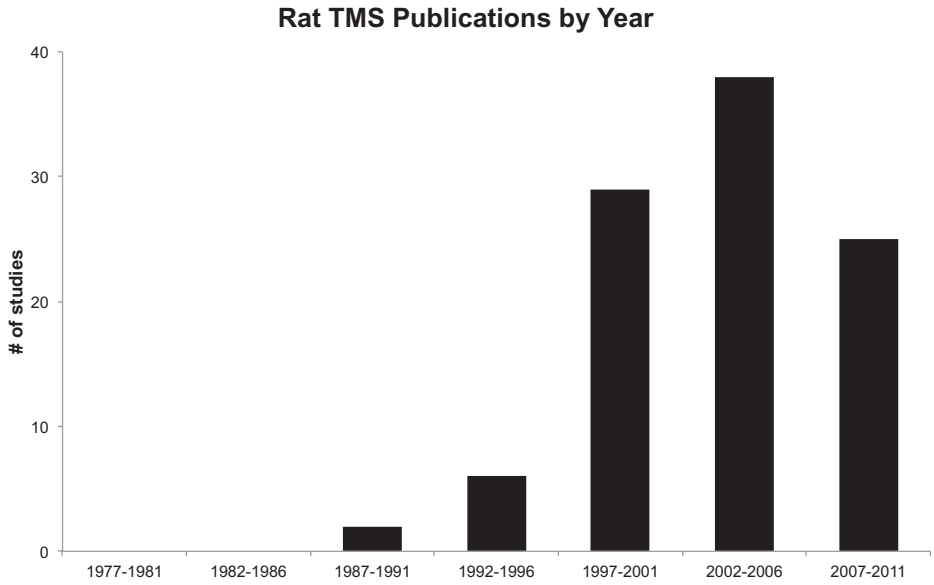

Figure 3. Recent growth in rodent transcranial magnetic stimulation (TMS) publications. PubMed search was performed from 1966 through December 2010 for "rat(s)," "tcMMEP," "TMS," "rTMS," "repetitive transcranial magnetic stimulation," and "transcranial magnetic stimulation." Publications from 2012 were not included as the year was not yet complete at the time of submission.

animal associated with experimentation (17). This only reiterates a major hurdle we must overcome in rodent TMS; namely, minimization of both experimentally induced stress and of the utilization of anesthesia. Furthermore, recent animal studies have also helped to elucidate the target neuronal populations of theta-burst stimulation; namely, fast-spiking interneurons and GABAergic interneuronal synapses (65). As we learn more about the specific effectors of rTMS we may optimize the research and clinical applications of these techniques.

Other rat rTMS studies have focused on the longer lasting effects of several rTMS sessions, perhaps more relevant to the clinical approach in humans (36). As mentioned above, Gersner and colleagues (36) looked at the long-term effects of rTMS using BDNF and the GluR1 AMPA receptor subunit expression as markers of neuronal plasticity. They found that ten daily treatments of high-frequency rTMS induced lasting change (e.g., three days) in these markers, the direction of which was dependent upon whether the animal was awake vs. anesthetized (36). The sharp contrast in their findings between these states underscores the fact that these states are unequal and that caution must be taken when interpreting results obtained under anesthesia. This study also highlights a potential benefit of such animal studies; namely, the capacity to measure brain levels, as opposed to peripheral/serum levels, of plasticity markers. Such capacity may improve the sensitivity and validity of studies as well as the consistency of findings across reports to better inform clinical translation. Overall, these findings obtained in animal studies support the hypothesis that changes in cortical excitability induced by rTMS are accompanied by acute and lasting alterations in molecular markers for neuroplasticity.

TRANSLATIONAL TMS LITERATURE

At the time of this writing, there are more than one hundred published reports that contain rat TMS data (Supporting Information Tables S1, S2, and S3) (17–19,21,22,24,31–33,36,37,55–59,61–63,66–150). Our MEDLINE search strategy to gather these publications was performed for reports from 1966 through March of 2012 that contained keywords "rat(s)," "tcMMEP" (transcranial magnetic MEP), "TMS," "rTMS," "repetitive transcranial magnetic stimulation," or "transcranial magnetic stimulation." Notably, from the first rat TMS publication in 1990 there has been an exponential increase in published rat TMS studies (Fig. 3), which underscores the TMS community's growing interest in the subject.

TMS Safety

Since TMS in humans has an exceptionally favorable safety profile and is relatively easy to apply, the technique has become widespread in clinical literature (10,151–155). Because of its wide safety margin, the development and application of TMS in humans has been less reliant upon foundational findings from animal models. In fact, TMS in rats, particularly if coupled with EMG or electroencephalogram, has required specialized technical adaptation, contributing to a lag in basic science data acquisition from rodent experiments. Nevertheless, rat studies have proliferated to meet investigators' need to explore TMS safety, basic TMS mechanisms, and TMS efficacy in high-throughput rat disease models with neuroscience techniques that are not available in humans. Safety, beyond mechanism, is arguably highest among the concerns of TMS users. To date, a number of studies have used rat models to provide a deeper evaluation of the safety of rTMS. Chalet de Sauvage and colleagues (37) implemented an rTMS protocol in rats similar in design to the protocol for treatment of depression in humans. They demonstrated that the brain current density profiles were similar between rat and human rTMS and that rTMS (2000 pulses at 100% MT) did not cause measurable genotoxicity when compared with sham-treated rats (37). Additional studies have evaluated the safety of more chronic rTMS exposure and failed to demonstrate an alteration in the cerebral metabolite profile or any associated brain or brainstem lesions (58,132,156). These safety reports emphasize only a fraction of the beneficial knowledge gained from rat TMS work to date and highlight their potential for great contribution to the field of noninvasive brain stimulation.

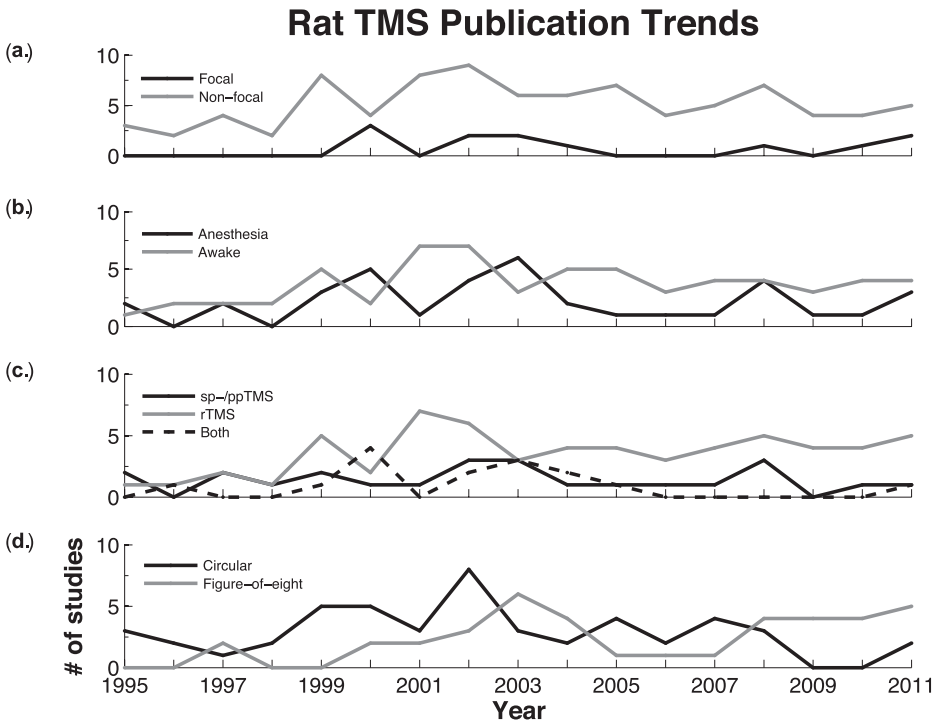

Figure 4. Trends in stratified rat transcranial magnetic stimulation (TMS) publications. The articles from the above-mentioned PubMed search were used with the exception of those that did not detail their TMS protocol. One hundred articles were used in total. a. Number of publications regarding focal and non-focal TMS over time. b. Number of publications that used TMS on anesthetized vs. awake rats. c. Number of publications using single- or paired-pulse TMS (sp-/ppTMS), repetitive TMS (rTMS), or some combination thereof. d. Number of publications stratified by their usage of a circular vs. figure-of-eight coil.

TMS Functional, Diagnostic, and Therapeutic Applications

The articles reviewed were stratified on a number of key principles in rat TMS work; namely, whether TMS was applied in a focal or non-focal manner, whether it was applied to awake or anesthetized animals, and whether single-/paired-pulse, repetitive TMS, or some combination of protocols was utilized. Only those articles detailing their stimulation protocol were used in this analysis. One hundred were included dating back to 1995. Focal TMS was utilized in 12% of these studies while the remainder was unconcerned for its localized application. Among these publications, the first report with focal TMS occurred in 2000 with a steady interest in its application ever since, although of a less productive magnitude when compared with non-focal TMS studies (Fig. 4a). As we are beginning to learn more about our capacity to achieve focal rat TMS, a growing trend for the future is anticipated. Figure 4c demonstrates a general increasing trend in the number of rTMS reports annually since 1995. With increasing clinical applications, we anticipate the maintenance of this trend for the foreseeable future. For specific details and citations of these reviewed articles organized by TMS protocol and year see Supporting Information Tables S1, S2, and S3.

Rat TMS Study Flaws

Over time coil shape usage has been almost evenly split between circular and figure-of-eight types, 51% vs. 42%, respectively (Fig. 5). However, looking at the distribution of coil shapes utilized over time there has been a recent shift, from 2009 to present, away from circular coils toward predominantly figure-of-eight coils (Fig. 4d). The predilection to a more focal coil in recent years would suggest that studies are beginning to place a greater emphasis on stimulation specificity. Surprisingly, this does not appear to be the case as non-focal TMS studies continue to dominate the field. While circular coils and non-focal stimulation have and continue to provide valuable information on TMS mechanisms, their overall scientific utility is more limited. Stimulation of the majority of the rodent brain provides too much background precluding our ability to draw more

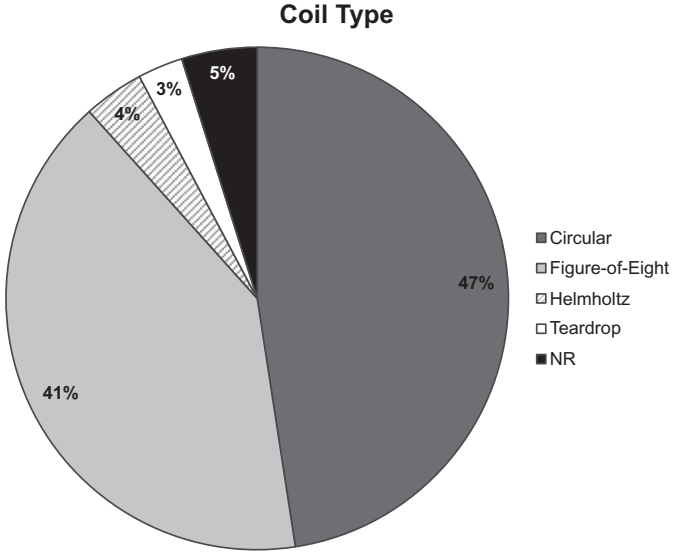

Figure 5. Distribution of coil type utilized in rat transcranial magnetic stimulation (TMS) reports. This pie chart depicts the percentage of reports from among the 100 studies analyzed (Supporting Information Tables S1, S2, and S3), which utilized different types of TMS coils; namely, circular, figure-of-eight, Helmholtz, or teardrop coils. Since 1995, studies have mainly used the circular or figure-of-eight type coils at a near equal frequency. NR, not reported.

spatially relevant and mechanistic conclusions. In order to truly uncover underlying biological processes in TMS, it is the opinion of our group that studies should dedicate more effort to refining and utilizing focal stimulation protocols whether via TMS or by focal electrical stimulation as an approximation of focal TMS in humans.

The use and type of anesthesia in both therapeutic and diagnostic TMS applications is highly variable among publications. A wide range of anesthetics have been used, including ketamine, pentobarbital, isoflurane, propofol, and urethane. Any anesthesia will play a significant role in altering steady-state biochemistry and neuronal activity as demonstrated recently by our group (36). Different anesthetics have differing effects on excitability, as demonstrated in our recent study where ketamine significantly depressed MT compared with pentobarbital and enhanced LI-ppTMS inhibition (24). As previously stated, TMS effects are inherently state-dependent (34,35,157), and both the use and variability of anesthesia selection warrant caution in the interpretation of data and comparison across studies. Studies dedicated to the effect of each anesthetic on TMS outcome measures are needed, and standardization of anesthetic use would benefit the field. Overall, the use of anesthesia remains a considerable difference between human and rat TMS studies, which merits further attention hopefully leading to novel and creative solutions.

Animal restraint is another area in which rat TMS publications offer inconsistent methods. Restraint techniques are in some cases elegant, utilizing fixation screws attached to the occipital bone followed by subsequent fixing to a stereotactic frame (56,80) or the use of a stereotactic frame with a TMS coil attached to a manipulator (22,24). However, in the majority of studies animals and TMS coils are simply held and approximated by hand. Use of a proper restraint in order to facilitate reproducible stimulation is imperative to TMS experimental design. While global changes induced by TMS can be analyzed with less intricate methods, one cannot assume that stimulation is identical between animals. Thus, an effort should be made to immobilize the head in a maximally humane and efficient manner and provide a method for accurately placing the stimulation coil to reduce stimulation and outcome variability.

While EMG use is not a required readout, its use in determining stimulation strength should be considered indispensible. Among the publications analyzed in this review, EMG is used in 47% of reports without a discernable chronological trend. Approximation to human protocols alone would require that stimulation strength be standardized to a percentage of MT. Crude observation of limb twitching, while an improvement, cannot replace the sensitivity provided by EMG recording. Whether MT is unreliably determined, or a set machine output is provided, each will contribute to variable stimulation among animals and likely introduce error.

Stimulation protocol is one final area of inconsistency among rat TMS studies. It is clear that rTMS is the more popular form of TMS used in these studies to date (Fig. 4c). However, the use of single-pulse TMS and ppTMS, in this groups' opinion, should be used in combination with rTMS as both a readout and method of ensuring that the desired excitation or depression of targeted cortical regions has been achieved. Additionally, there is a lack of rationale concerning the choice of rTMS frequency, as the field is without a comprehensive overview of low- and high-frequency rTMS effects. Although great strides have been made in the study of TMS in rats, a more concerted effort is needed to refine our tools and techniques.

CONCLUSIONS AND PRACTICAL SIGNIFICANCE

The growing body of translational TMS research in rodents has realistic potential to complement the already established field of clinical TMS. Although limited by stimulation focality, specialized technical requirements, and essential differences in neuronal circuitry between rat and human, rat data have provided important insights into basic TMS mechanisms and its prospects in the treatment of neuropsychiatric disease. For instance, a demonstration of seizure suppression by rTMS in the rat (21) has influenced the design of an ongoing clinical trial for rTMS in patients with epilepsy. In the authors' view, as with other translational fields, basic science exploration of TMS, particularly rTMS and ppTMS protocols, will continue to direct clinical trial development. The steady increase in rodent TMS research over the recent years seems to uphold this prospect. As rodent TMS models continue to be refined, we can expect to learn more about TMS and the physiologic underpinnings of neurologic and neuropsychiatric disease, while expanding upon our diagnostic and therapeutic armamentarium.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Organizes retrieved articles that utilized an rTMS protocol.
Table S2. Organizes retrieved articles that utilized a single- or paired-pulse TMS protocol.
Table S3. Organizes retrieved articles that utilized a single- or paired-pulse TMS protocol as well as an rTMS protocol.

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COMMENTS

Applications of rTMS have become commonplace across the cognitive sciences and now increasingly in clinical practice and psychiatry. The sensible use of the rTMS treatment modality, however, requires a more sophisticated understanding of its action. Animal models have the potential to substantially contribute to this and in this manuscript the authors have brought together the majority of the literature conducted in this manner to date. Hopefully this will stimulate further research and interest in this area, contributing substantially to the advancement of this field.

Paul Fitzgerald, MBBS, MPM, PhD
Melbourne, Australia

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Investigating standard medical interventions in animal models is always a challenge—it has to do not only with difference in pathological conditions and clinical disease manifestations, but also with difference in anatomy and the size of most laboratory species. As an example, studies of transcranial magnetic stimulation (TMS) in rodent models are difficult because of the striking difference in size of human and rat brain. Nevertheless, there is a large and constantly growing body of literature focusing on various aspects of TMS based on rat TMS model. The paper by Vahabzaghdeh-Hagh and colleagues presents a critical overview of this literature and attempts to draw parallels between animal and human research endeavors.

For most of us in the world of neuromodulation who are not aware of recent developments in this area, this thorough review provides detailed information regarding multitude of completed and ongoing research projects. Among other things, this information may spark some interest in the clinical community and facilitate additional human TMS applications translating basic science into medical interventions.

Konstantin V. Slavin, MD
Chicago, IL, USA

Comments not included in the Early View version of this paper.

