TMS in animal models: Methods and Applications

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Conflict of Interest Disclosure

Alexander Rotenberg

Current:
- Neuro’motion Inc. (technology for improving emotional control; co-founder)
- NeuroRex (medical advisor)
- Brainsway Inc. (research support [equipment and personnel])
- Soterix Medical Inc. (research support [equipment])
- Neuroelectrics Inc. (research support [equipment])
- Journal of Central Nervous System Diseases (EIC)
- NIH NIMH, DoD, CIMIT, ERF, TRP (research grants)

Past:
- Neuropace Inc. (research grant and equipment)
- Nexstim Inc. (consultant)
- Sage Therapeutics Inc. (consultant)
- Fisher Family Fund and Fisher-Wallace Inc. (research support [unrestricted gift and equipment])
TMS in animals

- Cat: 40
- Dog: 20
- Pig: 10
- Rabbit: 5
- Rat: 160
- Mouse: 30

PubMed Citations
Why TMS studies in animals?

– Basic Science

– Translational Research

Poma et al., 2006
Advantages of animal subject

- Subject homogeneity
- Available histology
- Genetic / disease models

Liebetanz et al., 2003
Translational Relevance

- Disease modeling
- TMS safety
- Neuronal connectivity
- Synaptic plasticity
- Cortical organization
Induced dysfunction: neglect-like syndrome in cats

Valero Cabre et al., 2005
Frequency-Dependent $^{14}$C-2DG uptake modulated in cat

- 20 Hz on-line
- 1 Hz on-line
- 20 Hz off-line

Valero-Cabre et al. 2006
No injury after prolonged TMS

• Counter, 1995:
  – No deleterious effect on AEP after 1000 pulses at 1Hz in rabbits
• Nishikiori, 1996:
  – No cortical or brainstem lesions after ~1 month of daily TMS in rabbits
• Liebetanz et al., 2003:
  – No MRS or histologic changes after 5 days of 1 Hz rTMS
• Charlet de Sauvage et al., 2007
  – No DNA damage after 2000 TMS pulses
Most translational research is with rodents

- Well-described disease models
- Inexpensive
- Experiments may be translated to clinical care
- TMS effect can be examined at multiple levels: whole animal, brain slice, single cell, etc.

Kistsen et al., in progress
Disadvantages of rat model

– Compromised stimulus focality
– Slightly more difficult EEG
– Required restraint or anesthesia

Luft et al., 2001
Kamida et al., 1998

Luft et al., 2001
Stimulation protocols

**Single Pulse (sTMS)**
- Stimulation
- Response
- MEP
- Latency
- Time

**Paired Pulse (ppTMS)**
- Stimulation
- Response
- ISI
- Time

**Repetitive (rTMS)**
- Stimulation
- Response
- ITI
- Time

Off-Center Coil

Rotenberg et al., 2009
Lateralized brachioradialis MEP
Lateralized TMS in Rats

A. Integrated MEP Amplitude

B. Peak MEP Amplitude

- Integrated MEP Amplitude and Peak MEP Amplitude across different TMS intensities. The graphs show a significant increase in MEP amplitude with increasing TMS intensity, indicating a dose-response relationship. The data is analyzed for contralateral and ipsilateral MEPs, with statistical significance marked by asterisks (*) and double asterisks (**) for p-values below 0.05 and 0.01, respectively.
Stimulation protocols

(A) Single Pulse (sTMS)
Stimulation | Response
---|---
Time | MEP Latency

(B) Paired Pulse (ppTMS)
Stimulation | Response
---|---
Time | ISI

Repetitive (rTMS)
Stimulation | Response
---|---
Time | ITI

Measures of Cortical Excitability by Paired-Pulse TMS (ppTMS)

Conditioning TMS

Test TMS

Control

SICI; 2 ms ISI

ICF; 12 ms ISI

LICI; 200 ms ISI

Rotenberg and Pascual-Leone, 2010
Paired-Pulse Inhibition in rats

Vahabzadeh et al., 2011
Inhibition in rats preserved with anesthesia

MEP Inhibition as a Function of ISI and Anesthesia

% Unconditioned MEP V(peak-to-peak)

ISI (ms)

Vahabzadeh et al., 2011
Inhibition lost with GABA-A antagonist / seizures

Vahabzadeh et al., 2011
PTZ Effects on MEP Inhibition by ppTMS

A

preSaline – SP

postSaline – SP

B

prePTZ – SP

postPTZ – SP
Detection of cortical inhibition by MMG and ppTMS in unanesthetized rats

MMG (Mechanomyography)
EMG v.s MMG

Input–output curve of MMG

MMG

60%MO  70%MO  80%MO  90%MO  100%MO

50ms

EMG (Tibia anterior m.)

MMG

9.3 ms

EMG

7.4 ms

DO NOT COPY
MMG testing during TMS

Awake rat

TMS: Single pulse

Muscle twitch

Mechanomyogram (MMG)

Accelerometer
GABA_A-mediated cortical inhibition following pentobarbital (PB) and pentylenetetrazole (PTZ)

- reduced inhibition with PTZ and increased inhibition with PB
TMS in Experimental Epilepsy

• Diagnostic
  – Measure of cortical excitability
  – Assessment of drug (or other intervention) effect

• Therapeutic
  – Anticonvulsant (seizure termination)
  – Antiepileptic (seizure prevention)
Fluid Percussion Injury: a post-traumatic epilepsy model

Nature Protocols, 2011

McIntosh et al., 1989
Reduced cortical inhibition in TBI: a marker for epileptogenesis?

- Rats with TBI show less ppTMS-MMG inhibition relative to sham-TBI controls 6 weeks after injury, when post-traumatic epilepsy develops.
Gradual decrease in LICI reaches significance at 1 week after TBI as compared to pre-values. More detailed data compared between sham and TBI group in LICI at 100 ms (C) and 200 ms ISI (D) following TBI. (*p<0.05, **p<0.01)
General cortical architecture was not affected by TBI.

Sham control

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>2</th>
<th>4</th>
<th>6</th>
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<tbody>
<tr>
<td>NeuN</td>
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TBI (lesion)

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TBI (contra-lesion)

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<th>Time (weeks)</th>
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Layer V thickness

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<th>Time (weeks)</th>
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<tbody>
<tr>
<td>Thickness</td>
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</table>
Parvalbumin (PV) interneurons are the major sub-type of cortical inhibitory neuron... and vulnerable to oxidative stress

Gonchar et al., 2007, Front Neuroanat.
Gradual loss of parvalbumin (PV)-cells after TBI

Sham control  

Post-TBI (peri-lesion)  

Post-TBI (contra-lesion)  

<table>
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<tr>
<th>2</th>
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Peri-lesion

Cell count (% Sham)

2 weeks | 4 weeks | 6 weeks

Contra-lesion

Cell count (% Sham)

2 weeks | 4 weeks | 6 weeks

* n.s. n.s. **

PV
Delayed increase in oxidative stress after TBI

**Peri-lesion**

- Sham control: n.s.
- Post-TBI (peri-lesion):
  - 2 weeks: n.s.
  - 4 weeks: ***
  - 6 weeks: ***

**Contra-lesion**

- Sham control: n.s.
- Post-TBI (contra-lesion):
  - 2 weeks: n.s.
  - 4 weeks: n.s.
  - 6 weeks: **
Implications for Therapy

Antioxidant (N-acetylcysteine)

Oxidative stress

↓ Perineuronal nets

Impaired inhibition

↓ Otx2

Neuroprotection (Otx2)

TBI

PTE

Epileptic seizure

Loss of PV-cells

Lee et al., 2013
Ceftriaxone Treatment after Traumatic Brain Injury Restores Expression of the Glutamate Transporter, GLT-1, Reduces Regional Gliosis, and Reduces Post-Traumatic Seizures in the Rat

Grant S. Goodrich, Anatoli Y. Kabakov, Mustafa Q. Hameed, Sameer C. Dhamne, Paul A. Rosenberg, and Alexander Rotenberg.
Ceftriaxone treatment prophylaxes against posttraumatic seizures
ppTMS as a biomarker in TBI treatment

Hameed et al., 2014
Stimulation protocols

(A)

Stimulation protocols

(B)

Therapeutic TMS

• Three potential targets:
  – Seizure
  – Epilepsy
  – Epileptogenesis
Rat TMS-EEG methods

Rotenberg, et al., 2005
Ives et al., 2006
Spike Provocation by TMS in Rats

Rotenberg, Brain Topogr 2010
Rat “deep” TMS during seizure

EEG analysis (seizure detection)
Kainate (KA) Model Status Epilepticus

• Three-Stage Effect:
  – Acute 2-3 hour prolonged seizure
  – Subacute 6-9 week seizure-free period
  – Chronic daily recurrent seizures
Terminated KA seizure
Refractory KA Seizure
rTMS during KA seizure

Rotenberg et al., Clin Neurophys 2008
rTMS during KA seizures

Relative Average Seizure Duration (% untreated control)

0.25 Hz

0%
25%
50%
75%
100%
125%
150%

0.5 Hz

untreated active sham untreated active sham untreated active sham

0.75 Hz

untreated active sham

Rotenberg et al., 2008
Reduced c-Fos expression (and excitotoxicity?) with 0.5 Hz rTMS

Rotenberg et al., AES abstr 2005
Mixed results in controlled trials

– Theodore et al., Neurology 2002
  • N=24; 1 Hz X 900 BID X 1 week
  • Mild and short-lived seizure reduction

– Fregni et al., Annal Neurol 2006
  • N=21; 1 Hz X 1200 X 5 days
  • Significant seizure reduction and EEG improvement

– Cantello et al., Epilepsia 2007
  • N=43; 0.3 Hz X 1000 X 5 days
  • Significant EEG improvement; no change in seizures

– Sun et al., Epilepsia 2012
  • N=64; 0.5 Hz X 1500 X 14 days
  • Significant seizure reduction and EEG improvement
Better effect with 1 Hz

% reduction in Seizure Frequency After rTMS

Rotenberg et al., unpublished data
Frequency-response *in vitro* LTD approximates rTMS data

Nakano et al., 2004
Molecular Basis: Does rTMS induce LTP/LTD?

Kandel, 2001

[Diagram showing the molecular pathways involved in LTP/LTD, including Schaffer collateral, mossy fiber, and perforant pathways.]
rTMS mechanisms

A

Test stimulus (1 train or 4 trains)
Recording
Schaffer collateral pathway
Mossy fiber pathway
CA1
Dentate gyrus
Perforant pathway

B

EPSP slope (% of control)

Late LTP (4 trains)
Early LTP (1 train)

Time (min)

C

Protein phosphatase 1
Phosphatase inhibitor
Calcineurin

Modulatory input (dopamine)
Schaffer collateral
NMDA
Ca²⁺/calmodulin
Ca²⁺/calmodulin kinase
AMPA

CREB-2
CREB-1
CRE

Effectors for growth (IPA, BDNF)
Regulators (C/EBPβ)

MAPK
PKA

Kandel, 2001
MEP depression by rTMS in anesthetized rat

Muller et al., PLOS One 2014
rTMS mechanisms

[Diagram of brain regions and pathways]

Kandel, 2001
CREB phosphorylation by 20 Hz rTMS

pCREB (% control)

- 20 Hz rTMS
- Sham

20 Hz rTMS

Sham
rTMS mechanisms

Kandel, 2001
BDNF expression after rTMS

Gersner et al., J. Neursci 2011
GluR1 expression and phosphorylation after rTMS

Gersner et al., J. Neursci 2011
Functional Dopaminergic Neurons in Substantia Nigra are Required for Transcranial Magnetic Stimulation-Induced Motor Plasticity

Tsung-Hsun Hsieh\textsuperscript{1,2,4,\dagger}, Ying-Zu Huang\textsuperscript{6,\dagger}, Alexander Rotenberg\textsuperscript{7}, Alvaro Pascual-Leone\textsuperscript{8}, Yung-Hsiao Chiang\textsuperscript{1,2,9}, Jia-Yi Wang\textsuperscript{3} and Jia-Jin J. Chen\textsuperscript{4,5}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure.png}
\caption{Graph showing normalized amplitude of MEP over time for different groups.}
\end{figure}
Follow-up
Work in Progress:

- Baseline
- Time (min)
- Anesthesia
- Kainic acid injection

-10 -70 -85

-10 0 10 20 30

Lorazepam Sham/rTMS Follow-up
/rLorazepam

Gap in knowledge: how to combine neurostimulation with AEDS?
Spike suppression by 20 Hz rTMS

A  
Sham  
Baseline  
Treatment  
Follow-up  

30 sec

B

Normalized spike frequency (auto-count)

Baseline  
Treatment  
Follow-up  

***  
**  

C

Freq [Hz]

Time (sec)
The effect of Lorazepam and rTMS combination treatment on spike frequency

<table>
<thead>
<tr>
<th>Condition</th>
<th>Baseline</th>
<th>2nd Treatment</th>
<th>Follow-up</th>
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<tbody>
<tr>
<td>LZP + Sham</td>
<td><img src="image1.png" alt="Graph" /></td>
<td><img src="image2.png" alt="Graph" /></td>
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<tr>
<td>LZP</td>
<td><img src="image4.png" alt="Graph" /></td>
<td><img src="image5.png" alt="Graph" /></td>
<td><img src="image6.png" alt="Graph" /></td>
</tr>
<tr>
<td>LZP + LZP</td>
<td><img src="image7.png" alt="Graph" /></td>
<td><img src="image8.png" alt="Graph" /></td>
<td><img src="image9.png" alt="Graph" /></td>
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<tr>
<td>LZP + rTMS</td>
<td><img src="image10.png" alt="Graph" /></td>
<td><img src="image11.png" alt="Graph" /></td>
<td><img src="image12.png" alt="Graph" /></td>
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</tbody>
</table>

Normalized spike frequency (auto-count)

** ***

30 sec
Can we model TMs in rodents without magnetic coils?

Hsieh et al., work in progress
LTP-like potentiation after electrical iTBS

Hsieh et al., work in progress
Repetitive Magnetic Stimulation Induces Functional and Structural Plasticity of Excitatory Postsynapses in Mouse Organotypic Hippocampal Slice Cultures

Andreas Vlachos,1* Florian Müller-Dahlhaus,1,2* Johannes Rosskopp,1,2 Maximilian Lenz,1 Ulf Ziemann,2,3* and Thomas Deller1*