TMS in animal models: Methods and Applications

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Director, Neuromodulation Program
Boston Children’s Hospital
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

Charlet de Sauvage et al. 2007
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
Induced dysfunction: neglect following rTMS 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
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
Stimulation protocols

Off-Center Coil

Rotenberg et al., 2009
Lateralized brachioradialis MEP
TMS in a Deployable Automated Anesthesia Unit (DAAU)

Roteberg, Goldie, Leroy (Vivonics Inc., and Boston Children’s Hospital)
Proposed use: a closed loop autonomous analgesia system

Operator interface → Controller → Infusion pump → Patient

Alarm → Infusion command → Drug reservoirs

ppTMS/evoked response → Vital signs → Automated Mental Status Exam → Other feedback? (PCA, drug sensor, ...)
MEP response to propofol bolus

Gersner et al., in progress
MEP response to propofol rate change

MEP amplitude change (Log % baseline)

Log-transformed

Control

2to1
Stimulation protocols

GABAergic cortical inhibition measures by paired-pulse TMS (ppTMS)
A new measure of cortical inhibition by mechanomyography and paired-pulse transcranial magnetic stimulation in unanesthetized rats


MMG (Mechanomyography)
EMG vs MMG

Input–output curve of MMG

-0.15 -0.10 -0.05 0.00 0.05 0.10 0.15

MMG (V)

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

50ms

EMG (Tibia anterior m.)

MMG

9.3 ms

EMG

7.4 ms
GABA<sub>A</sub>-mediated cortical inhibition following pentobarbital (PB) and pentylenetetrazole (PTZ)

Reduced inhibition with PTZ and increased inhibition with PB
TBI: The most common cause of acquired epilepsy in young adults

Causes of Epilepsy:

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Annegers JF. Lippincott Williams & Wilkins, 2001:165-72.
Trajectory of Parvalbumin Cell Impairment and Loss of Cortical Inhibition in Traumatic Brain Injury

Tsung-Hsun Hsieh¹,²,³,†, Henry Hing Cheong Lee⁴,†, Mustafa Qadir Hameed¹,⁴,⁵, Alvaro Pascual-Leone⁶, Takao K. Hensch⁴,⁷ and Alexander Rotenberg¹,⁴,⁶

A

2 4 6

Sham control

2 4 6

Post-TBI (perilesion)

2 4 6

Post-TBI (contralesion)

B

%PV⁺ cells enwrapped by PNN

Perilesion : contralesion ratio

Perilesion

Sham level

Contralesion

n.s. n.s.

*
Fluid Percussion Injury: a post-traumatic epilepsy model

Nature Protocols, 2011

McIntosh et al., 1989
Loss of cortical paired-pulse inhibition after TBI

Min GABA inhibition

Max GABA inhibition

Ratio

200ms ISI

Sham control
TBI

Pre 1WK 2WKS 3WKS 4WKS 5WKS 6WKS

Time

PLEASE DO NOT COPY
General cortical architecture is not affected by TBI

Hsieh et al., Cerebral Cortex 2016
Parvalbumin (PV) interneurons are the major sub-type of cortical inhibitory neuron... and vulnerable to oxidative stress.
Progressive PV loss after TBI

Sham control  Post-TBI (peri-lesion)  Post-TBI (contra-lesion)
2  4  6  2  4  6  2  4  6

*  ***  n.s.  n.s.

Peri-lesion  Contra-lesion

Progressive PV loss after TBI

Cell count (% Sham)

0  20  40  60  80  100
2 weeks  4 weeks  6 weeks

Cell count (% Sham)

0  50  100  150
2 weeks  4 weeks  6 weeks

Please do not copy.
Delayed increase in oxidative stress after TBI
(8-oxo-DG)

Sham control

Post-TBI (peri-lesion)

Post-TBI (contra-lesion)

Peri-lesion

Contra-lesion

Signal intensity (% Sham)

2 weeks 4 weeks 6 weeks

2 weeks 4 weeks 6 weeks

n.s. *** n.s.

*** n.s. **

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PLEASE DO NOT COPY
Disruption of perineuronal nets (PNN) after TBI
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. Dharmas, Paul A. Rosenberg, and Alexander Rotenberg
Ceftriaxone treatment prophylaxes against posttraumatic seizures

A

(i)

(ii)

B

Seizures per 24 hrs

Sal-TBI  Cef-TBI

C

Seizures per 24 hours

Seizure duration (s)
ppTMS as a **biomarker** in TBI treatment

Hameed et al., in progress
Ipsilesional Parvalbumin Expression after TBI

2 weeks 4 weeks 6 weeks

* p<0.05
Implications for Therapy

- Antioxidant (N-acetylcysteine)
- Oxidative stress
- Perineuronal nets ↓
- Impaired inhibition
- Loss of PV-cells
- Neuroprotection (Otx2)
- Impaired inhibition ↓ Otx2

TBI → PTE → Epileptic seizure

Lee et al., 2013
Stimulation protocols

Seizure suppression by EEG-guided repetitive transcranial magnetic stimulation in the rat

Alexander Rotenberg a,b,*, Paul Muller a, Daniel Birnbaum a, Michael Harrington a, James J. Riviello c, Alvaro Pascual-Leone b, Frances E. Jensen a
Rat “deep” TMS during seizure

EEG analysis
(seizure detection)
rTMS during KA seizure

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

Relative Average Seizure Duration (% untreated control)

Rotenberg et al., 2008
Combination therapy: lorazepam + rTMS in seizure suppression

Gersner et al., 2016.
Better seizure suppression in humans with 1 Hz

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
Can 1 Hz TMS suppresses motor excitability rats anesthetized with *pentobarbital*?

**Reduced cortical excitability**

Muller et al., PLOS One 2014
Short Communication

Transcranial magnetic stimulation for refractory focal status epilepticus in the intensive care unit

Anli Liu\textsuperscript{a,b}, Trudy Pang\textsuperscript{c,e}, Susan Herman\textsuperscript{c,e}, Alvaro Pascual-Leone\textsuperscript{c,e}, Alexander Rotenberg\textsuperscript{d,e,*}

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A. Liu et al. / Seizure xxx (2013) xxx–xxx

\begin{figure}
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\includegraphics[width=\textwidth]{Figure.png}
\caption{Graph showing the impact of pentobarbital and transcranial magnetic stimulation (TMS) on seizure frequency and background frequency.}
\end{figure}

\textbf{Number of Seizures}

\textbf{Background Frequency (Hz)}
rTMS mechanisms

![Diagram of rTMS mechanisms with labels for various pathways and processes.]

Kandel, 2001
CREB phosphorylation by 20 Hz rTMS

![Graph showing CREB phosphorylation](graph.png)
rTMS mechanisms

Kandel, 2001
BDNF expression after rTMS

Gersner et al., J. Neurosci 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, Ying-Zu Huang, Alexander Rotenberg, Alvaro Pascual-Leone, Yung-Hsiao Chiang, Jia-Yi Wang and Jia-Jin J. Chen
Can we model TMIs in rodents without magnetic coils?

Epidural electric stimulation of motor cortex

Target motor cortex: unilateral forelimb (Brachioradialis muscle)
- anterior–posterior: 1.25 mm from the bregma;
- lateral: 3.3 mm with respect to the midline

Reference
Active (brachioradialis muscle)

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

Hsieh et al., work in progress
Development/Plasticity/Repair

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*
MEA recording and TRANSCRANIAL FISH STIMULATION

Meyer et al., SFN 2014
Single evoked potential
Stimulation: 50 µA (half max),
at channel 16
Location of channels with EPs relative to stimulation channel

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Jingpu Zhao

Support:
NIH NINDS; NIH NIMH
Department of Defense
CIMIT
Epilepsy Research Foundation
Citizens United for Research in Epilepsy
Boston Children's Hospital Translational Research Program
Children's Hospital Department of Neurology
Al Rashed family; Siegel family; Fisher Family

Thanks!

Colleagues and mentors
Narong Auvichayapat - Khon Kaen U., Thailand
Paradee Auvichayapat - Khon Kaen U., Thailand
Marom Bikson - CCNY
Blaize Bourgeois - BCH
Dana Ekstein – Hadassah, Israel
Felipe Fregni – Spaulding Rehab / MGH
Joseph Gonzalez-Heydrich - BCH
Takao Hensch - BCH
Frances Jensen – U Penn
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Tobias Loddenkemper – BCH
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Alvaro Pascual-Leone – BIDMC
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