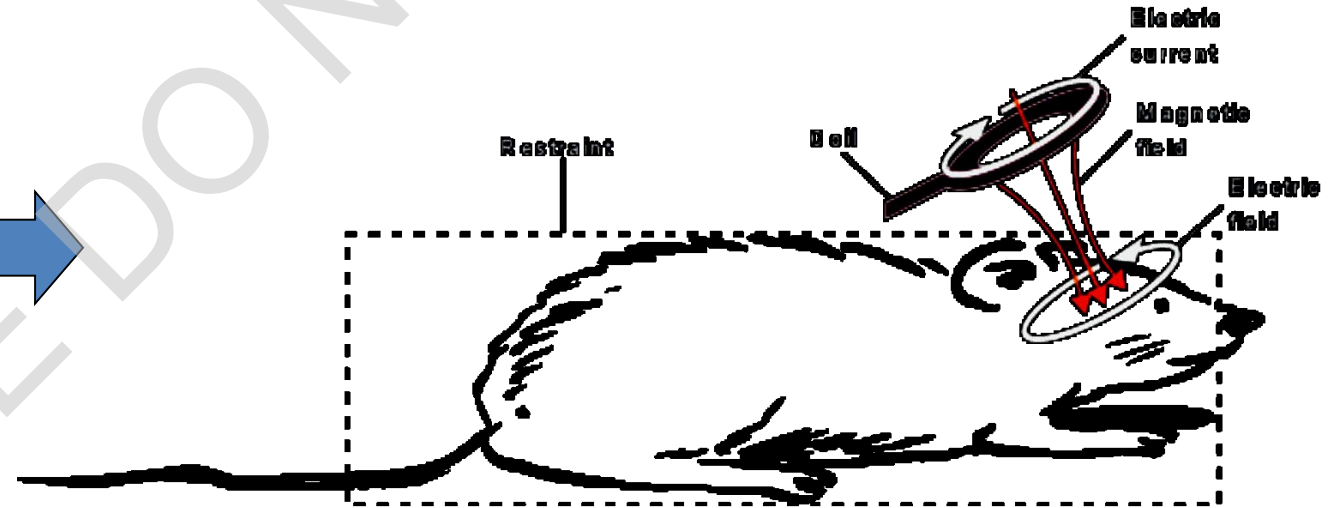
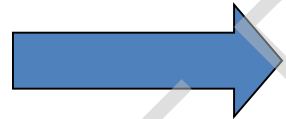
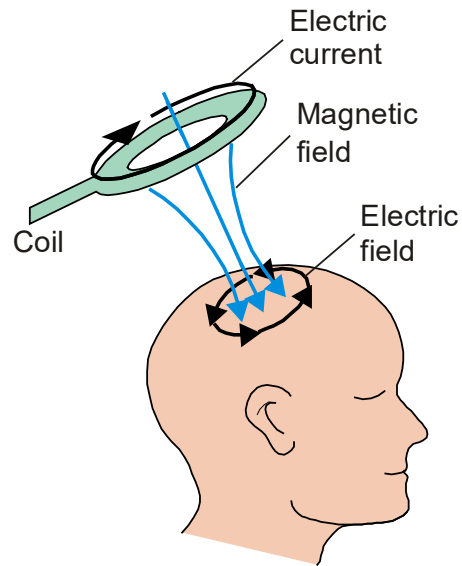
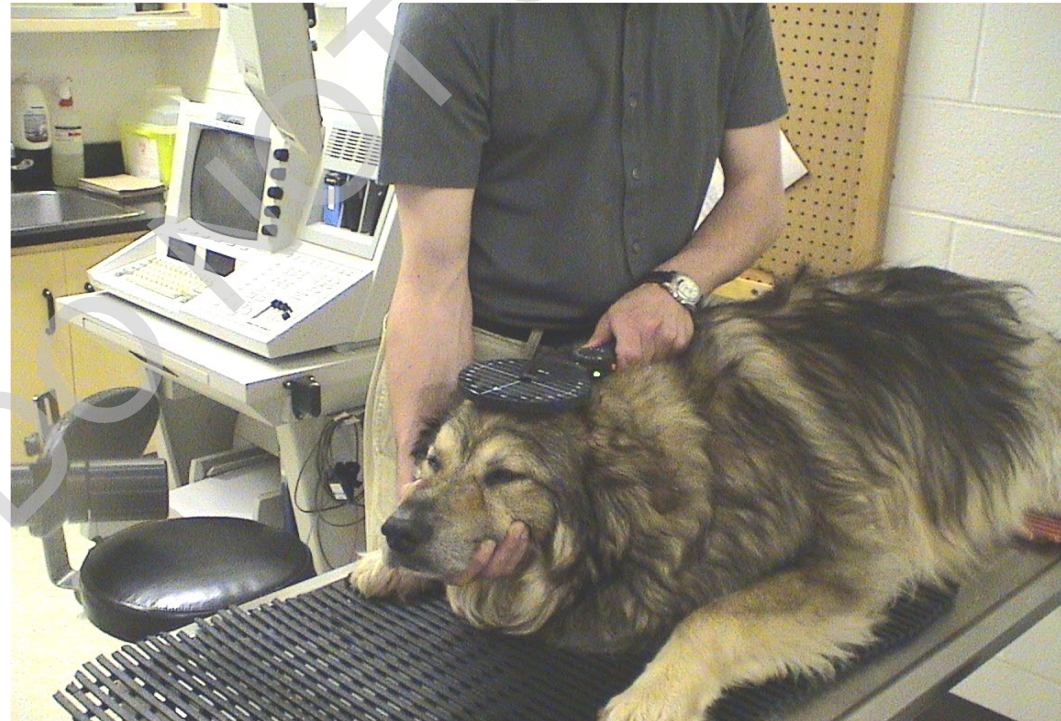


TMS in animal models: Methods and Applications



Why TMS studies in animals?

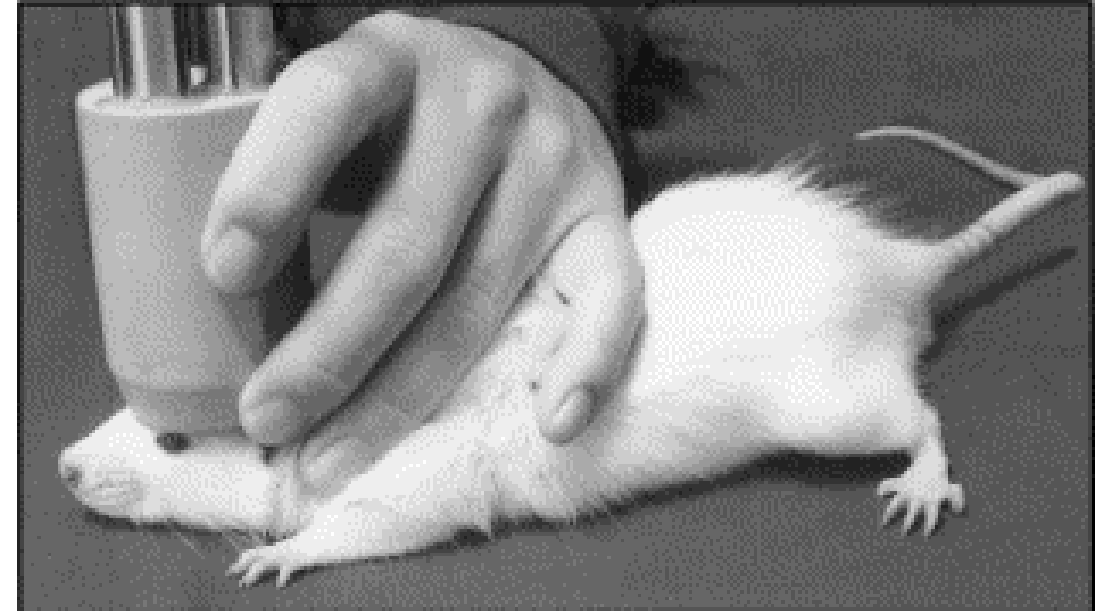
- Basic Science
- Translational Research



Poma et al., 2006

Advantages of animal subjects

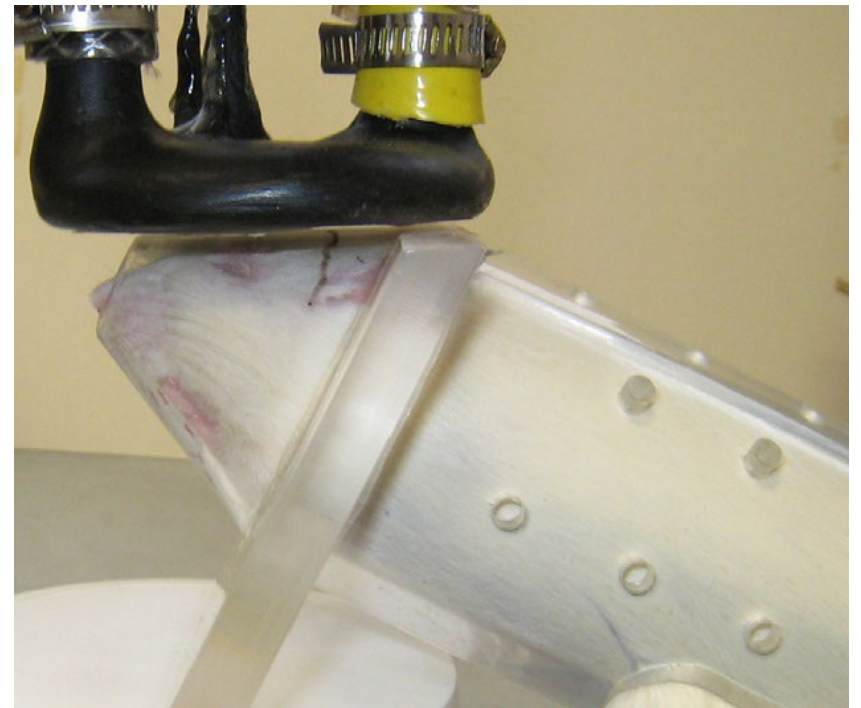
- Homogeneity
- Available histology
- Genetic / disease models



Liebetanz et al., 2003

Translational Relevance

- Disease modeling
- TMS safety
- Neuronal connectivity
- Synaptic plasticity
- Cortical organization

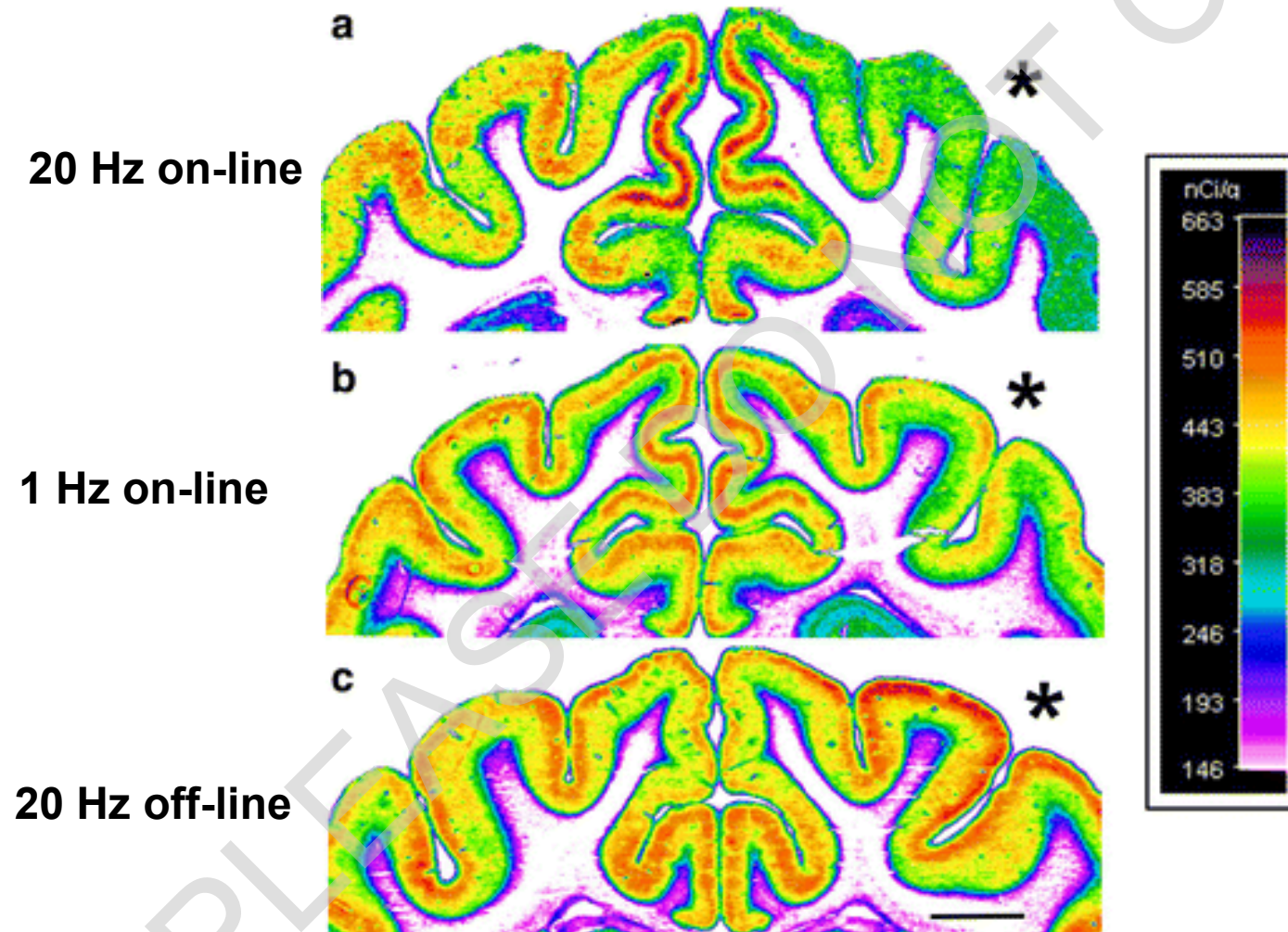


Charlet de Sauvage et al. 2007

Preclinical data indicate no injury after prolonged rTMS

- 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

Animal models enable insights into mechanisms (e.g. frequency-dependent ^{14}C -2DG uptake modulated in cat)



Most translational research is with rodents

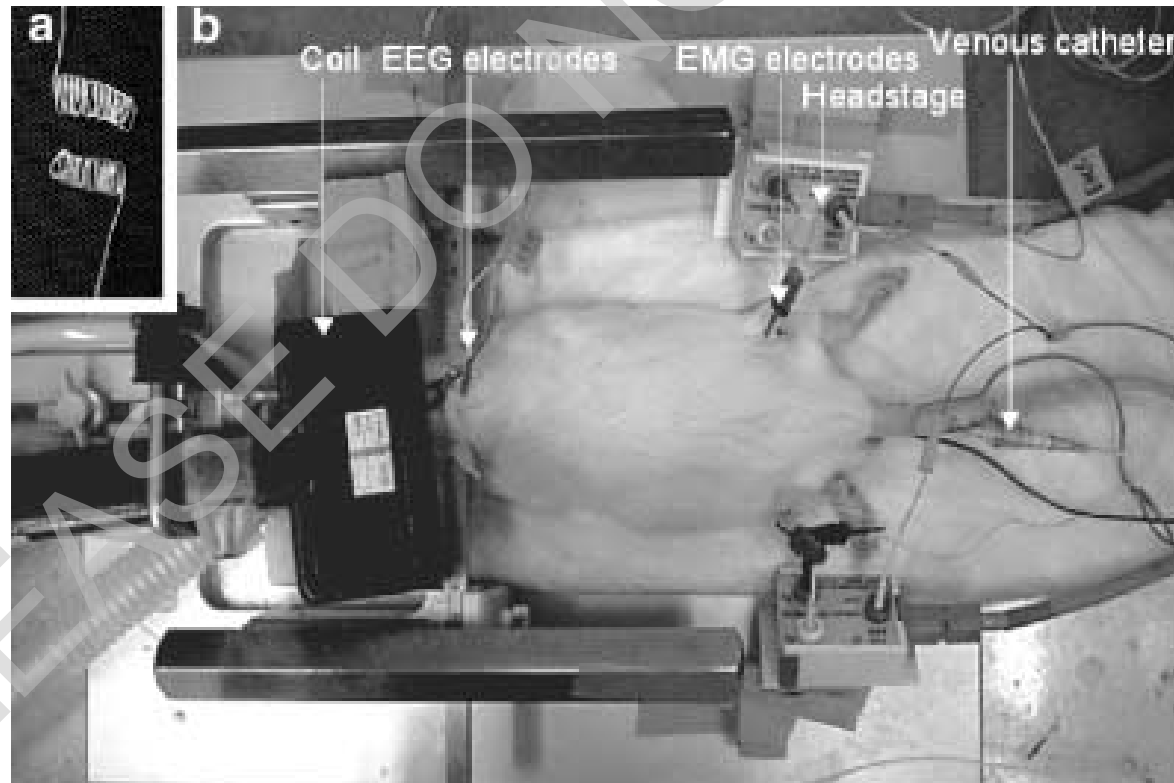
- Well-described disease models
- Inexpensive
- Experiments may be translated to clinical care
- TMS effect can be examined at multiple levels: whole animal, brain slice, single cell, etc.



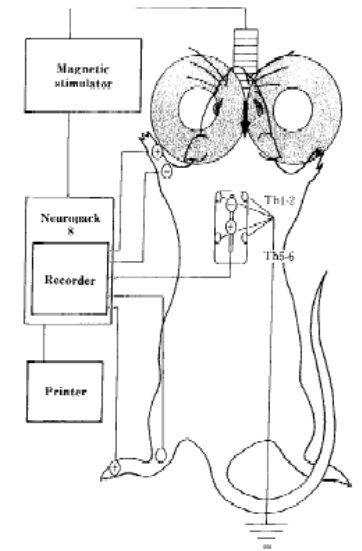
Kistsen et al., personal communication

Disadvantages of rat model

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

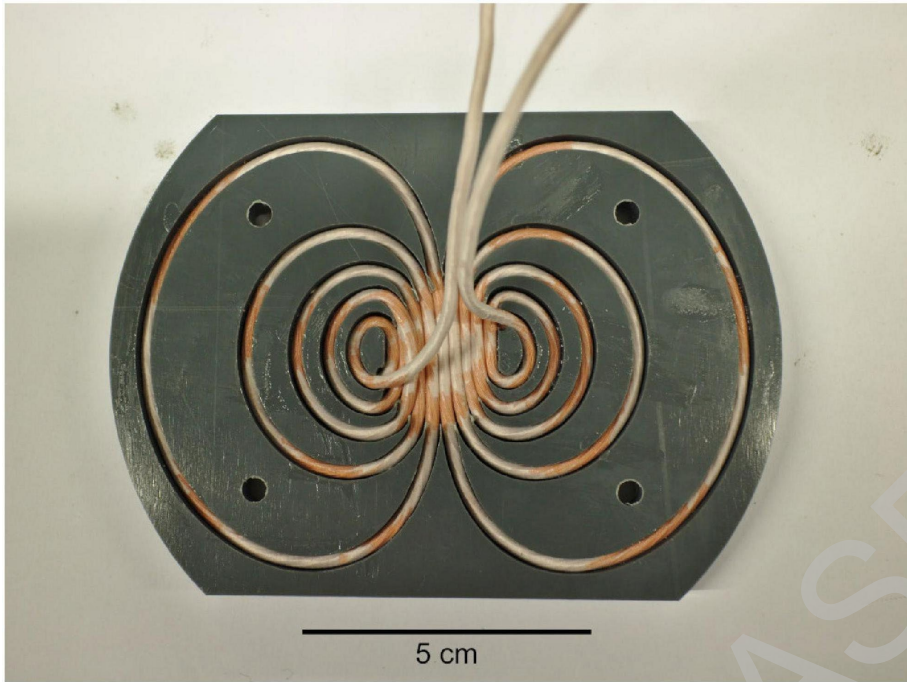


Luft et al., 2001

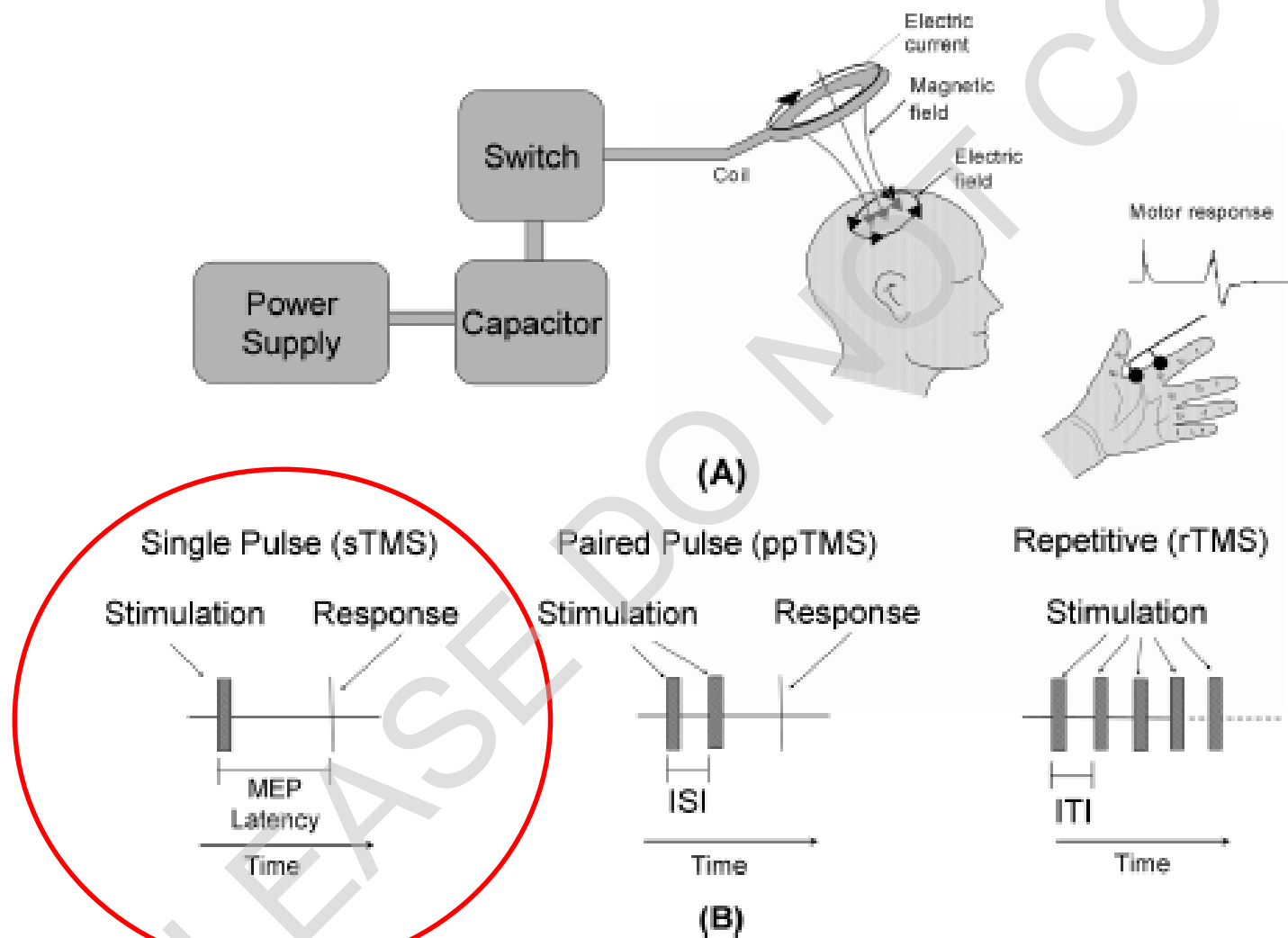


Kamida et al., 1998

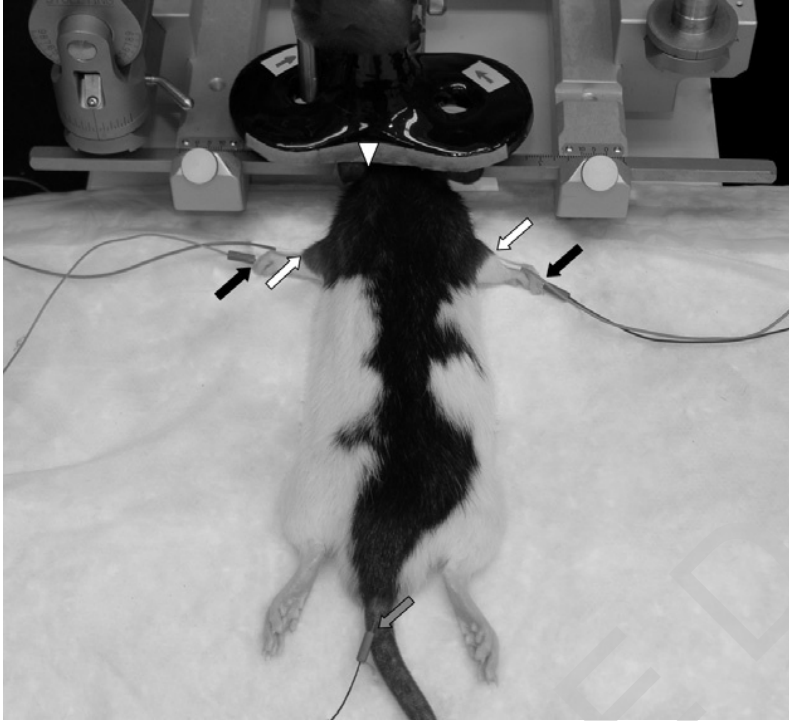
...but, some of these problems are surmountable
with adapted equipment



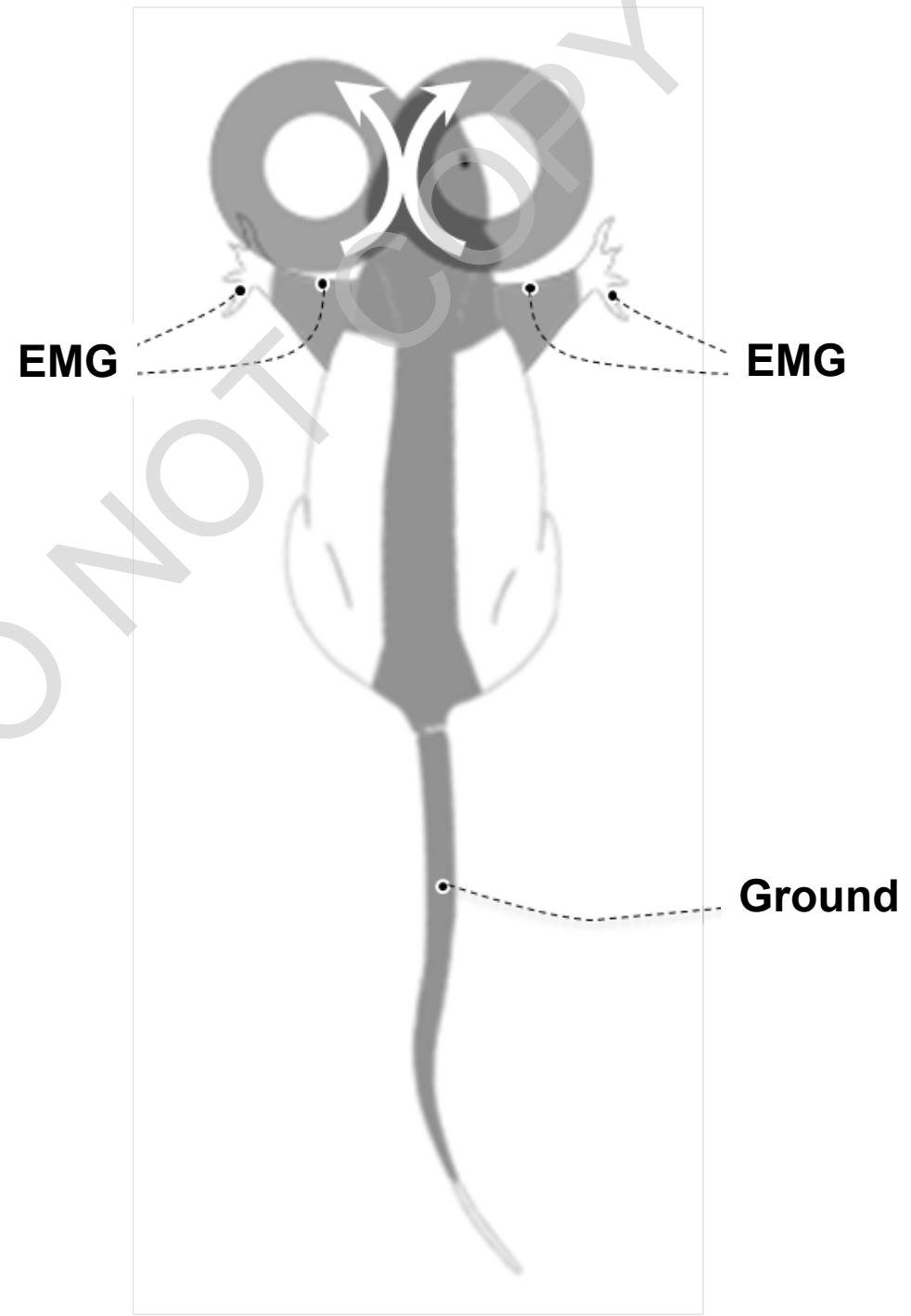
Stimulation protocols



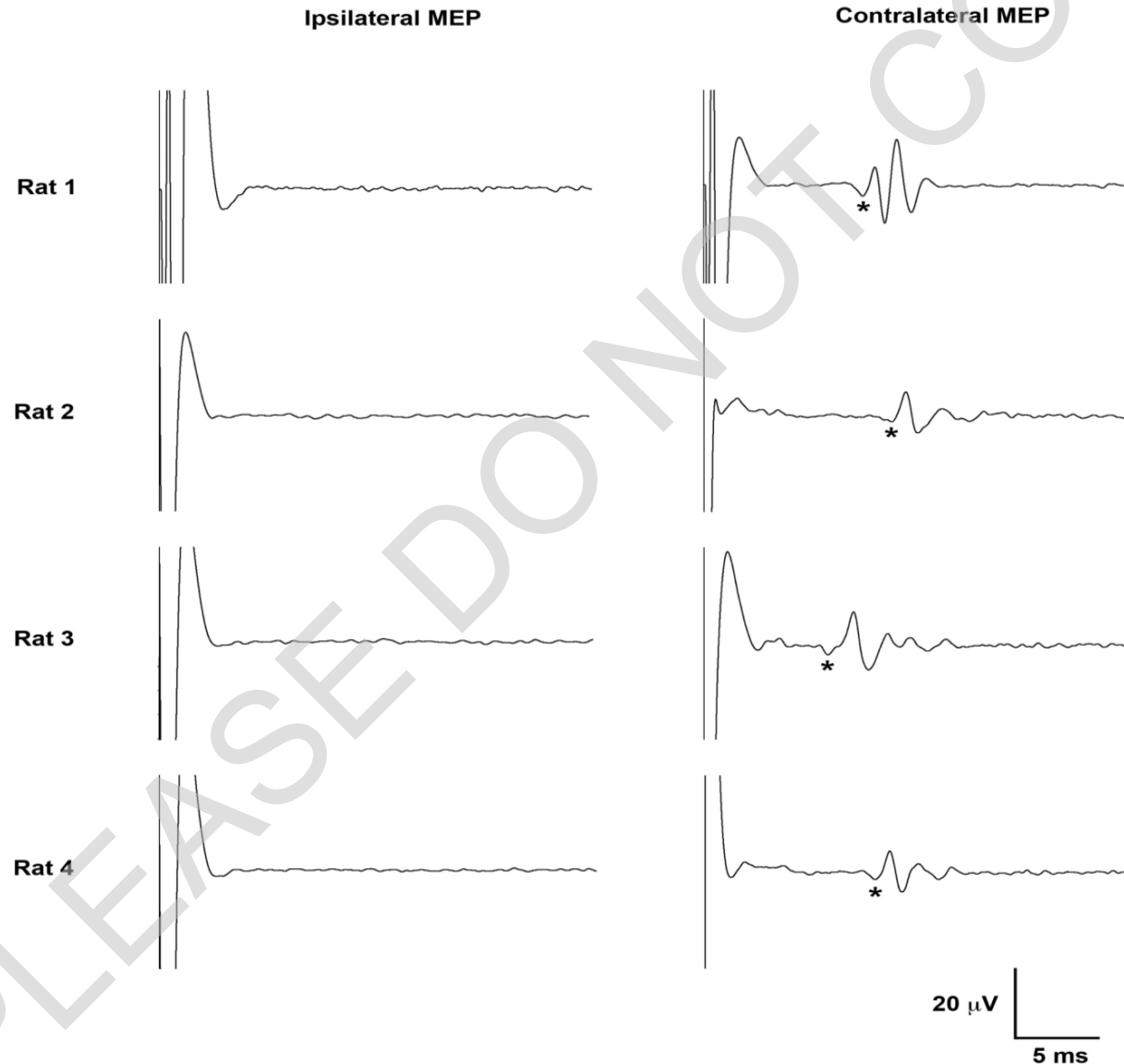
Off-Center Coil



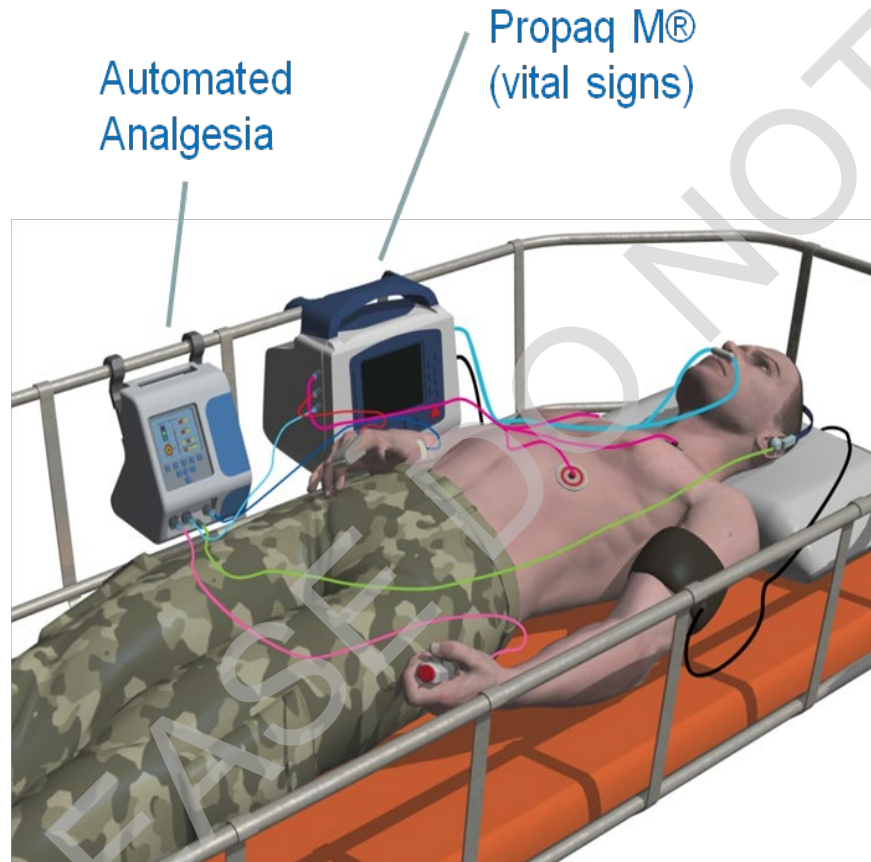
Rotenberg et al., 2009



Lateralized brachioradialis MEP in rat

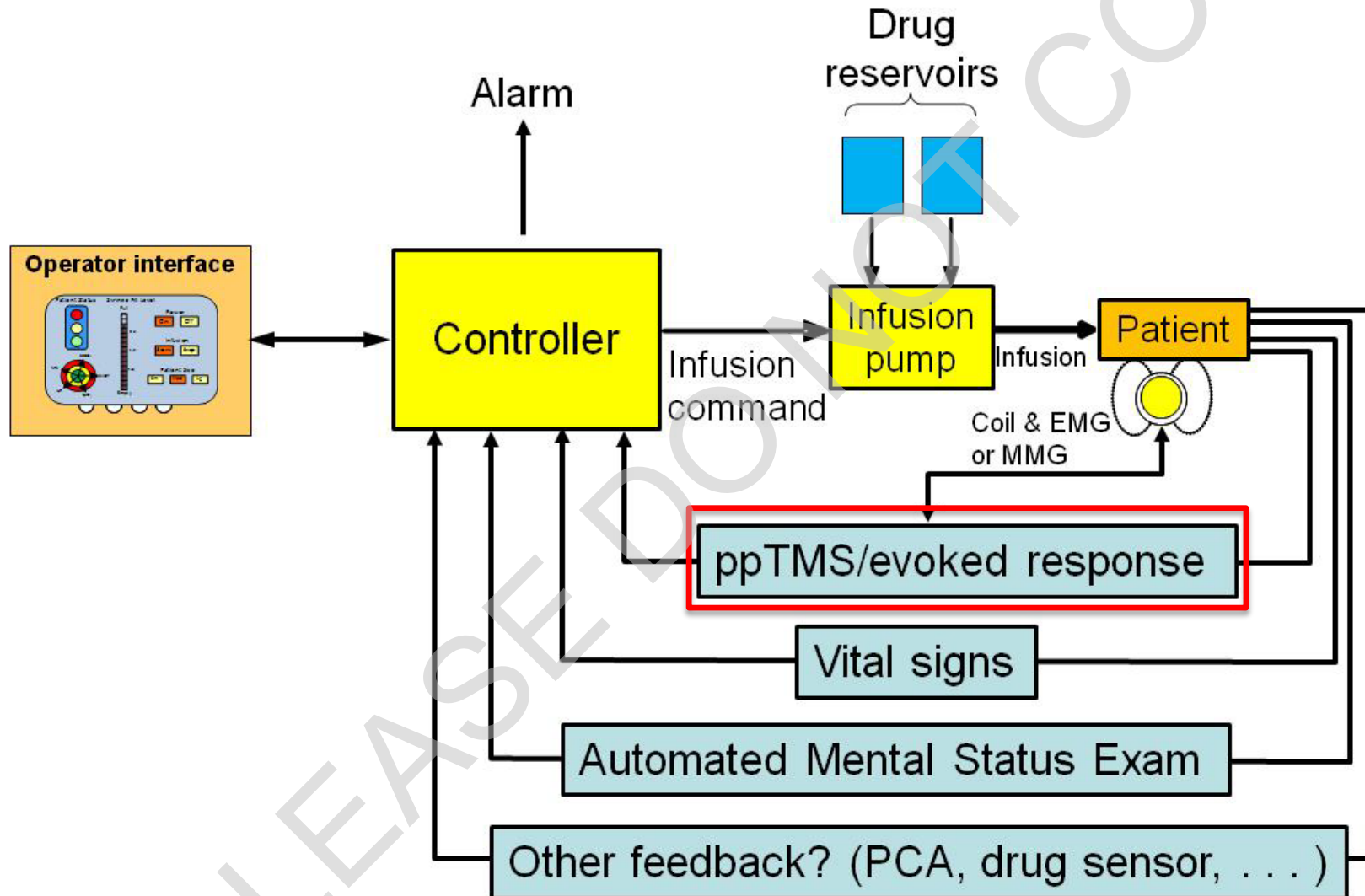


Use case: model TMS in a Deployable Automated Anesthesia Unit (DAAU)



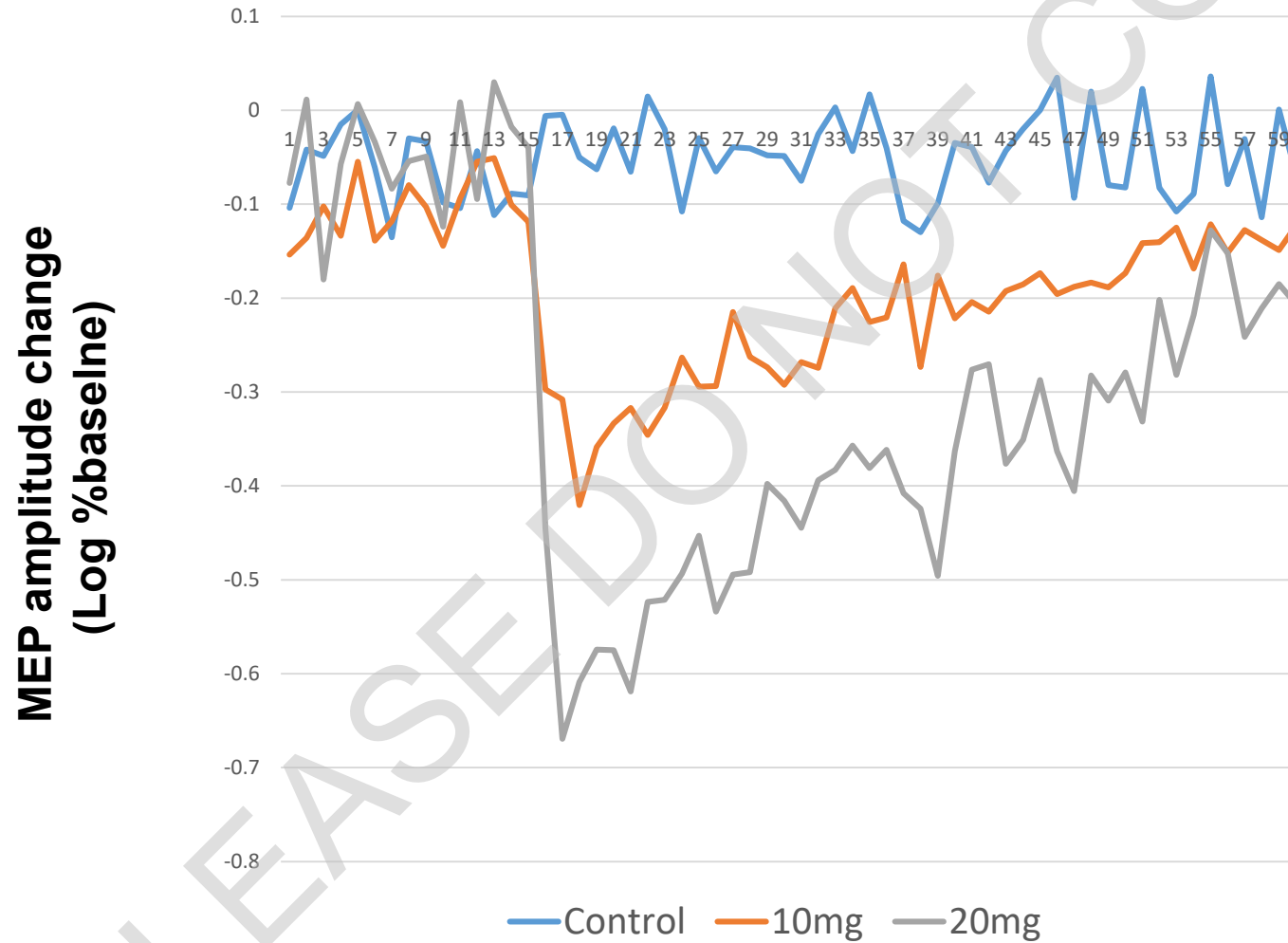
Rotenberg, Goldie, Leroy (Vivonics Inc., and Boston Children's Hospital)

Proposed use: a closed loop autonomous analgesia system

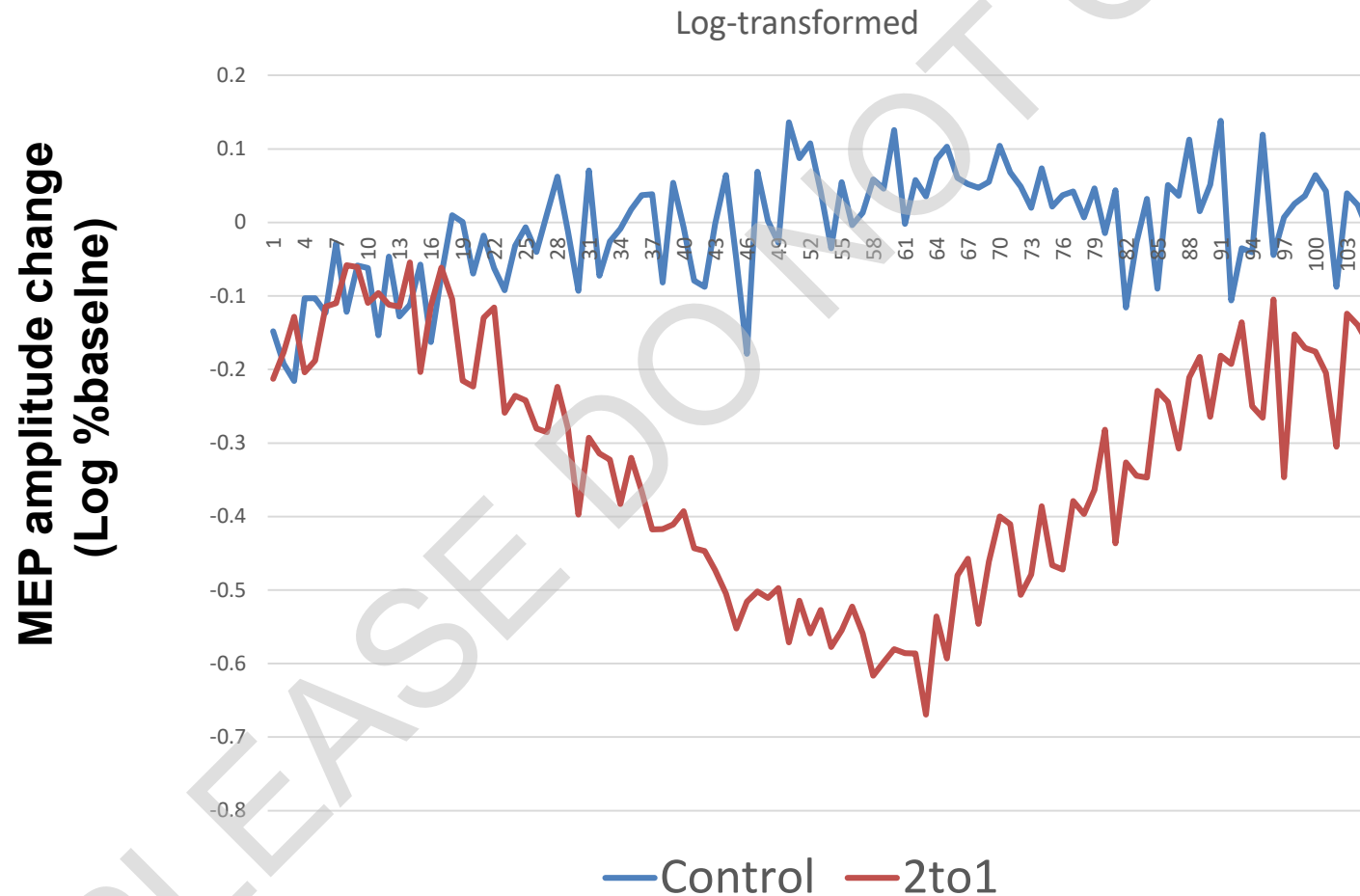


Rotenberg, Goldie, Leroy (Vivonics Inc., and Boston Children's Hospital)

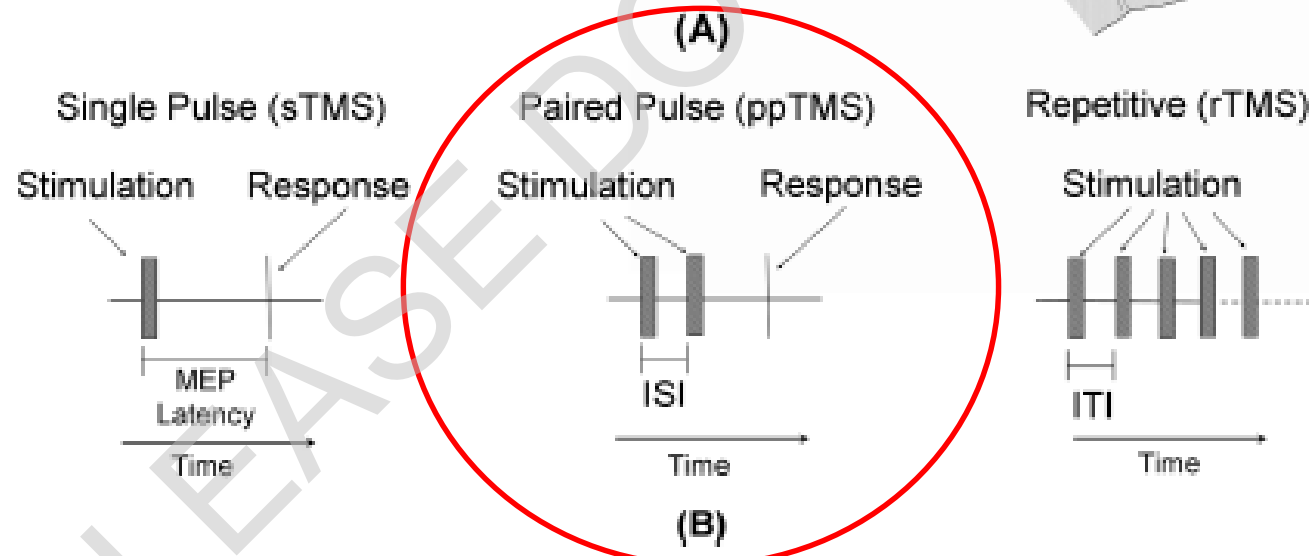
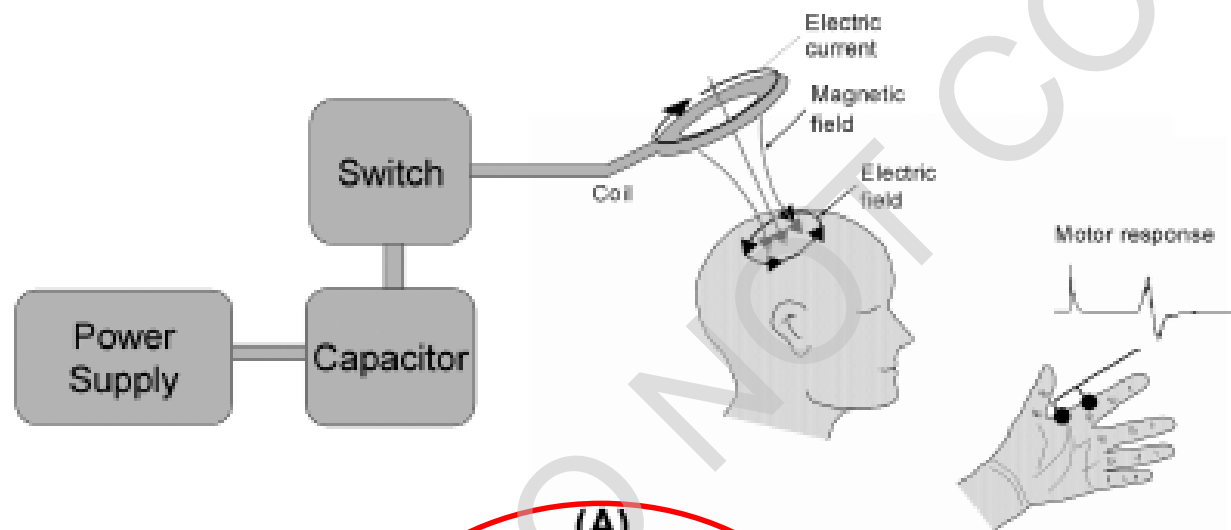
MEP response to propofol bolus



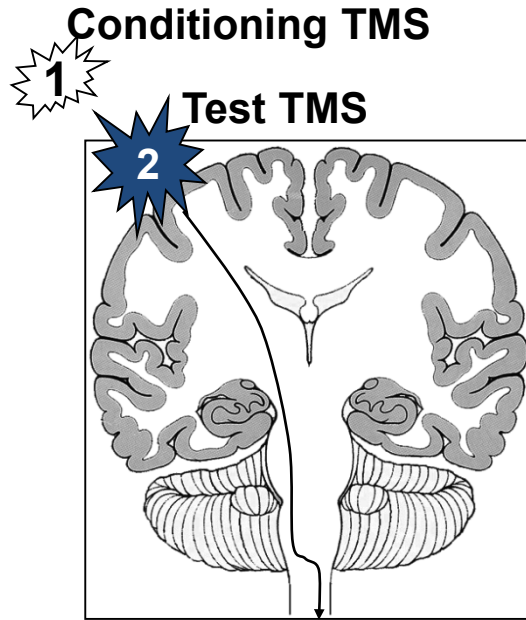
MEP response to propofol rate change



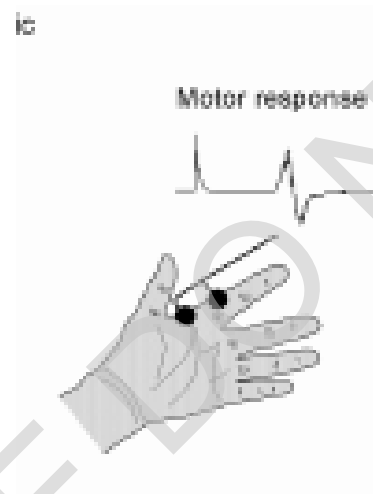
Stimulation protocols



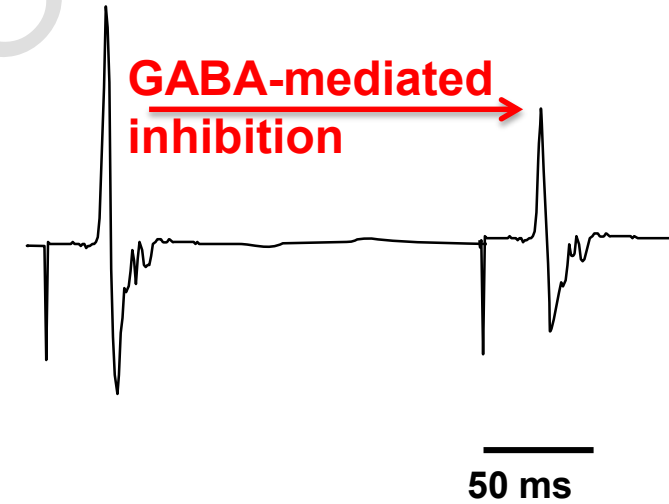
GABAergic cortical inhibition measures by paired-pulse TMS (ppTMS)



Rotenberg and Pascual-Leone, 2010



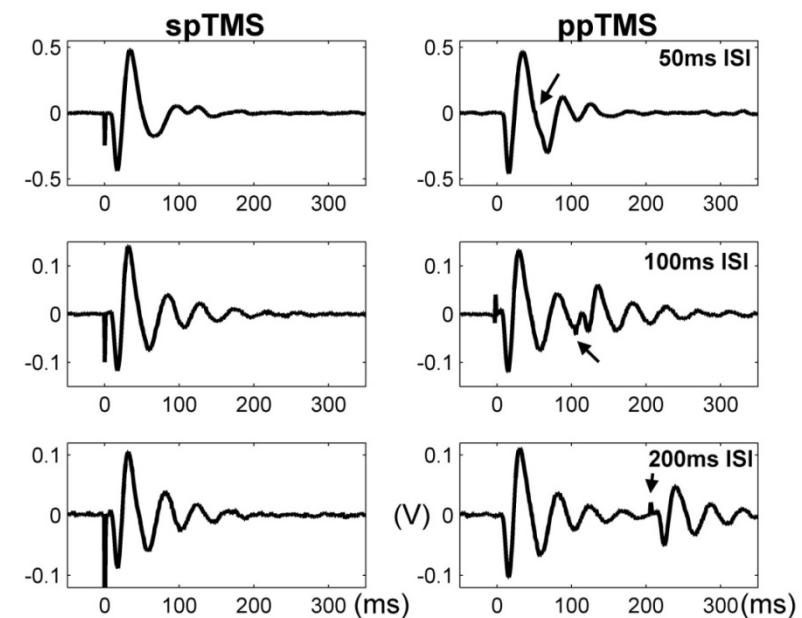
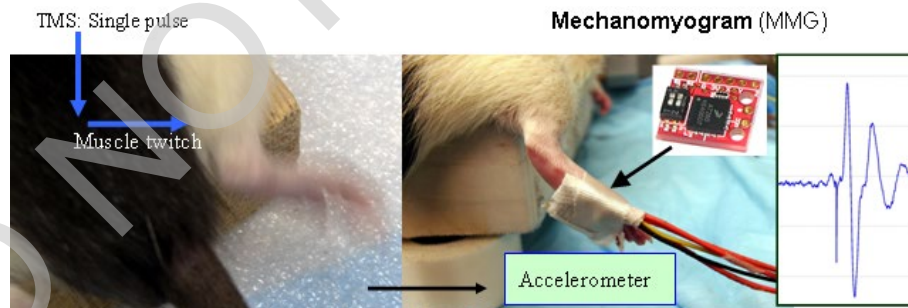
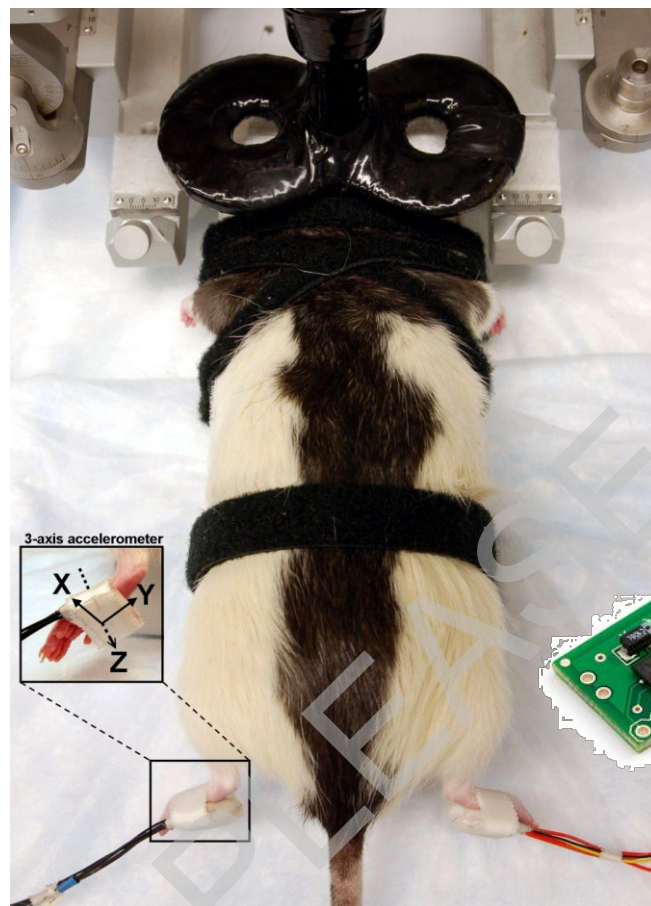
Paired-pulse MEP inhibition



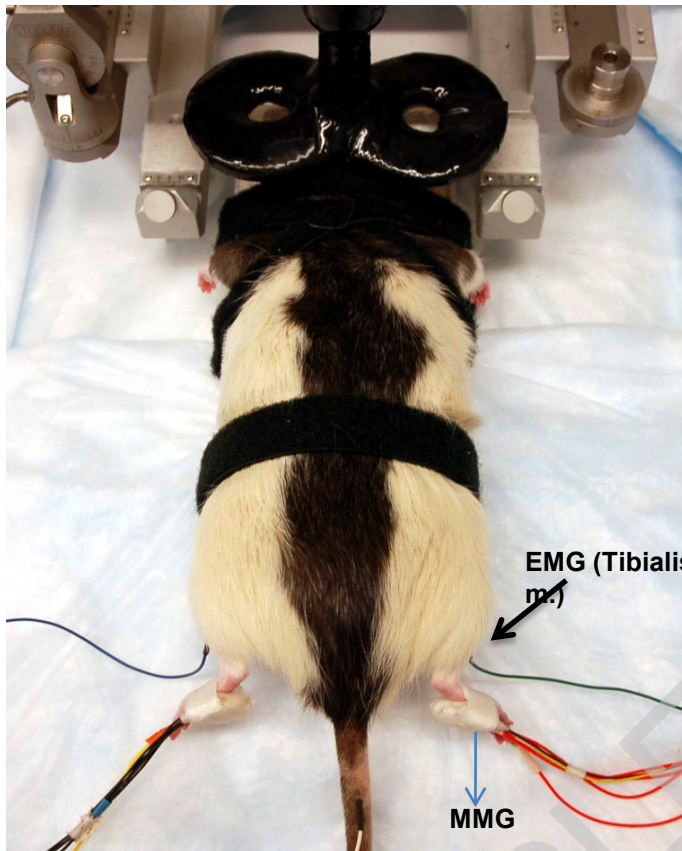
A new measure of cortical inhibition by mechanomyography and paired-pulse transcranial magnetic stimulation in unanesthetized rats

Tsung-Hsun Hsieh,^{1,2} Sameer C. Dhamne,¹ Jia-Jin J. Chen,² Alvaro Pascual-Leone,^{3,4}
Frances E. Jensen,^{1,5} and Alexander Rotenberg^{1,3}

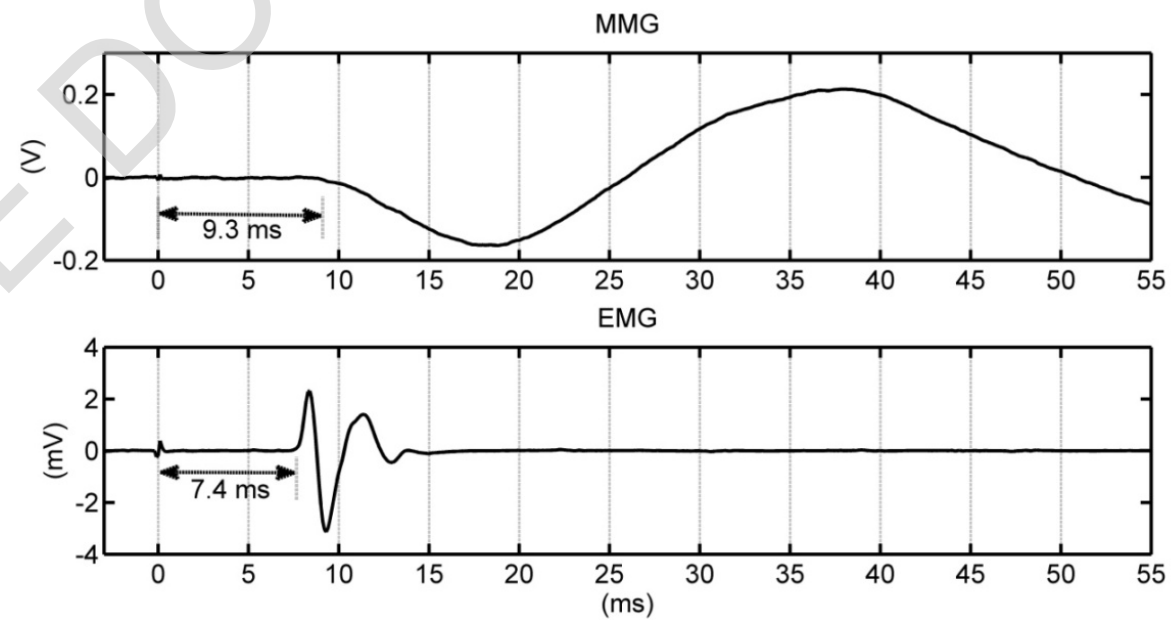
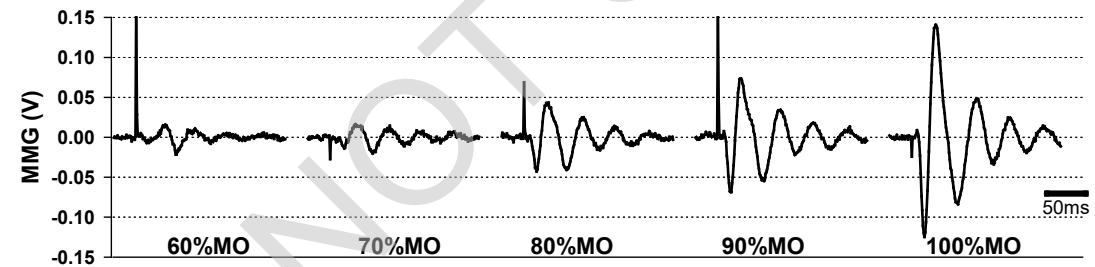
MMG (Mechanomyography)



EMG vs MMG

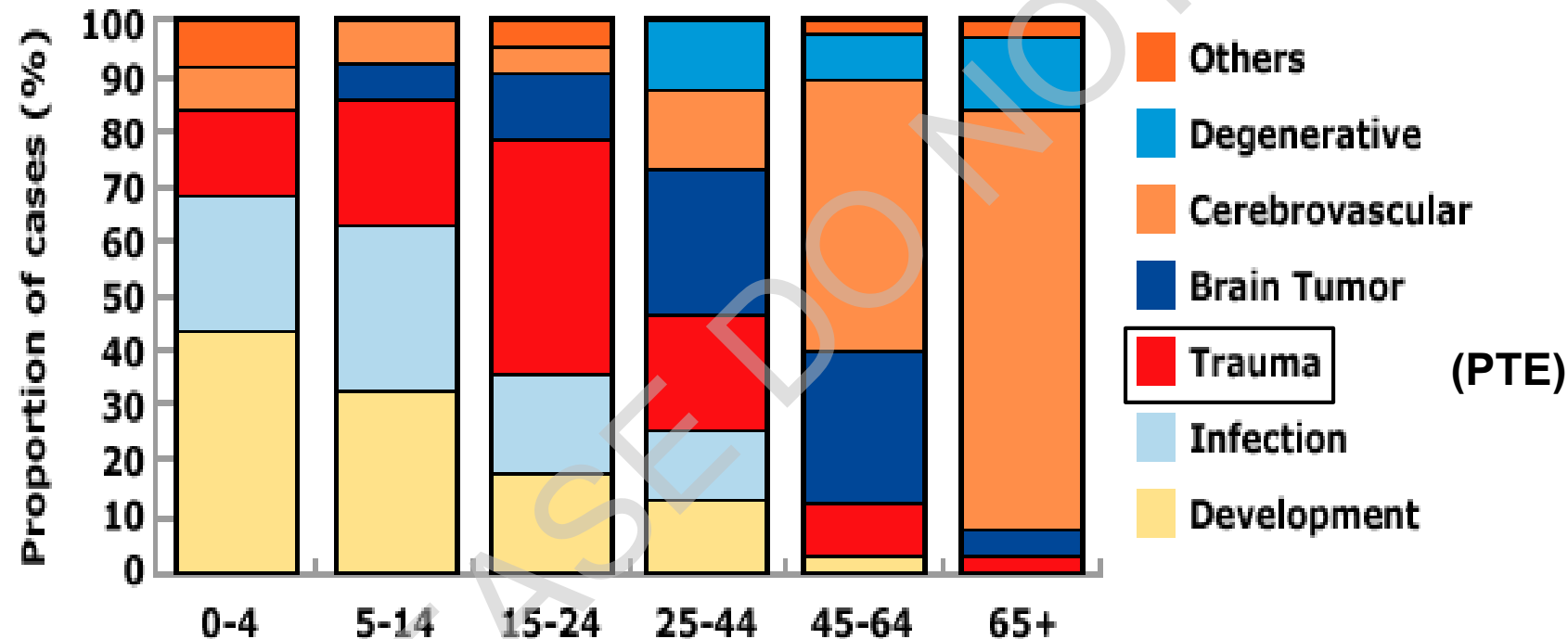


Input-output curve of MMG



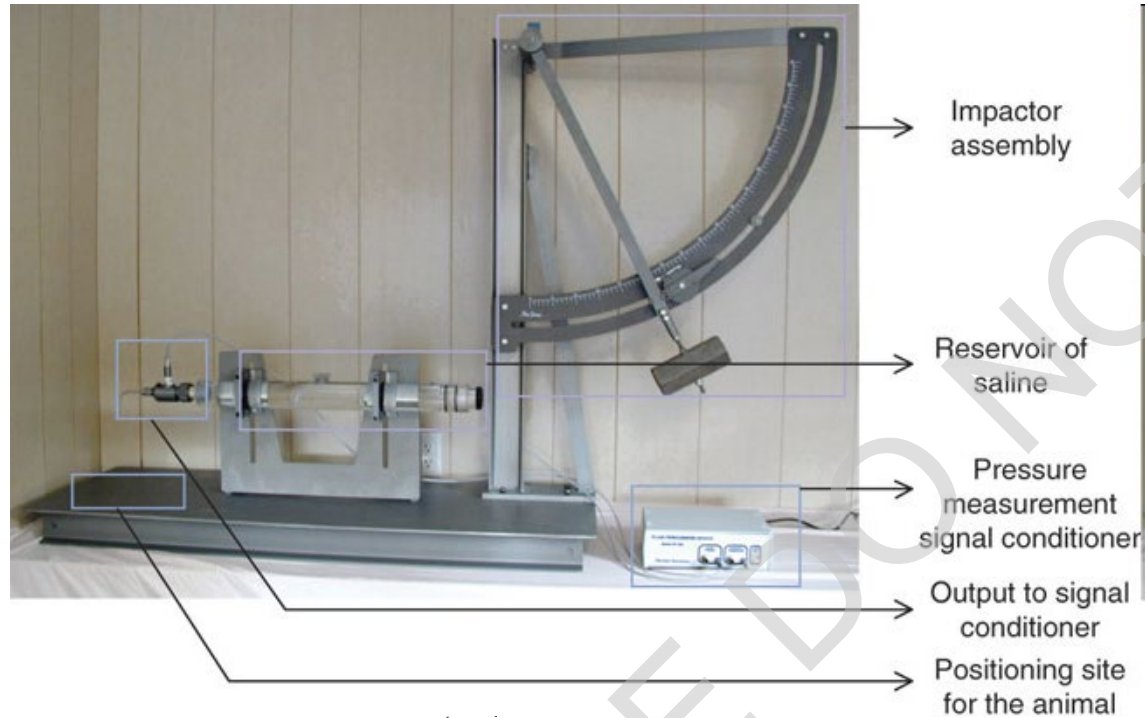
TBI: The most common cause of acquired epilepsy in young adults

- Causes of Epilepsy:

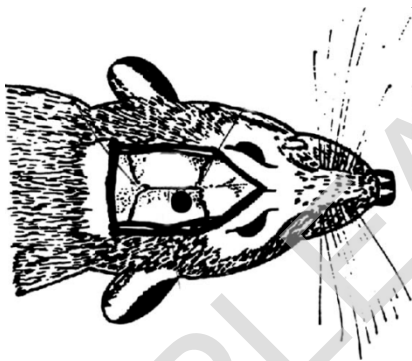


Annegers JF. Lippincott Williams & Wilkins, 2001:165-72.

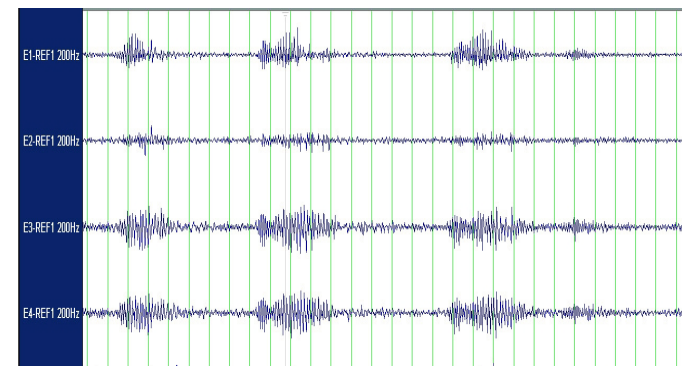
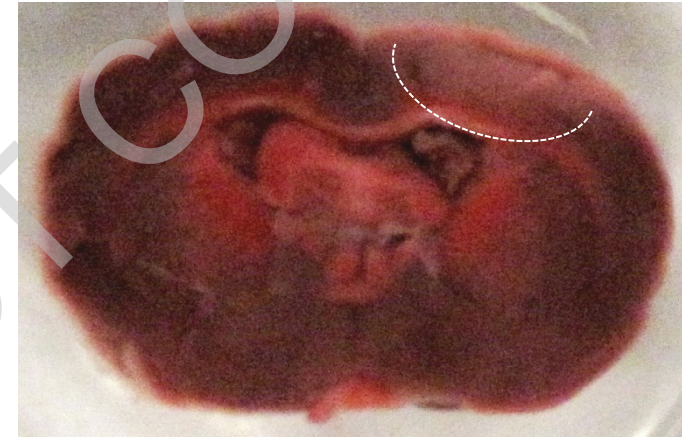
Fluid Percussion Injury: a post-traumatic epilepsy model



Nature Protocols, 2011



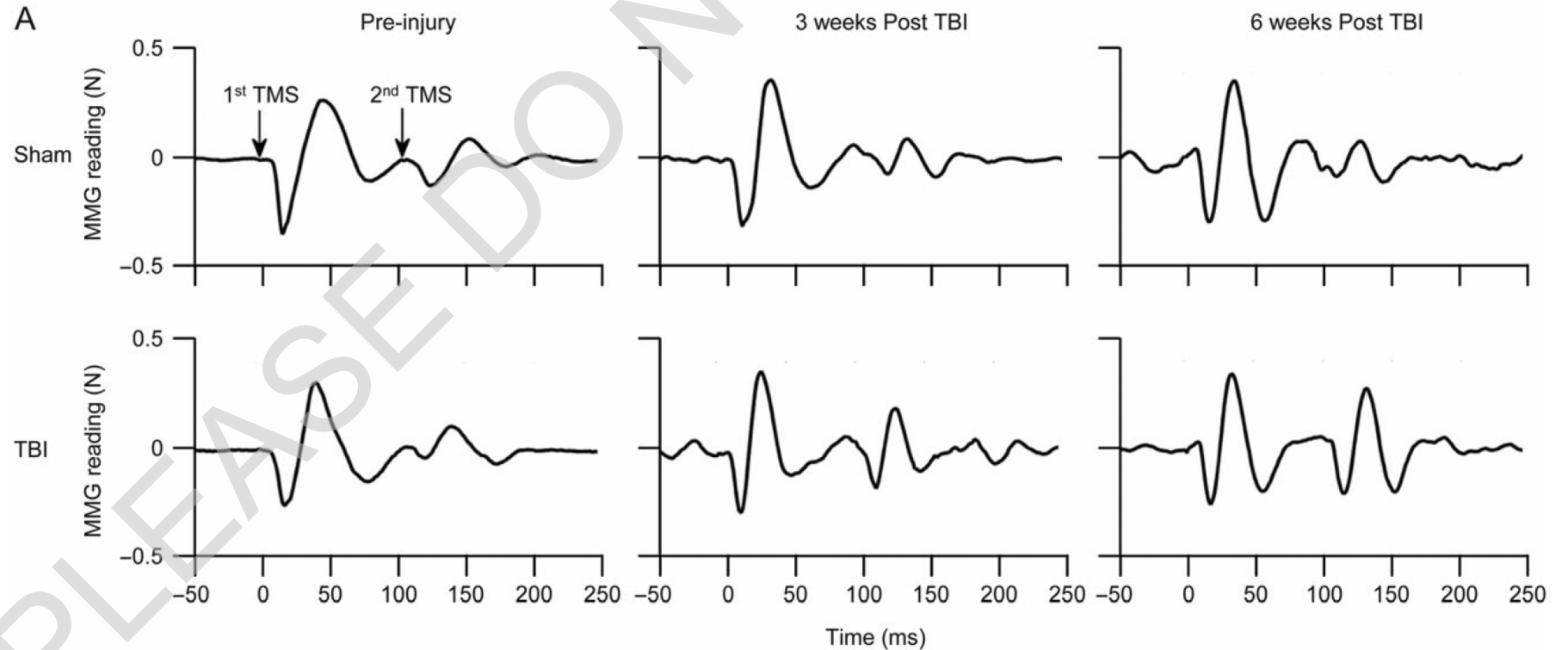
McIntosh et al., 1989



ORIGINAL ARTICLE

Trajectory of Parvalbumin Cell Impairment and Loss of Cortical Inhibition in Traumatic Brain Injury

Tsung-Hsun Hsieh^{1,2,3,†}, Henry Hing Cheong Lee^{4,†}, Mustafa Qadir Hameed^{1,4,5}, Alvaro Pascual-Leone⁶, Takao K. Hensch^{4,7} and Alexander Rotenberg^{1,4,6}

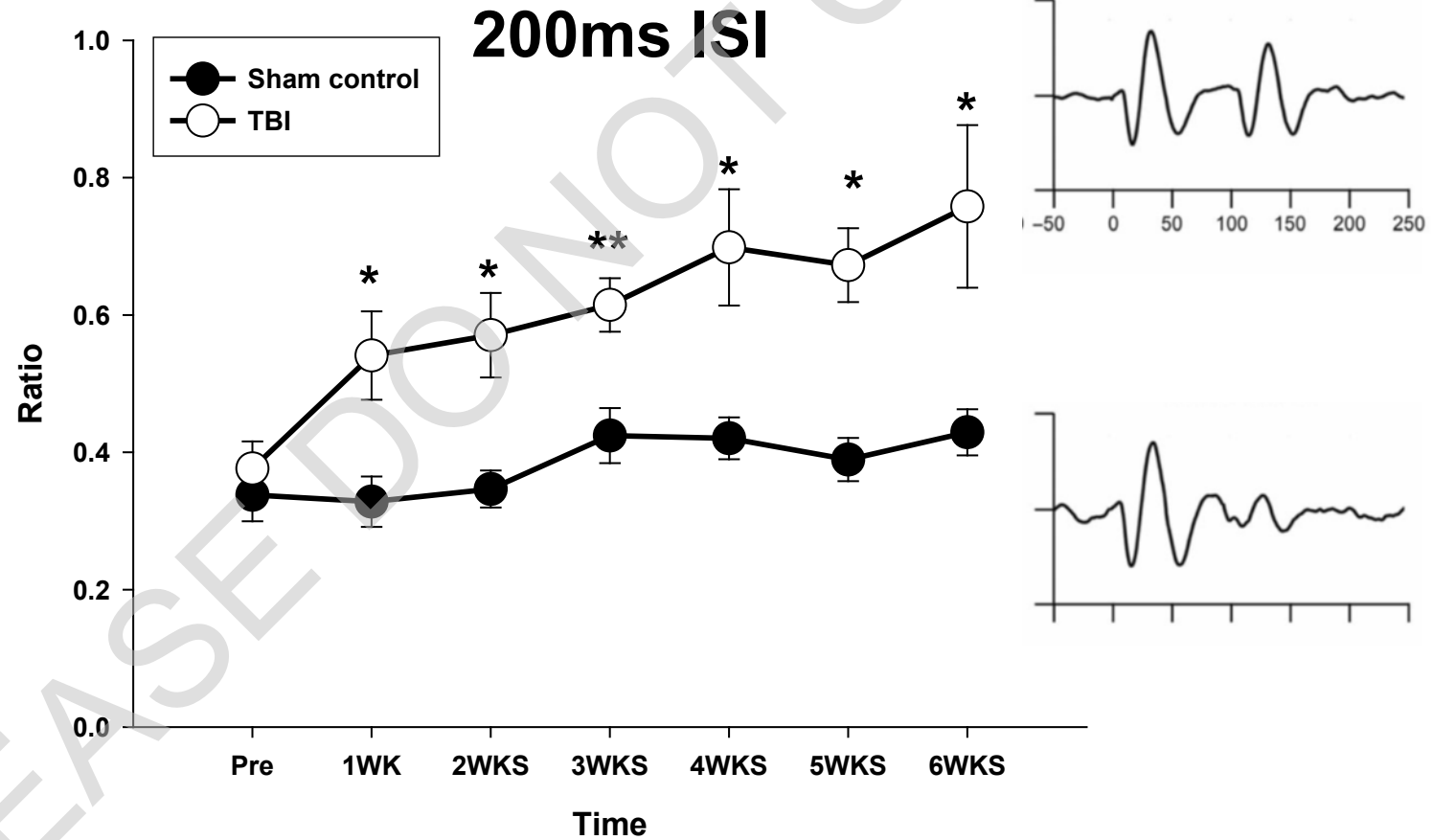


Loss of cortical paired-pulse inhibition after TBI

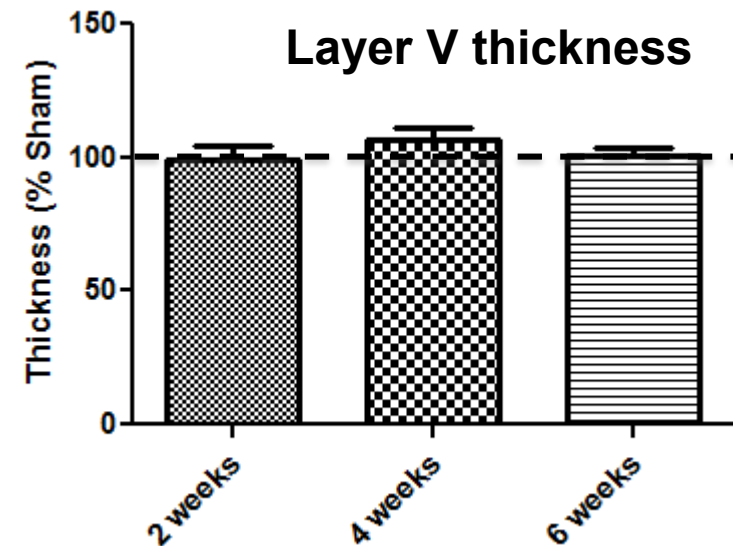
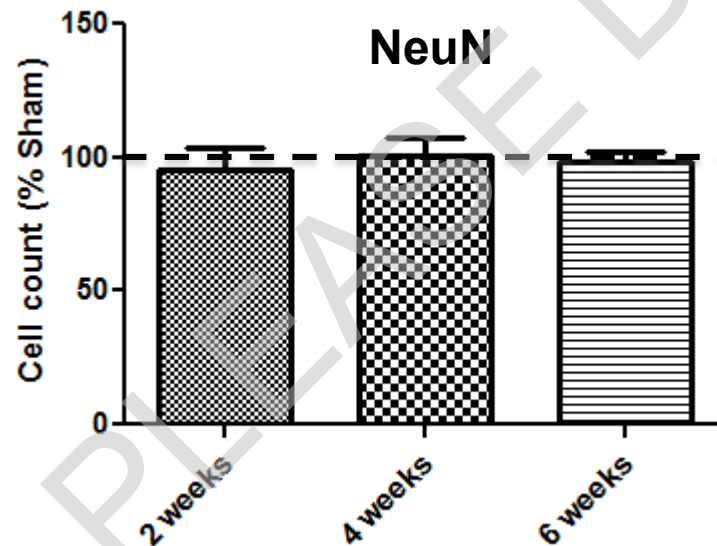
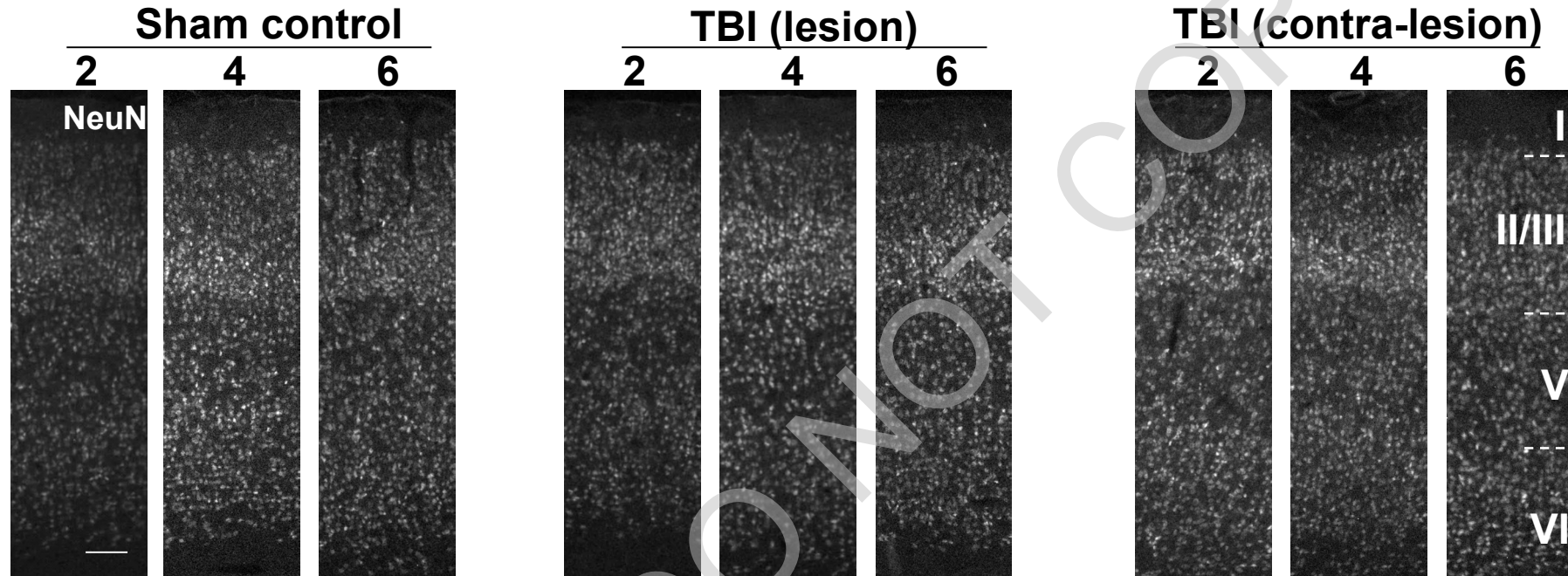
Min GABA inhibition



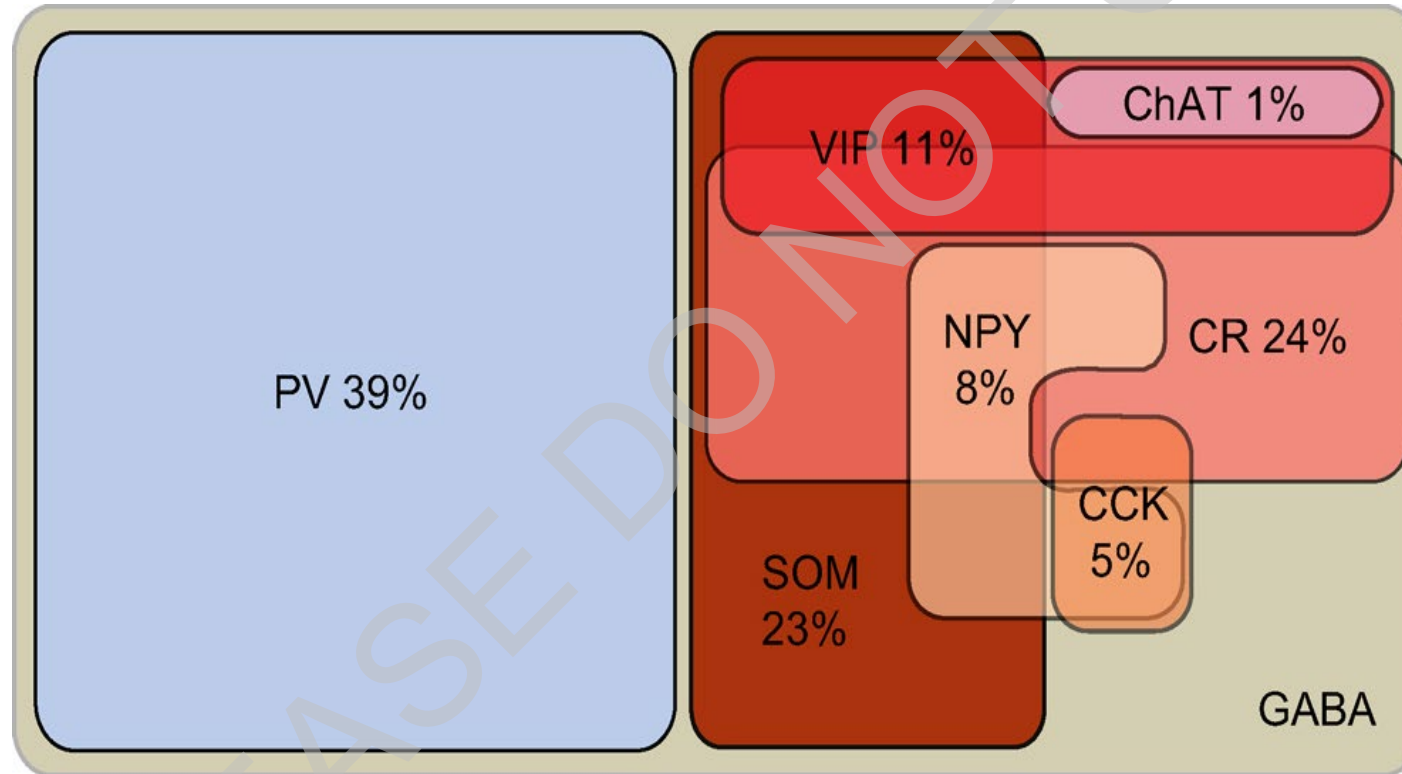
Max GABA inhibition



General cortical architecture was not affected by TBI



**Parvalbumin (PV) interneurons are the major
sub-type of cortical inhibitory neuron...
and vulnerable to oxidative stress**



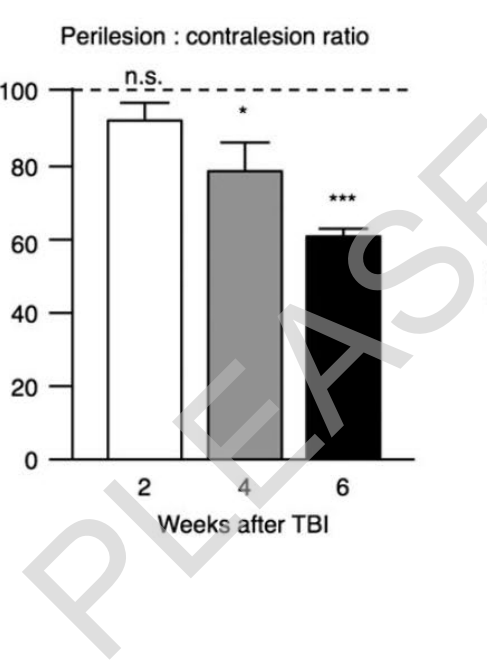
Gonchar et al., 2007, Front Neuroanat.

er TBI

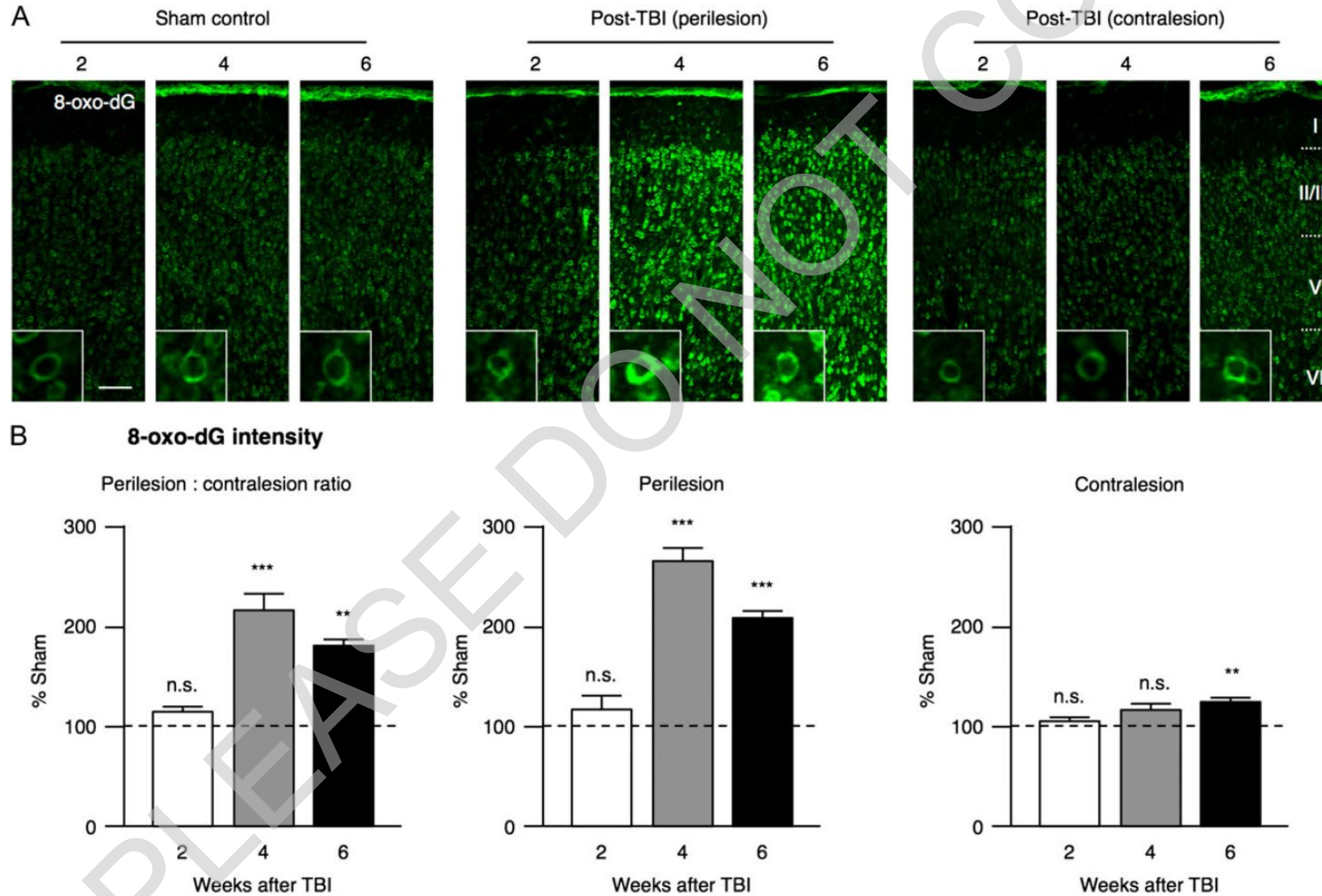
Post-TBI (contralateral)

2 4

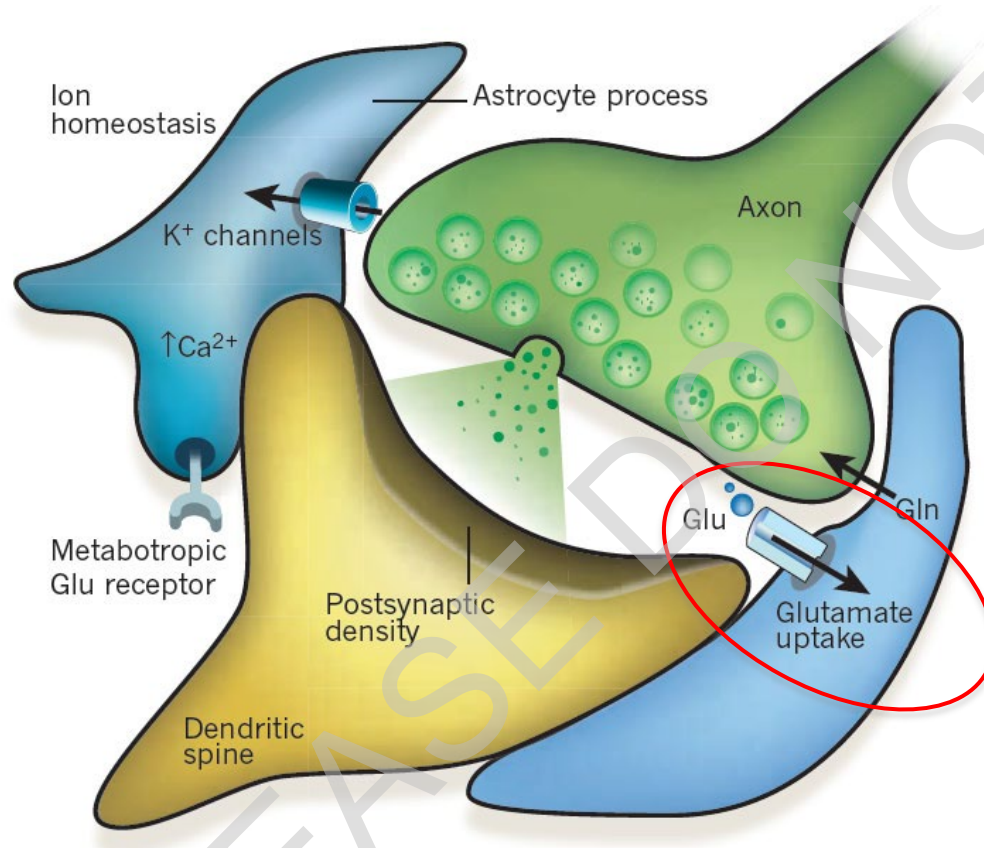
Fluorescence microscopy images of the hippocampus at 2 and 4 weeks post-TBI. The images show red fluorescence, likely representing a marker for neuronal damage or inflammation. The 2-week image shows a higher density of red spots compared to the 4-week image, indicating a more severe or persistent condition at the earlier time point.



Progressive oxidative stress after TBI



Astrocytic glutamate transporter as a druggable target in epileptogenesis

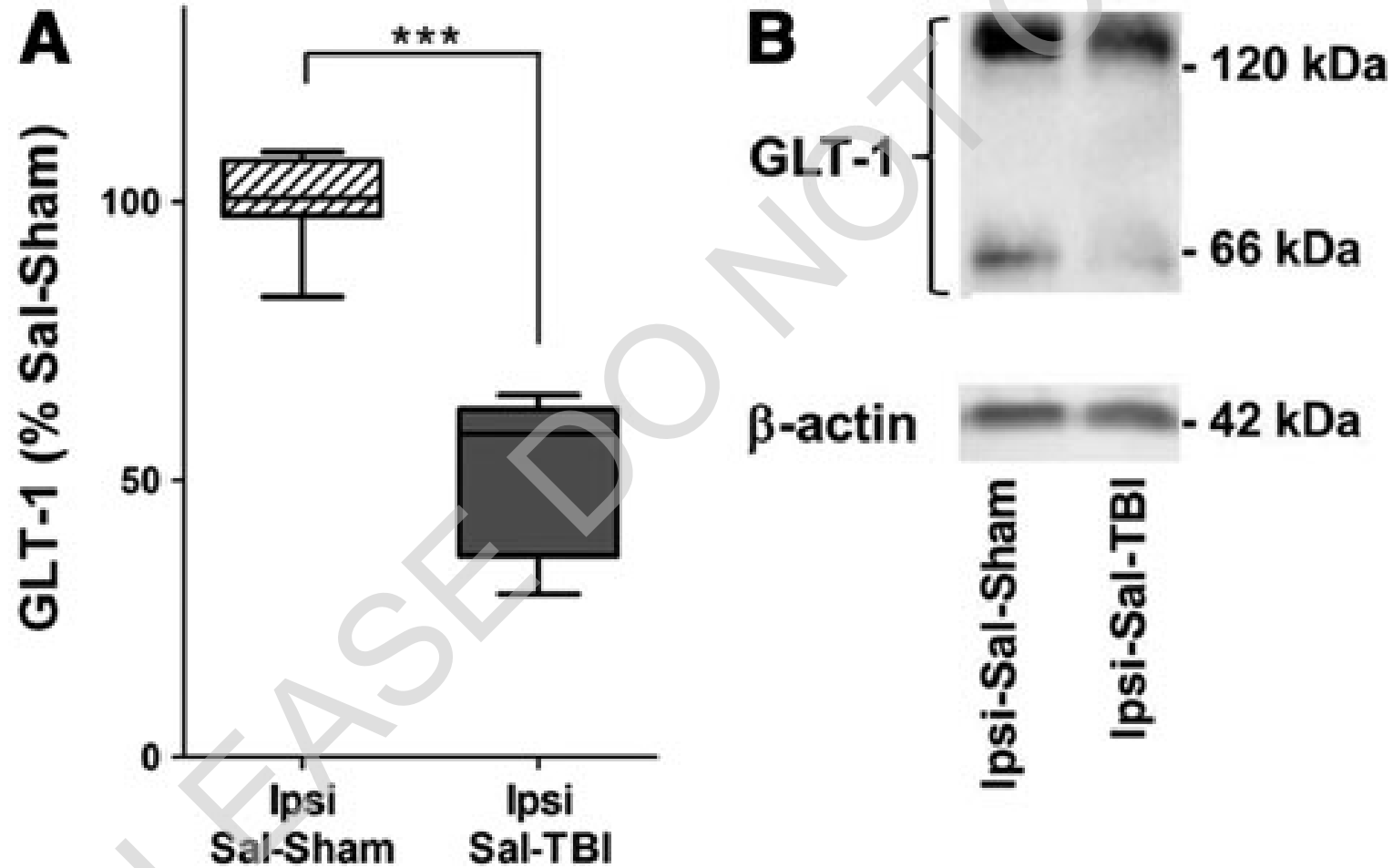


Astrocytes in the tripartite synapse

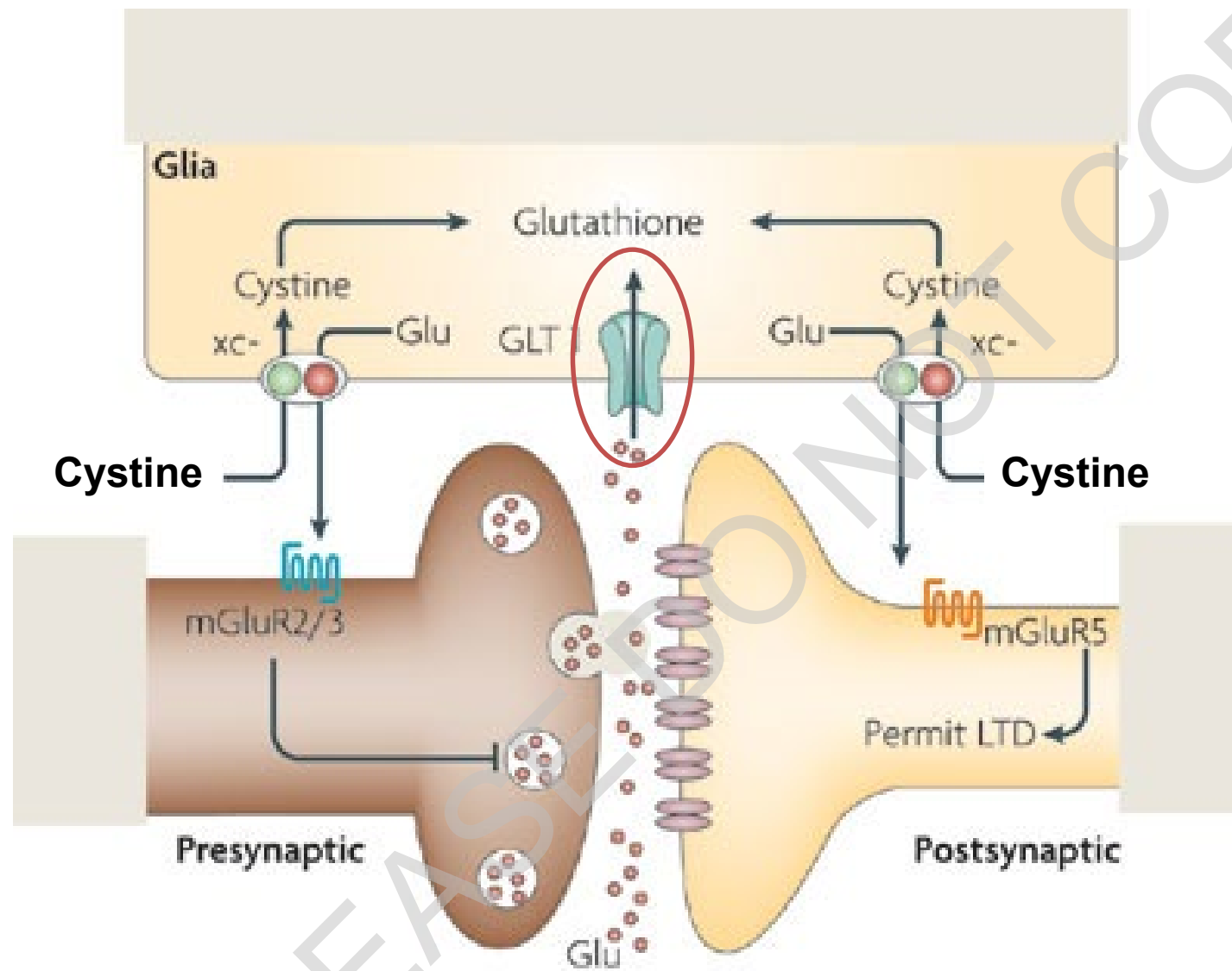
- K⁺ buffering
- **Glutamate homeostasis**
- GABA synthesis

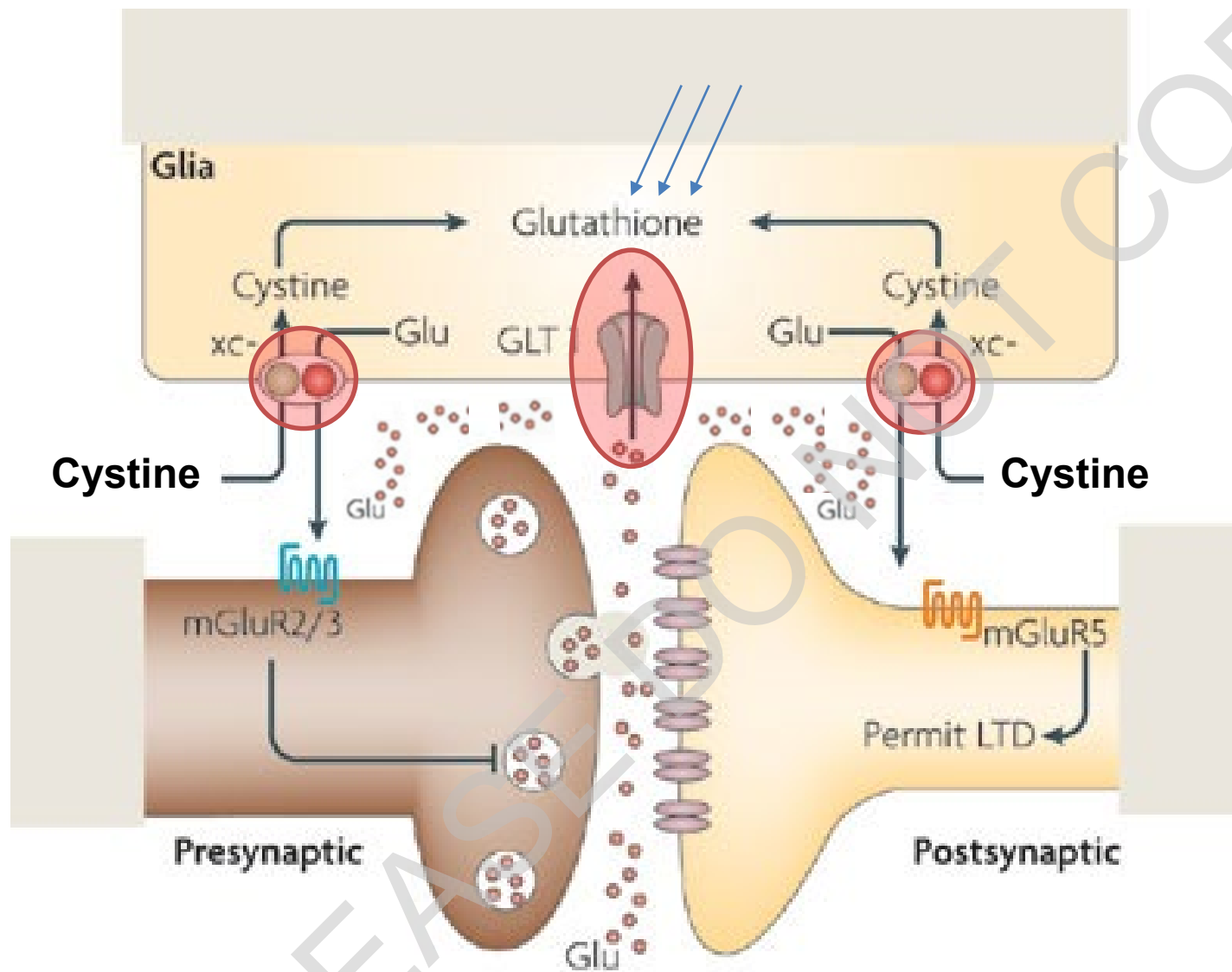
Courtesy of P. Rosenberg

Cortical GLT-1 is depressed after epileptogenic TBI

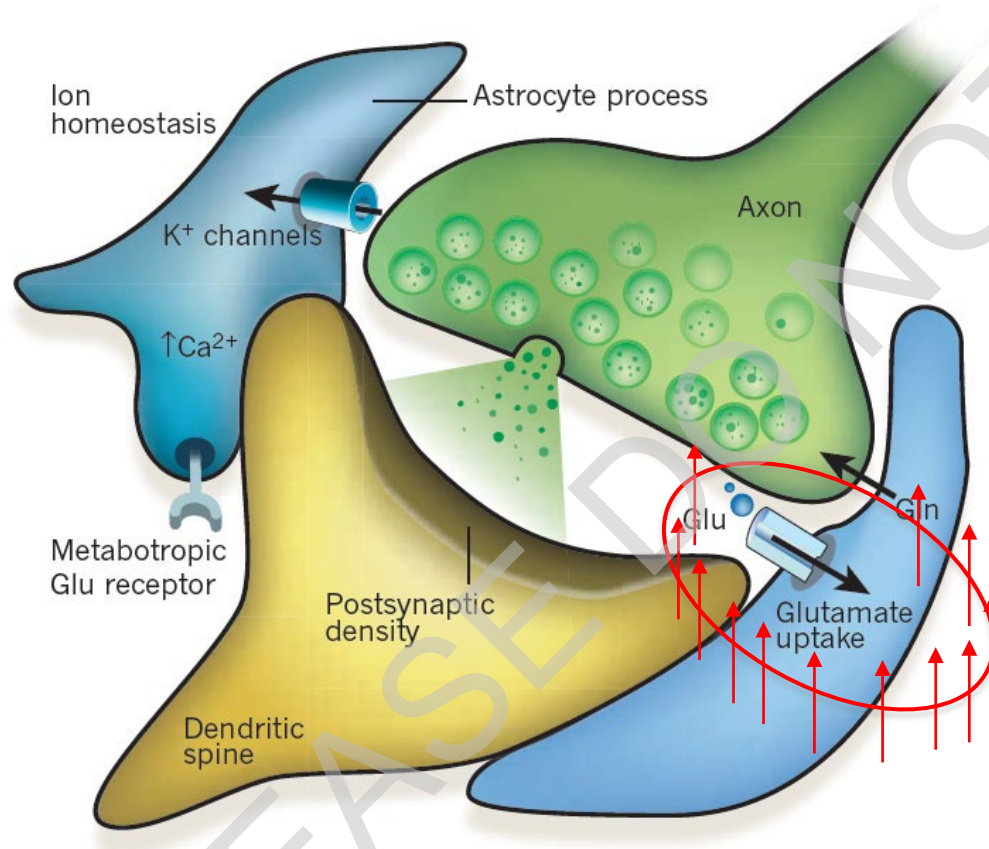


Goodrich et al., J. Neurotrauma 2013





Astrocytic glutamate transporter as a druggable target in epileptogenesis

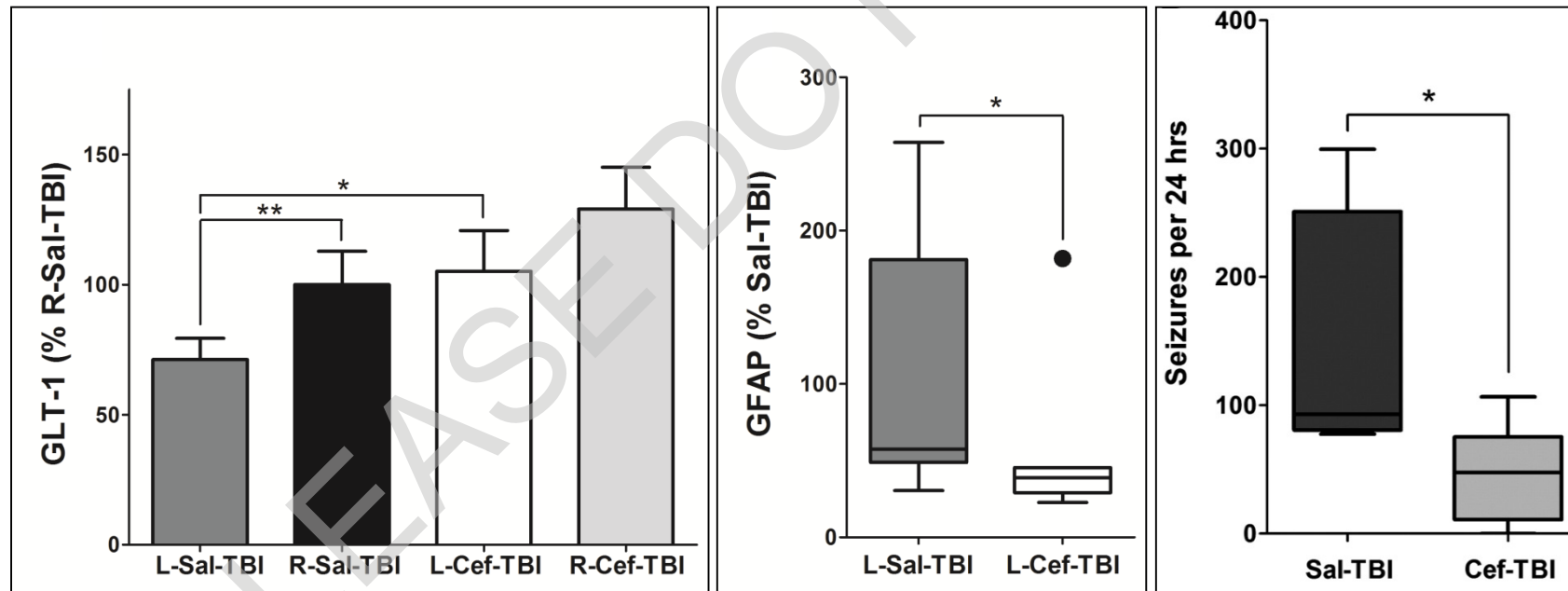


Glutamate clearance can be enhanced by **BETA LACTAMS (!)**

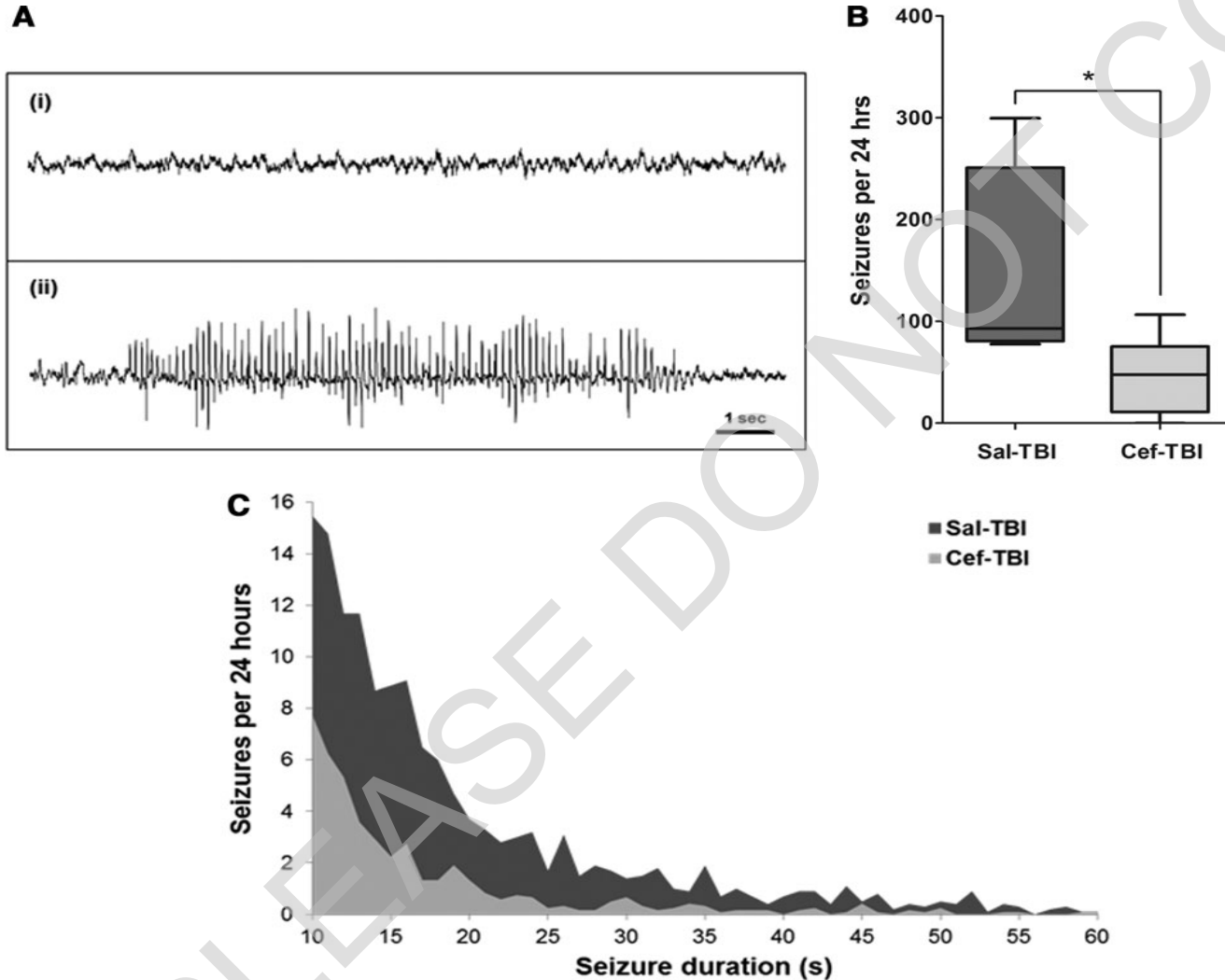
Courtesy of P. Rosenberg

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,^{1,*} Anatoli Y. Kabakov,^{1,*} Mustafa Q. Hameed,^{1,2} Sameer C. Dhamne,¹
 Paul A. Rosenberg,¹ and Alexander Rotenberg¹



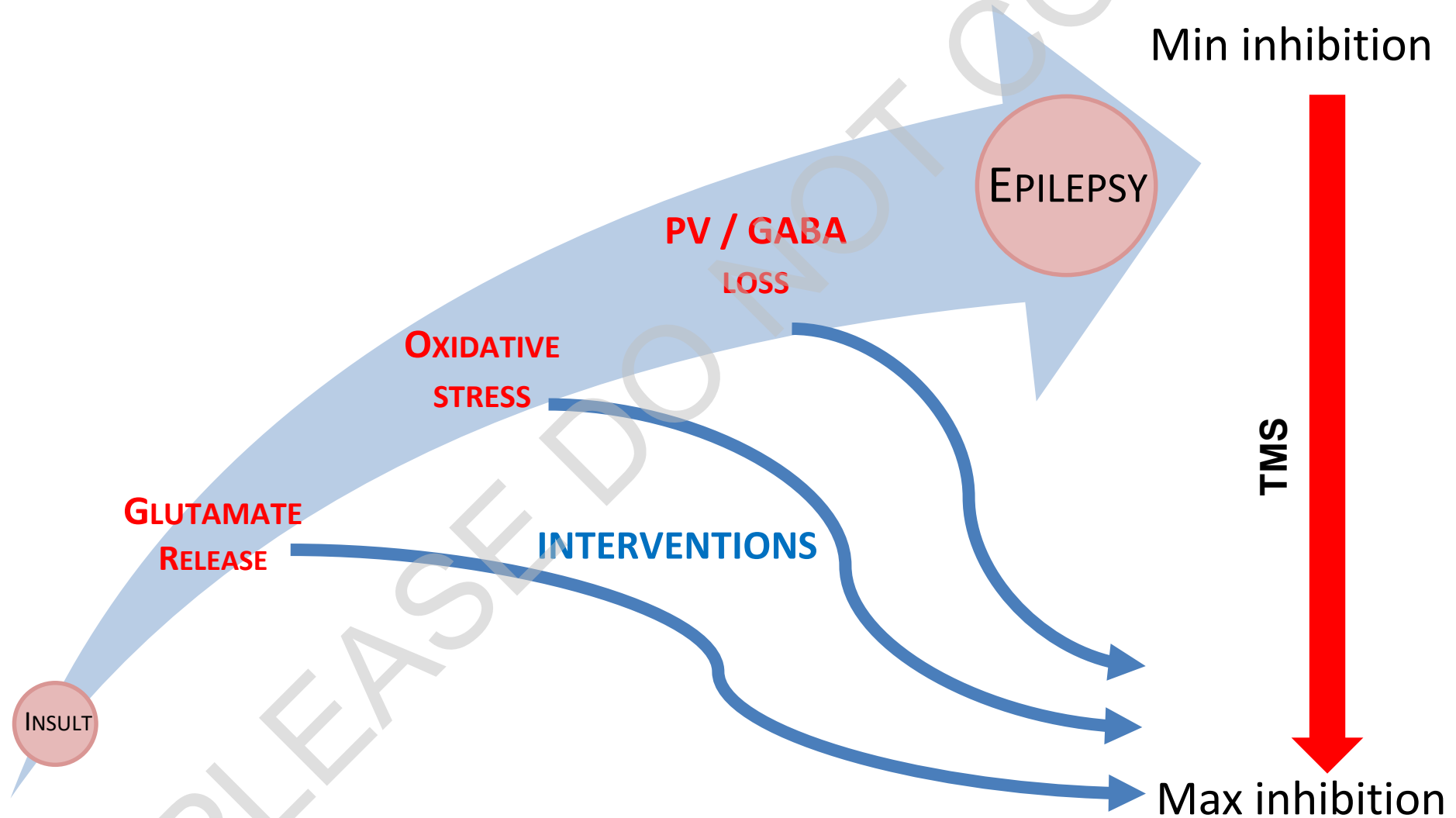
Ceftriaxone treatment prophylaxes against posttraumatic seizures



So why is this interesting to a TMS lab?



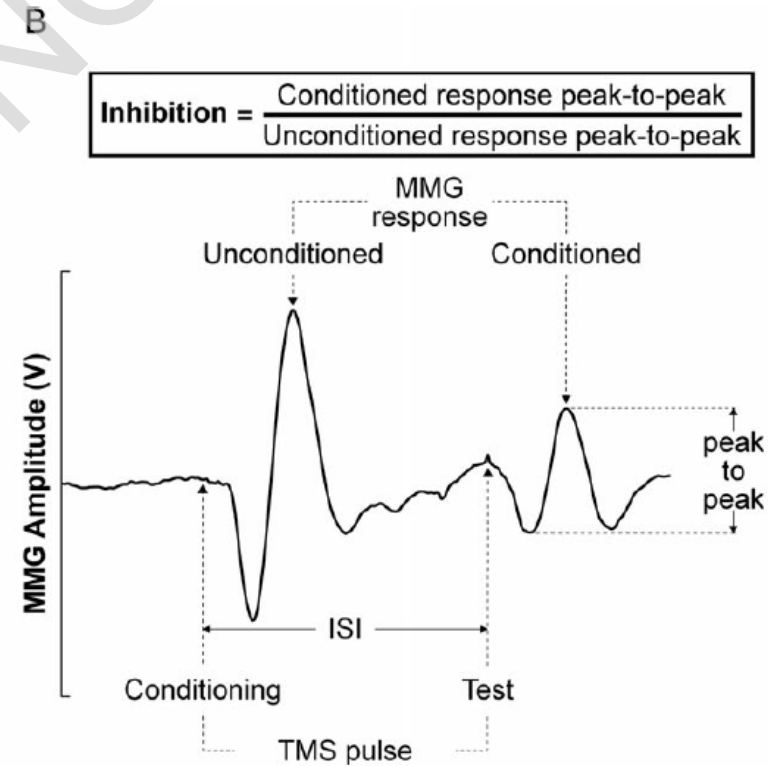
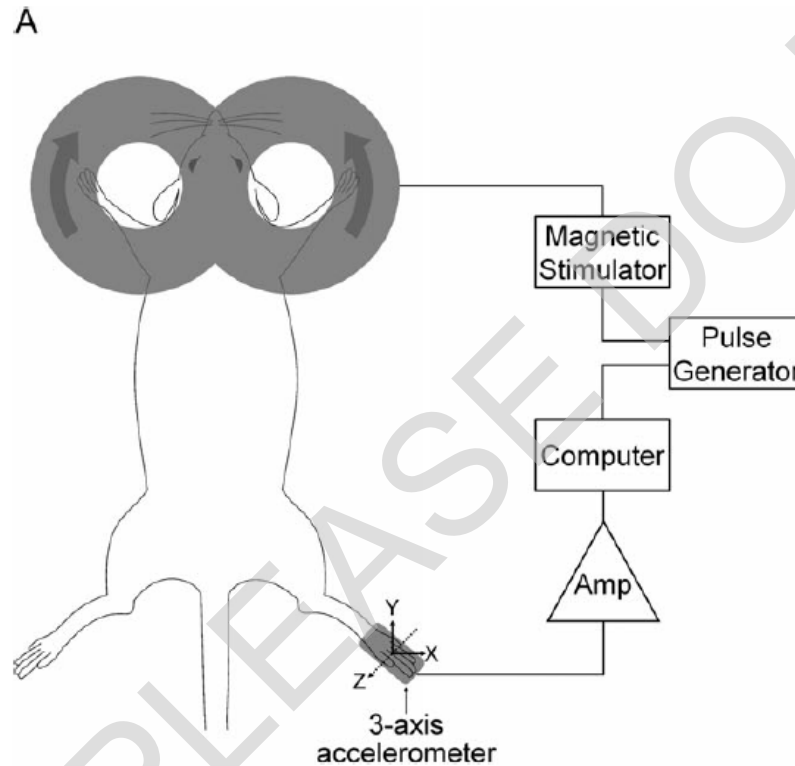
Post Traumatic Epileptogenesis



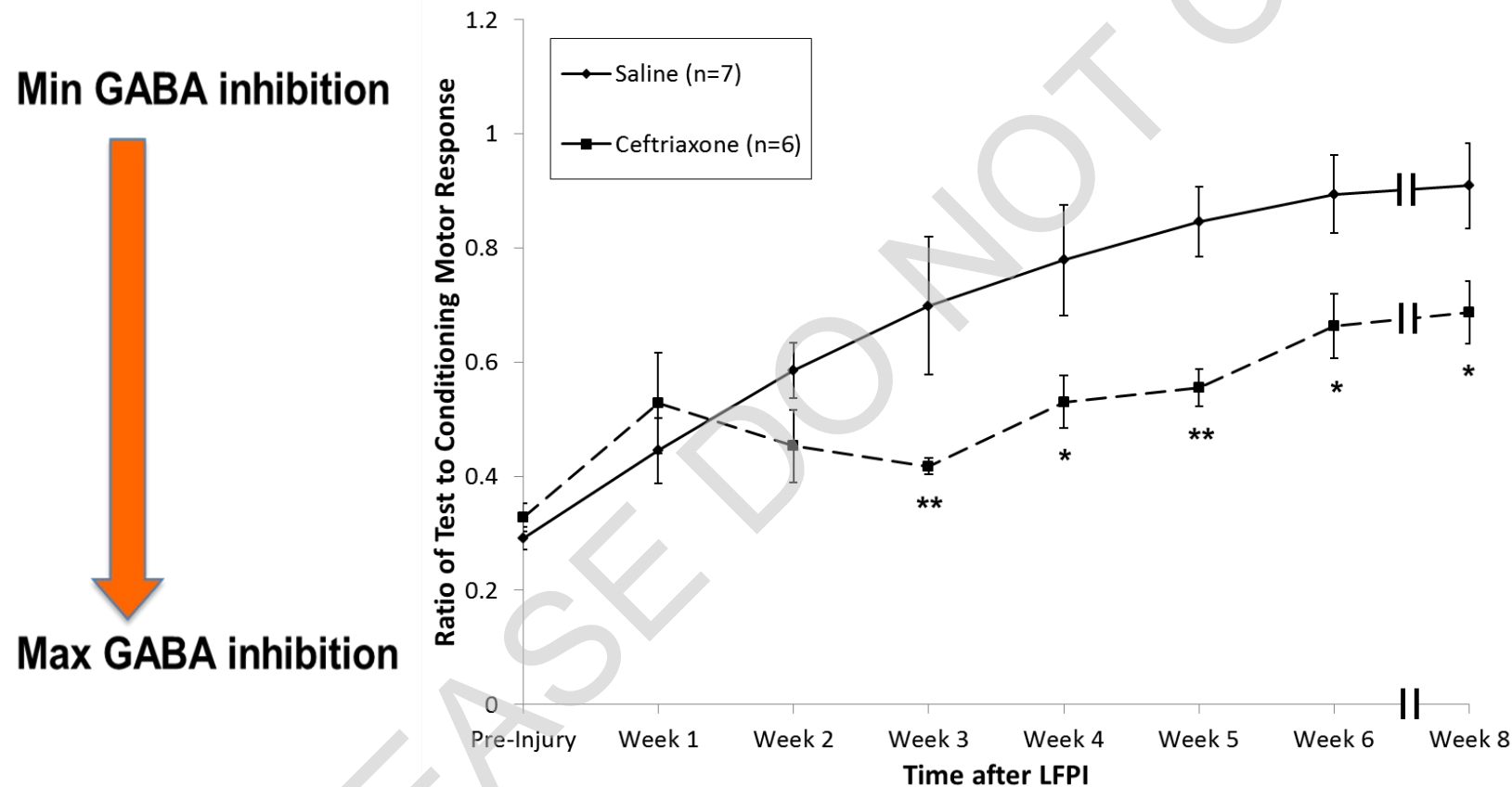
ORIGINAL ARTICLE

Ceftriaxone Treatment Preserves Cortical Inhibitory Interneuron Function via Transient Salvage of GLT-1 in a Rat Traumatic Brain Injury Model

Mustafa Q. Hameed^{1,2,3}, Tsung-Hsun Hsieh^{1,4,5}, Leon Morales-Quezada⁶, Henry H. C. Lee², Ugur Damar^{1,2}, Paul C. MacMullin^{1,2}, Takao K. Hensch^{2,7} and Alexander Rotenberg^{1,2}

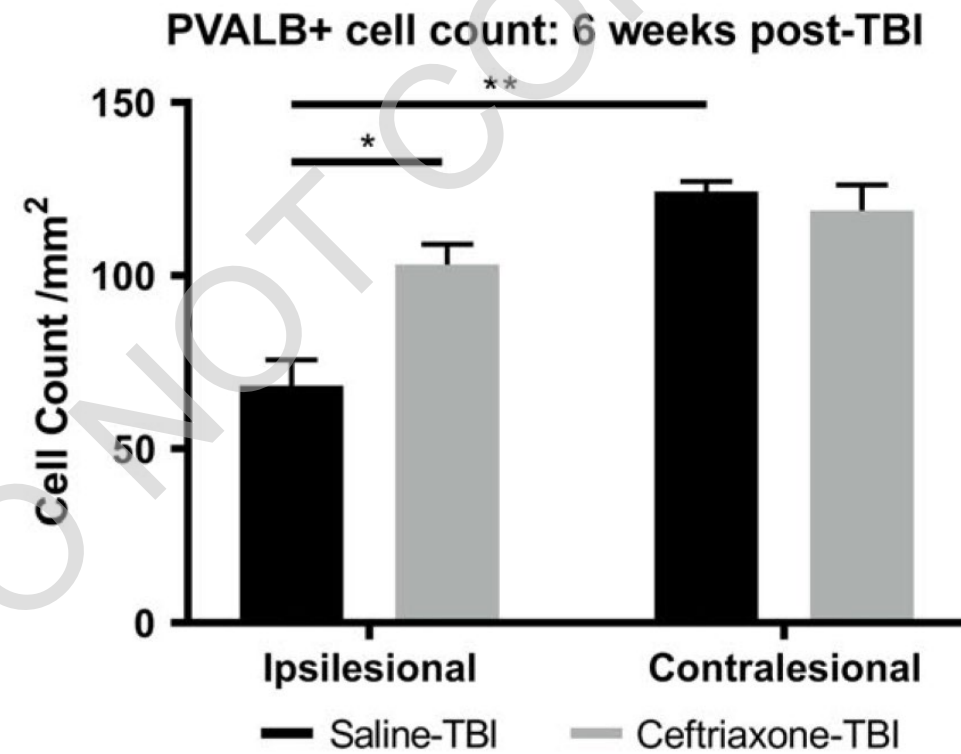
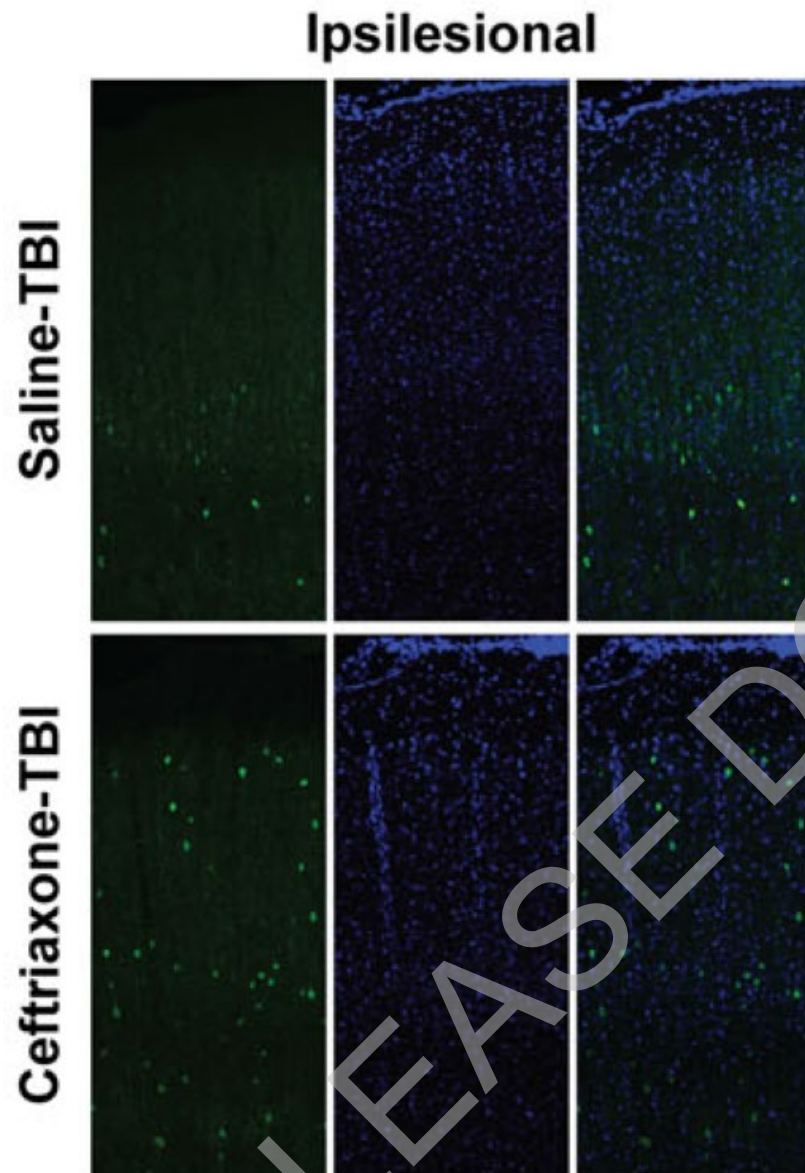


ppTMS as a biomarker and marker of target engagement in epileptogenesis

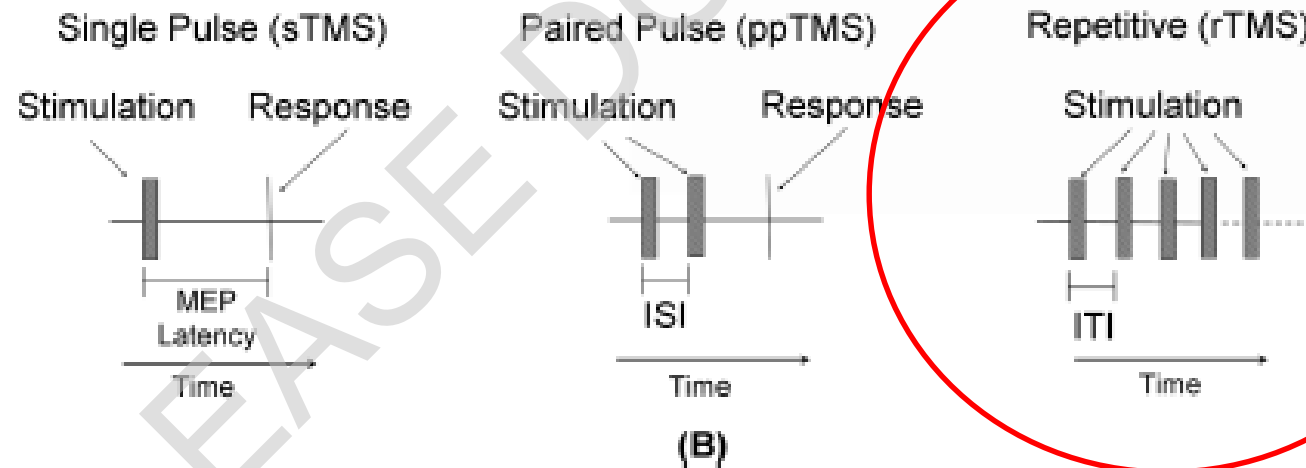
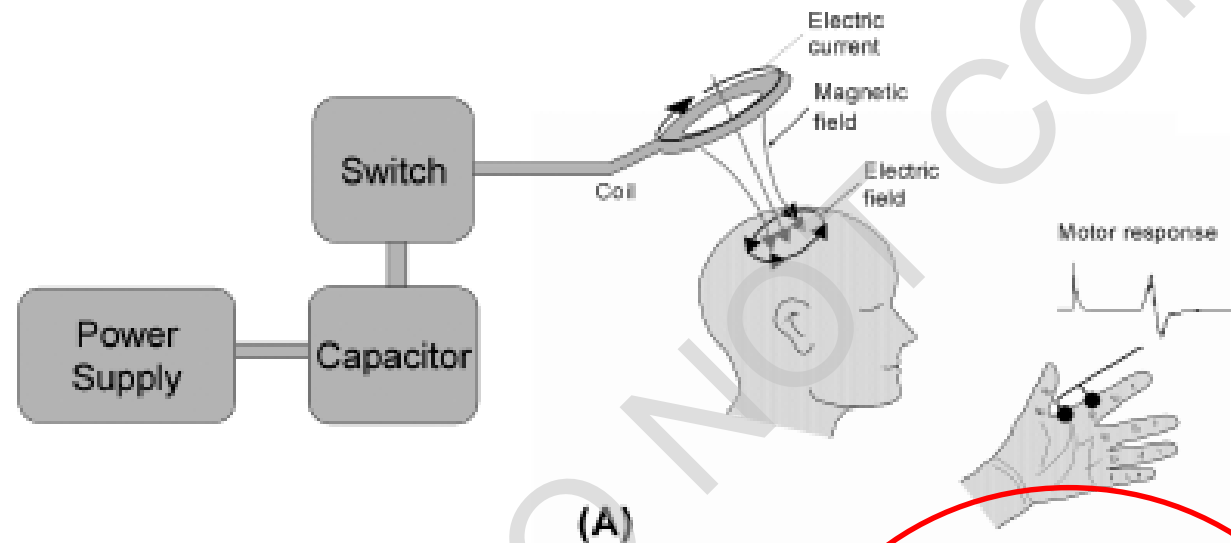


Hameed et al., SFN 2017

Hameed et al., Cerebral Cortex 2018



Stimulation protocols

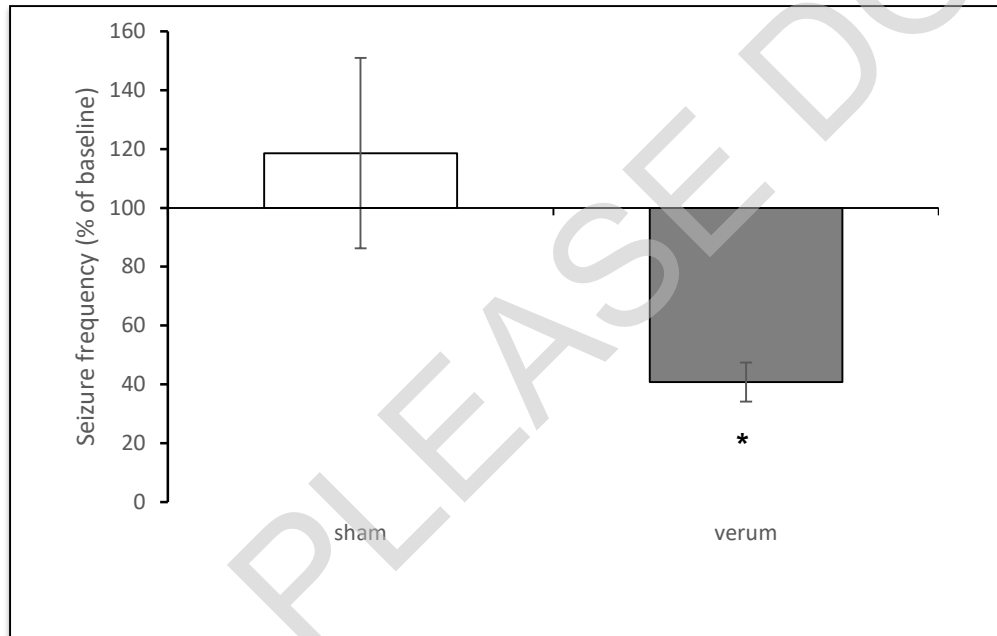


Why rTMS in rats?

1 Hz rTMS in temporal lobe epilepsy, trial in progress



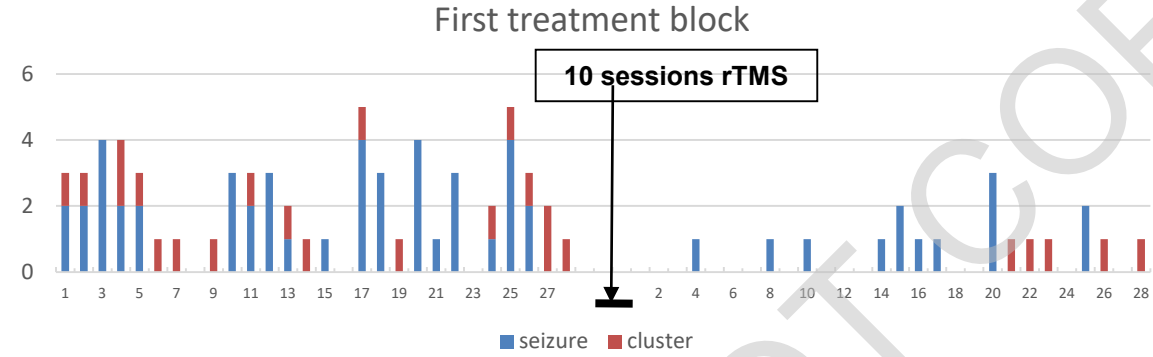
**Boston Children's Hospital
Neuromodulation Program**



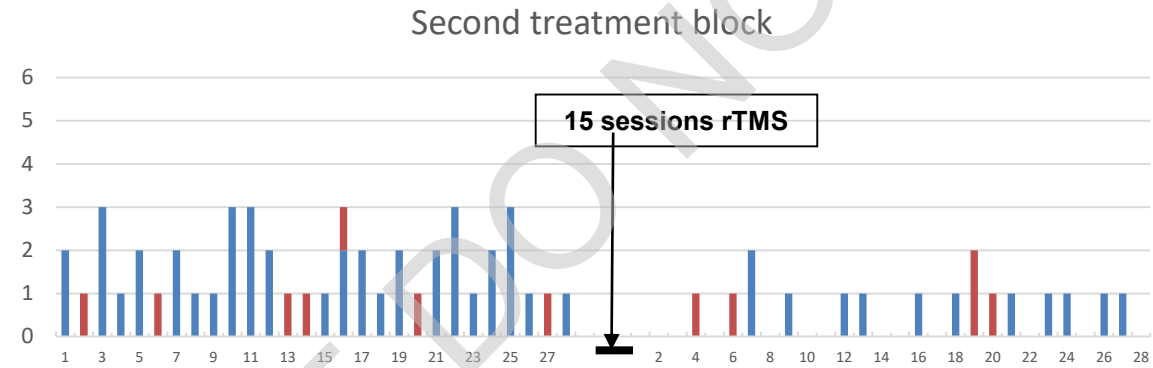
TMS H-Coil System

Figure 1

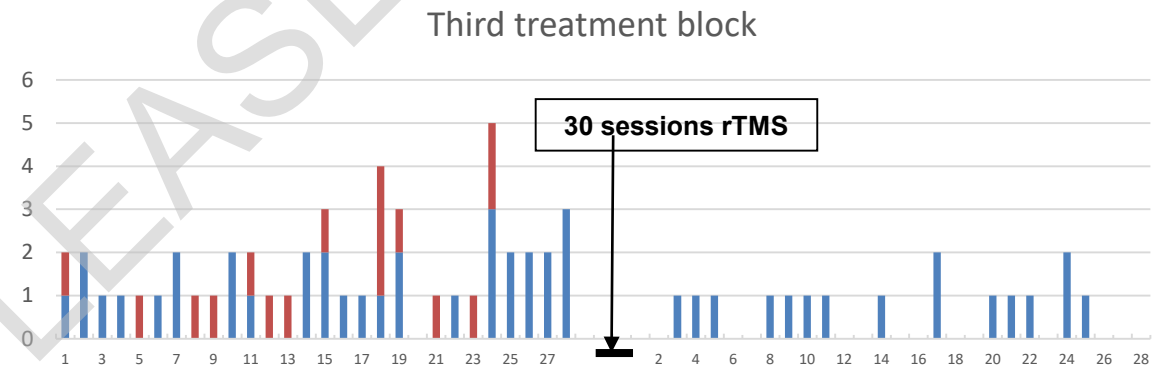
A



B



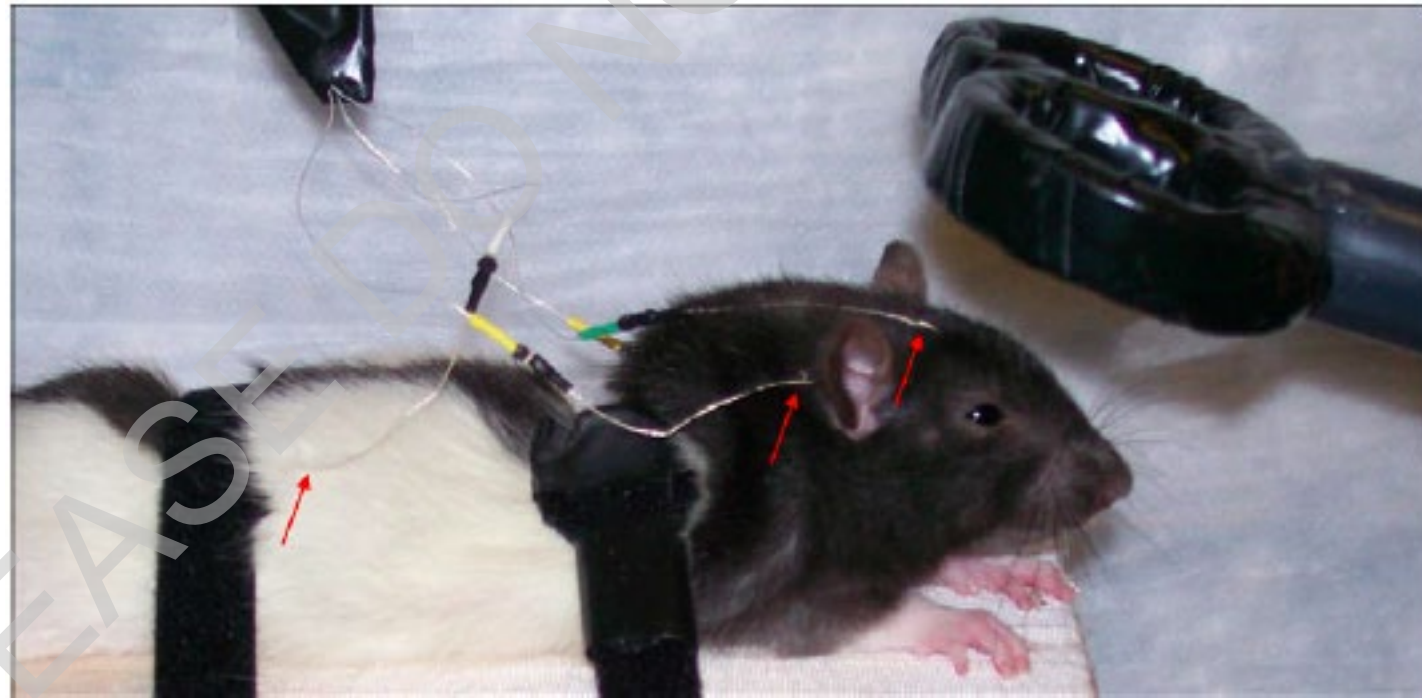
C



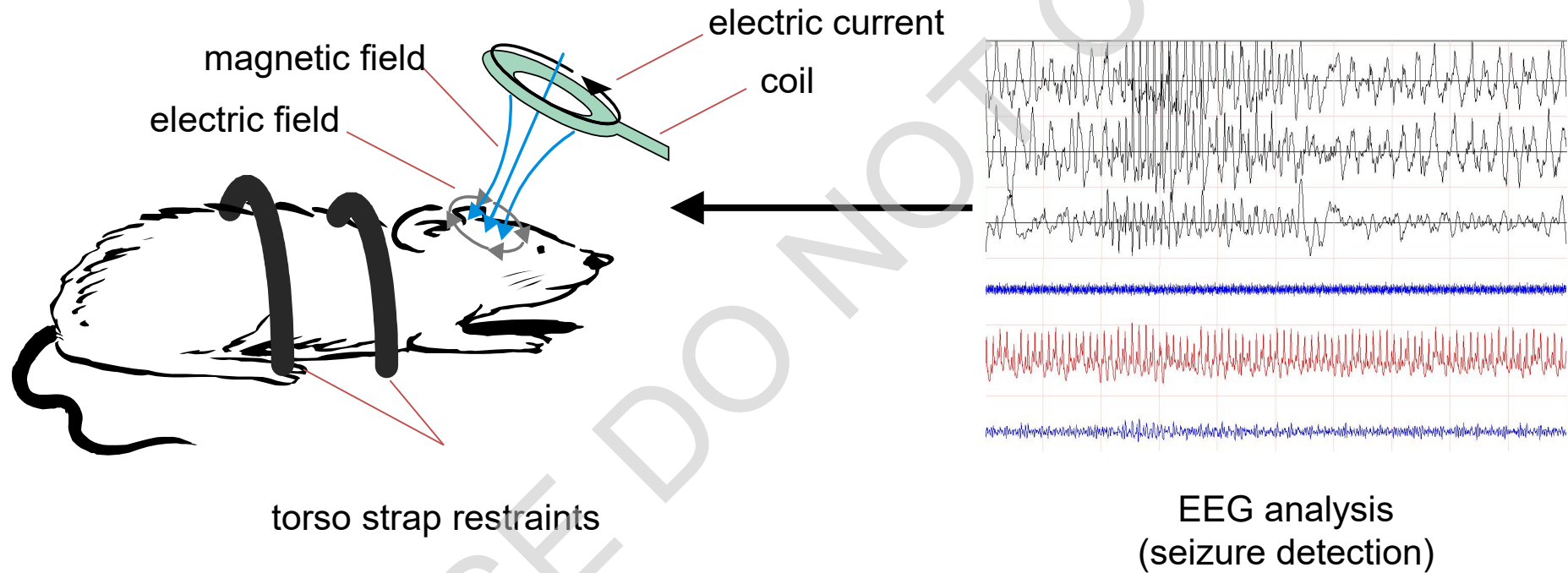


Seizure suppression by EEG-guided repetitive transcranial magnetic stimulation in the rat

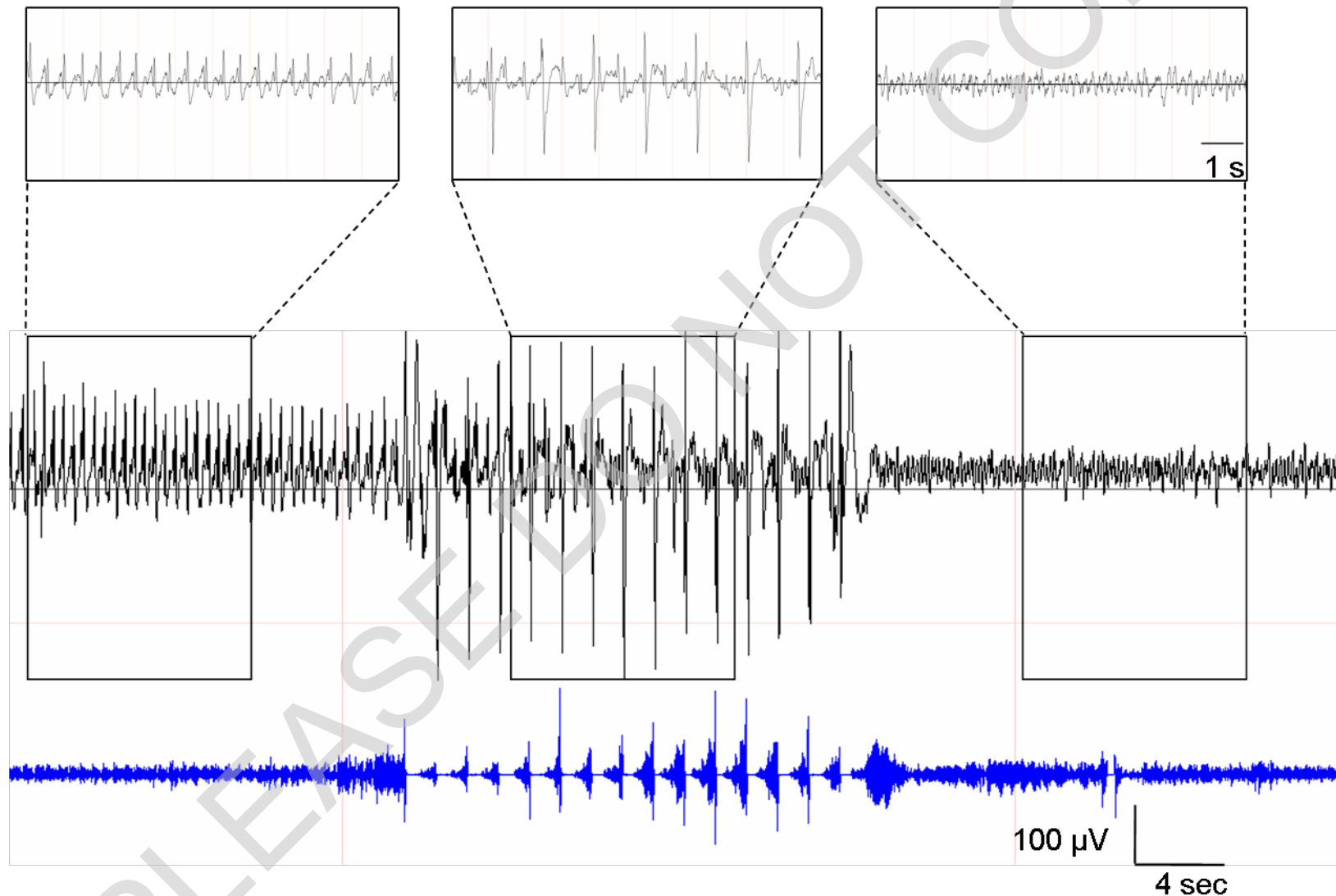
Alexander Rotenberg^{a,b,*}, Paul Muller^a, Daniel Birnbaum^a, Michael Harrington^a, James J. Riviello^c, Alvaro Pascual-Leone^b, Frances E. Jensen^a



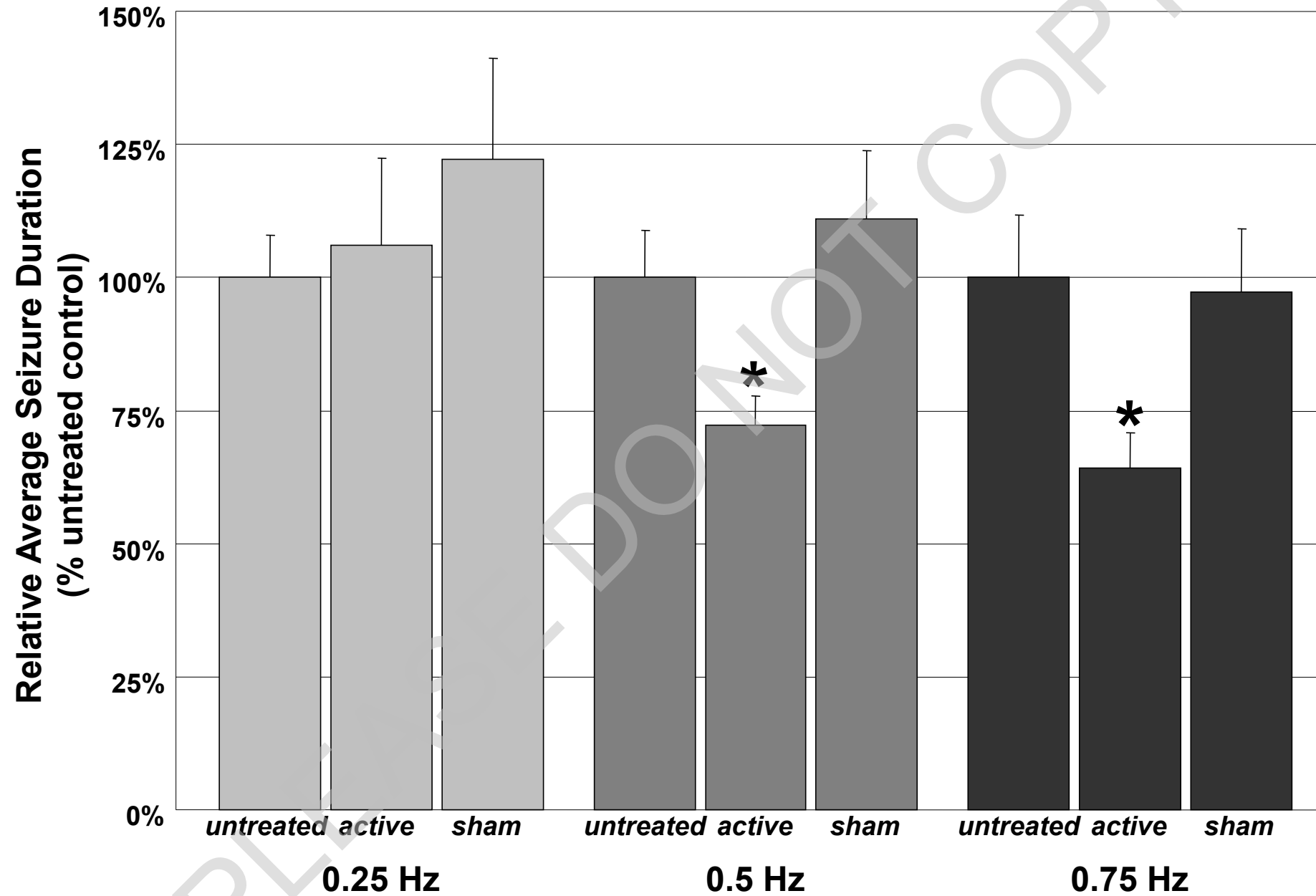
Rat “deep” TMS during seizure



rTMS during KA seizure

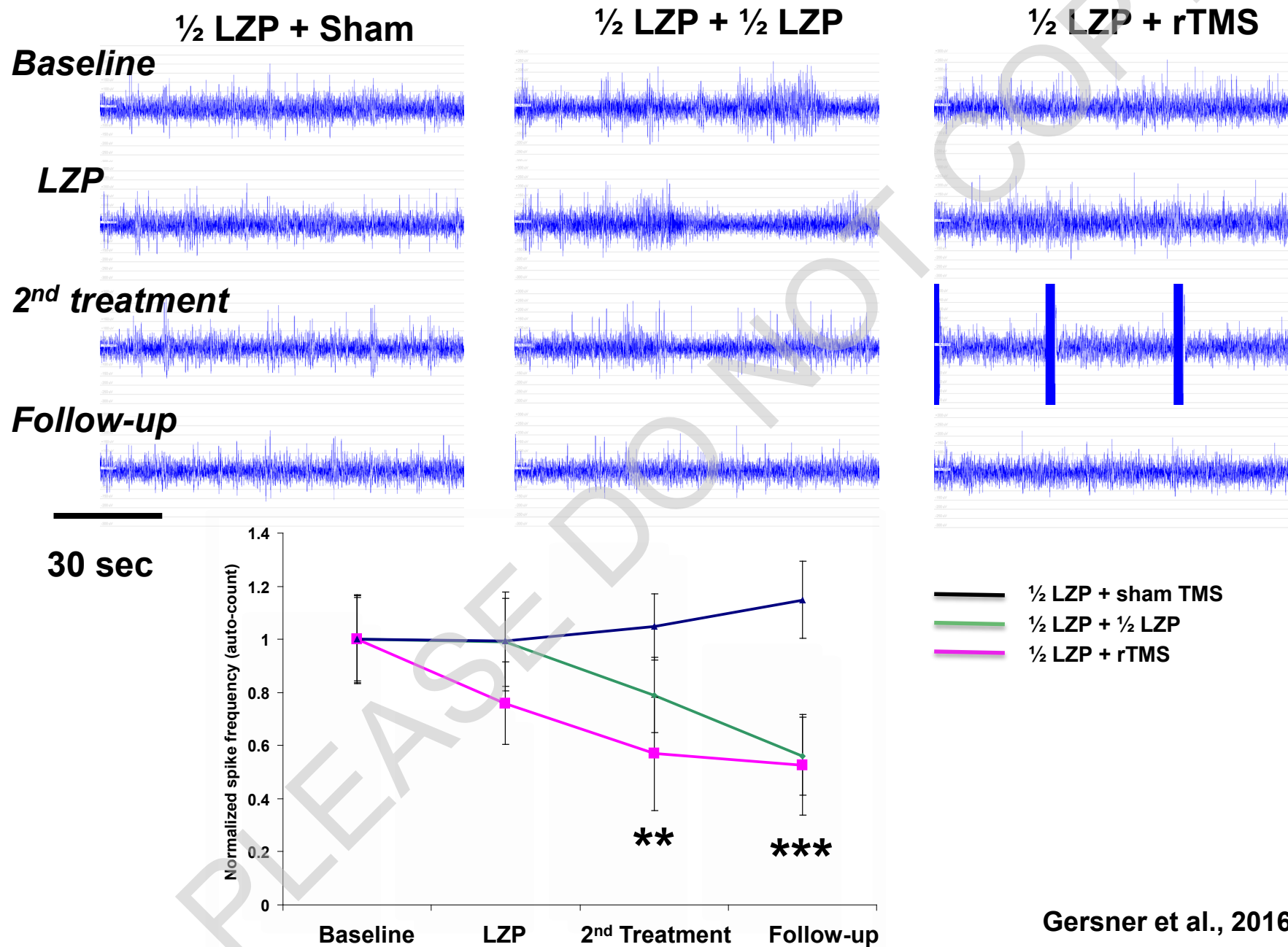


rTMS during KA seizures



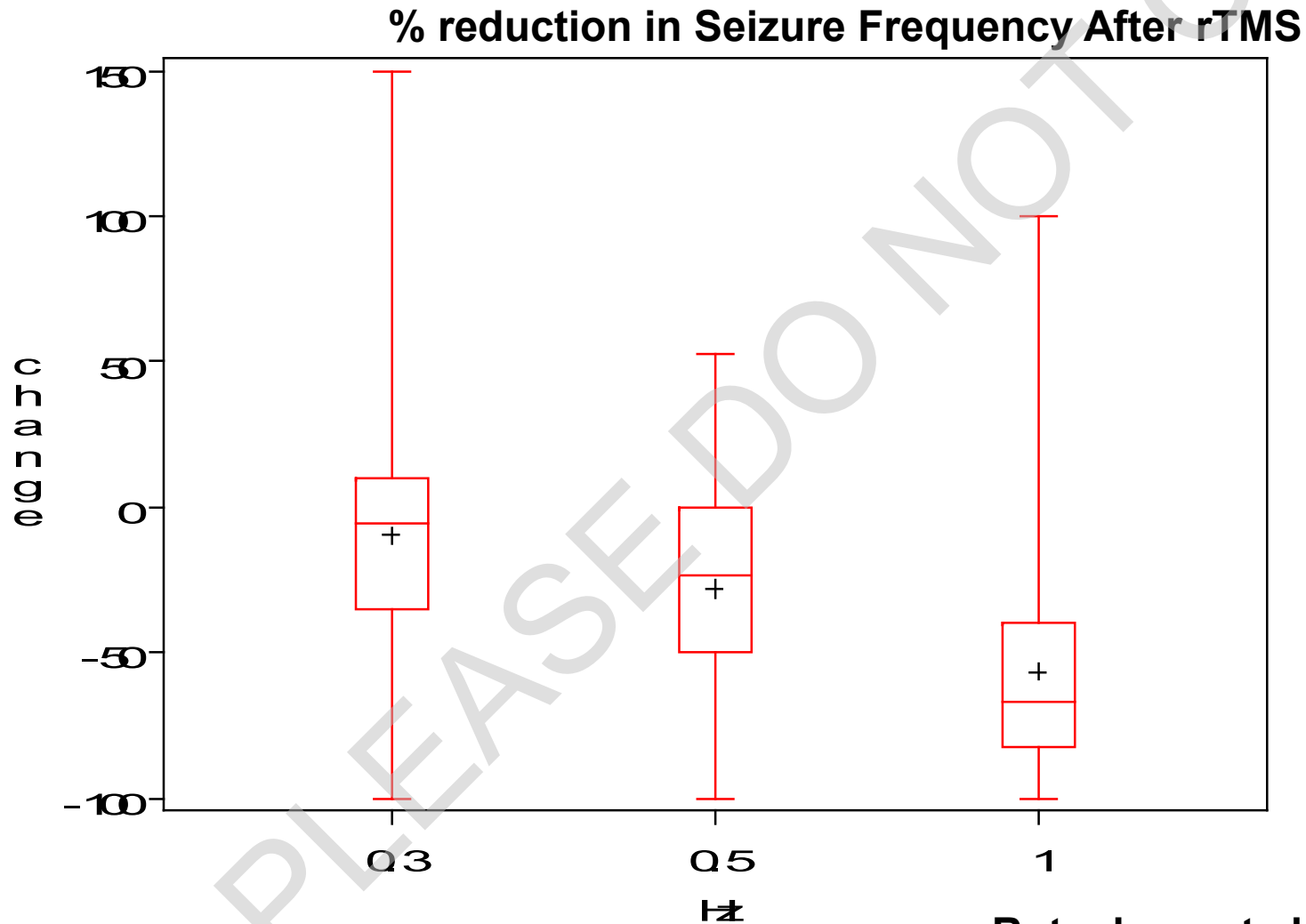
Rotenberg et al., 2008

Combination therapy: lorazepam + rTMS in seizure suppression



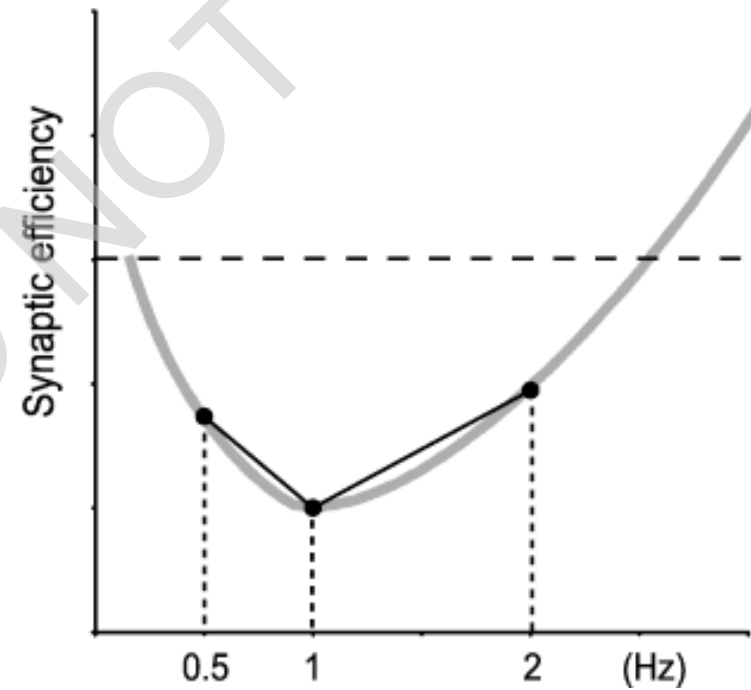
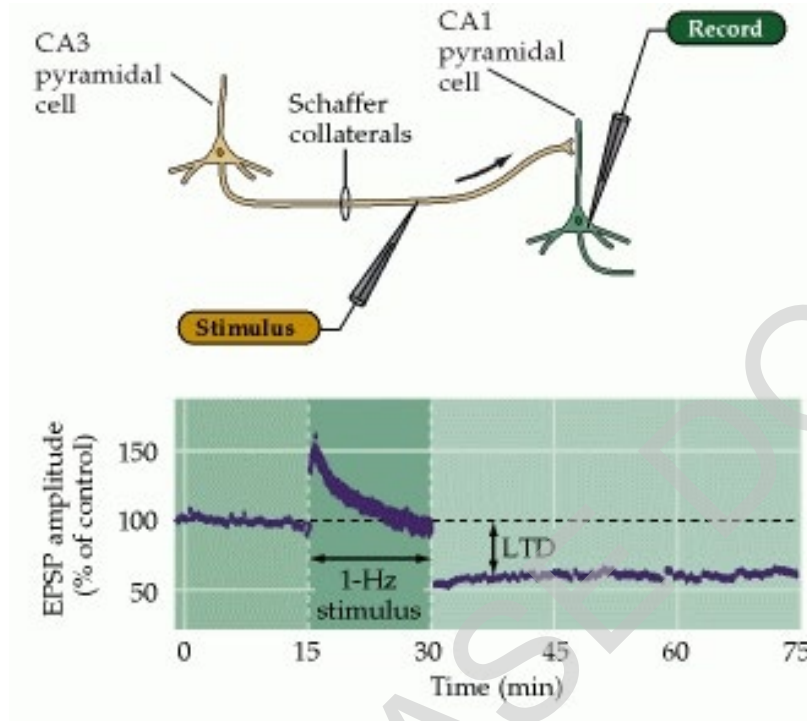
Gersner et al., 2016.

Better seizure suppression in humans with 1 Hz



Rotenberg et al., unpublished data

Frequency-response *in vitro* LTD approximates rTMS data



Molecular Basis: Does rTMS induce LTP/LTD

The diagram illustrates the molecular basis of LTP/LTD induction by rTMS, showing the relationship between electrical stimulation, neural activity, and intracellular signaling pathways.

Electrical Stimulation and Neural Activity: The top part shows a schematic of the hippocampus with the **Test stimulus** (1 train or 4 trains) applied to the **Perforant pathway**. The **Recording** is taken from the **CA1** region. The **CA3** region is also labeled. The **Dentate gyrus** is shown as part of the **Perforant pathway**.

Electrophysiological Traces: The bottom part shows two traces of **Test stimulus** (1 train or 4 trains) over **Time (min)** (0 to 210). The **Late LTP (4 trains)** trace shows a sustained increase in response, while the **Early LTP (1 train)** trace shows a transient increase.

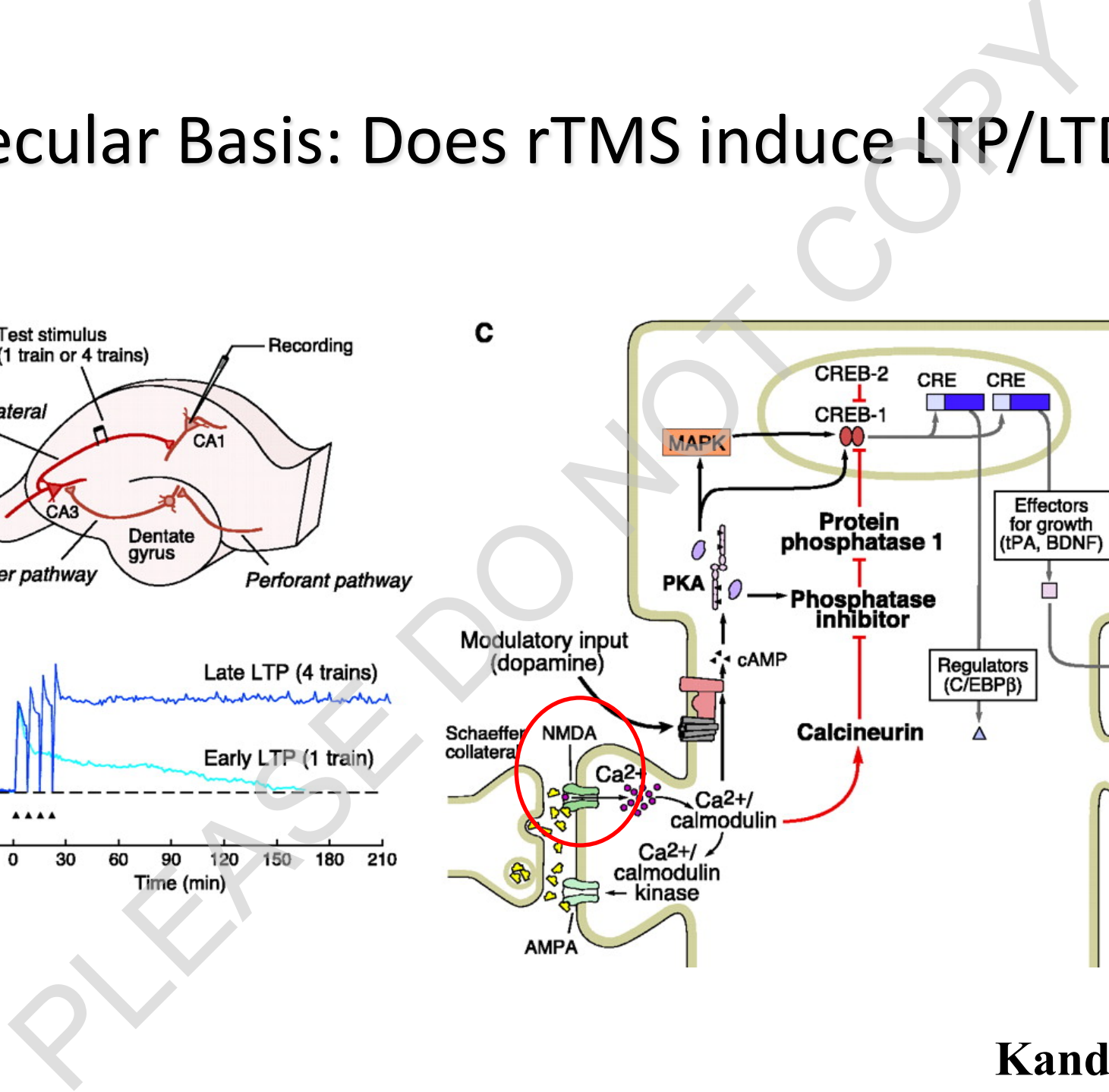
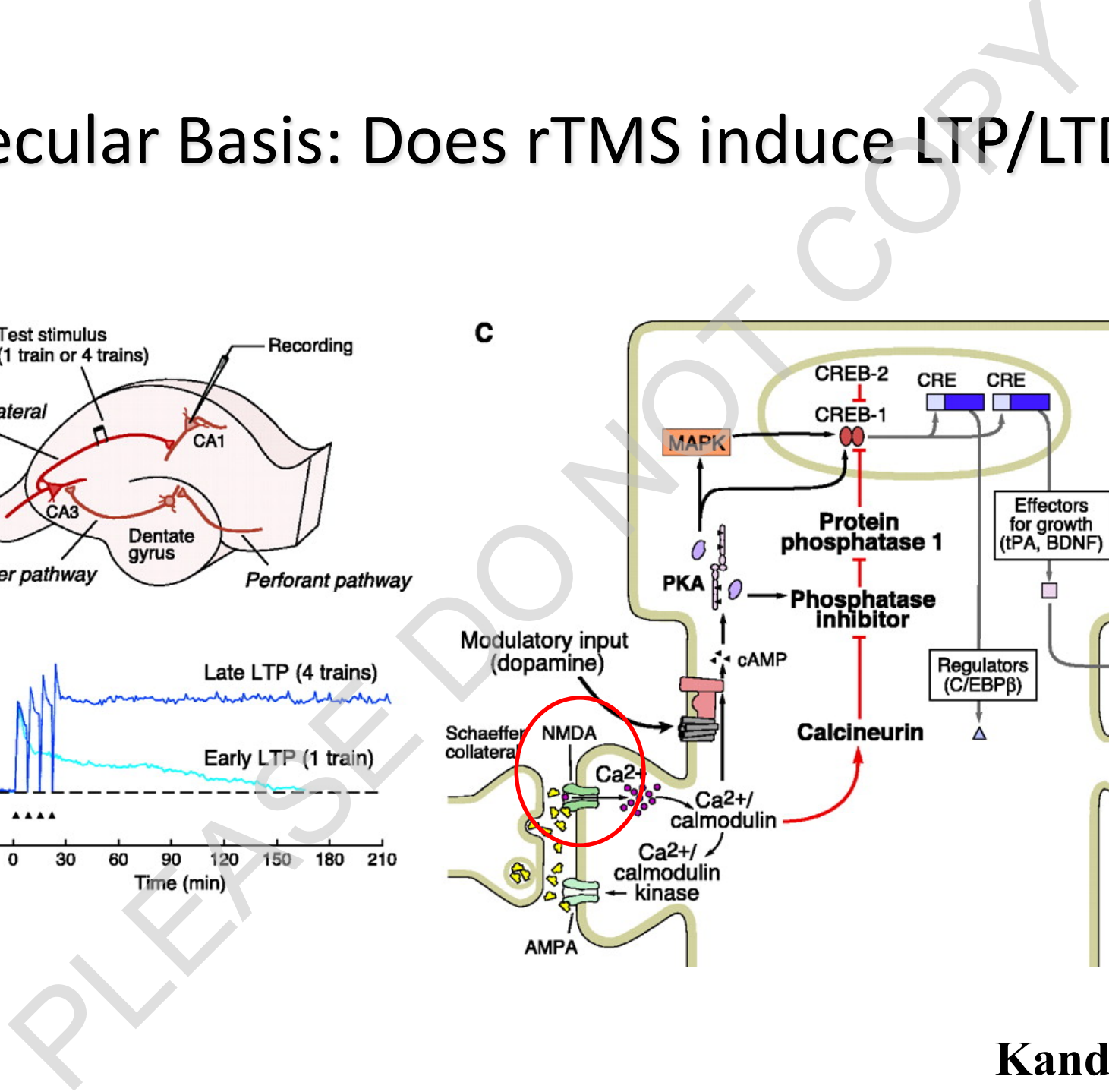
Molecular Signaling Pathways: The bottom part shows a detailed diagram of the molecular signaling pathways involved in LTP/LTD induction. The **Modulatory input (dopamine)** is shown acting on the **Schaeffer collaterals** and **NMDA** receptors. The **Ca²⁺/calmodulin** complex is shown activating **Ca²⁺/calmodulin kinase** and **Calcineurin**. The **Calcineurin** pathway is shown inhibiting **Protein phosphatase 1** and **Phosphatase inhibitor**. The **Protein phosphatase 1** pathway is shown inhibiting **MAPK** and **PKA**. The **PKA** pathway is shown activating **CREB-1** and **CREB-2**. The **CREB-1** and **CREB-2** pathways are shown activating **CRE** and **Regulators (C/EBP β)**. The **Regulators (C/EBP β)** pathway is shown activating **Effectors for growth (tPA, BDNF)**.

Legend: The legend indicates that the **Test stimulus** (1 train or 4 trains) is represented by a blue line, the **Late LTP (4 trains)** is represented by a red line, and the **Early LTP (1 train)** is represented by a green line.

References: The references listed are:

- Kandel ER (2007) *The Molecular Biology of Memory Storage*. New York: Oxford University Press.
- Kandel ER (2007) *The Molecular Biology of Memory Storage*. New York: Oxford University Press.
- Kandel ER (2007) *The Molecular Biology of Memory Storage*. New York: Oxford University Press.

Conclusion: The diagram illustrates the molecular basis of LTP/LTD induction by rTMS, showing the relationship between electrical stimulation, neural activity, and intracellular signaling pathways. The **Calcineurin** pathway is shown to be a key component of the molecular basis of LTP/LTD induction by rTMS.



Molecular Basis: Does rTMS induce LTP/LTD

The diagram illustrates the molecular basis of LTP/LTD induction by rTMS, showing the relationship between electrical stimulation, cellular signaling, and gene expression.

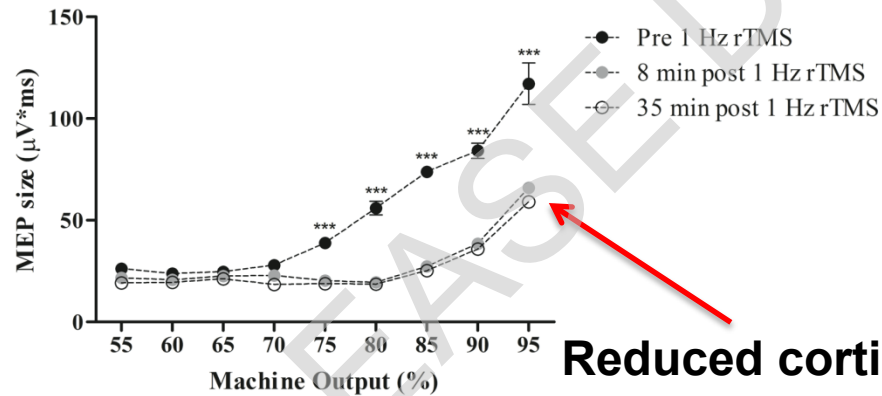
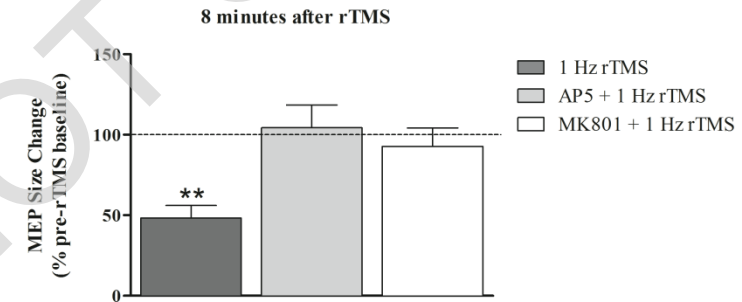
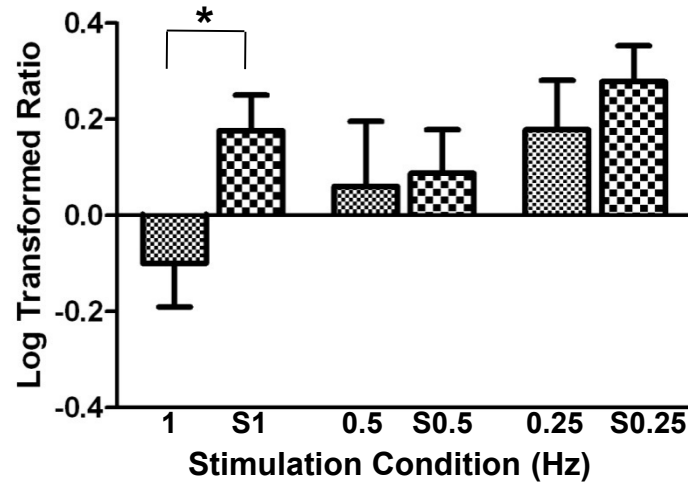
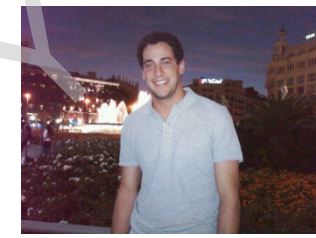
Electrical Stimulation and Recording: A schematic of the hippocampus shows the test stimulus (1 train or 4 trains) applied to the Schaeffer collateral, with recording electrodes in CA1, CA3, and the Dentate gyrus. The Perforant pathway is also indicated. Below the schematic, a graph shows the time course of LTP. The x-axis represents Time (min) from 0 to 210. The y-axis represents the amplitude of the response. The graph shows a sharp increase in response amplitude following the test stimulus (indicated by a dashed line at 0 min), which then decays over time. The 'Late LTP (4 trains)' curve (blue) shows a higher and more sustained response compared to the 'Early LTP (1 train)' curve (cyan).

Cellular Signaling Pathways: A detailed diagram of a dendritic spine shows the following components and pathways:

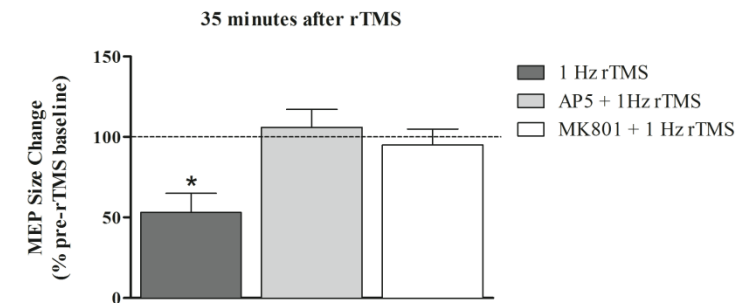
- Modulatory input (dopamine):** Dopamine (DA) binds to a receptor, activating a G-protein coupled receptor (GPCR) pathway that leads to the production of cAMP.
- Calcium Signaling:** NMDA receptor (NMDA) activation leads to an influx of Ca^{2+} into the spine. This Ca^{2+} binds to calmodulin, forming a Ca^{2+} /calmodulin complex. This complex activates a Ca^{2+} /calmodulin kinase (CaMK).
- PKA Pathway:** cAMP activates Protein Kinase A (PKA).
- MAPK Pathway:** PKA activates Mitogen-Activated Protein Kinase (MAPK).
- Protein Phosphatase 1 (PP1):** PKA and MAPK both activate PP1. PP1 is shown to inhibit the Ca^{2+} /calmodulin complex and the CaMK.
- Calcineurin Pathway:** The Ca^{2+} /calmodulin complex activates Calcineurin. Calcineurin is shown to inhibit PP1.
- Gene Expression:** The Ca^{2+} /calmodulin complex and Calcineurin both activate CREB-1. CREB-1, in turn, activates CREB-2, which then activates CRE. CRE is shown to activate Effectors for growth (tPA, BDNF) and Regulators (C/EBP β).

Summary: The diagram shows that rTMS-induced LTP/LTD involves a complex interplay of electrical stimulation, calcium signaling, and gene expression. The late LTP (4 trains) is characterized by a higher and more sustained response compared to the early LTP (1 train). The molecular pathways involve the activation of PKA, MAPK, and PP1, which in turn regulate the Ca^{2+} /calmodulin complex and Calcineurin, leading to the activation of CREB-1 and subsequent gene expression.

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



Reduced cortical excitability



Muller et al., PLOS One 2014



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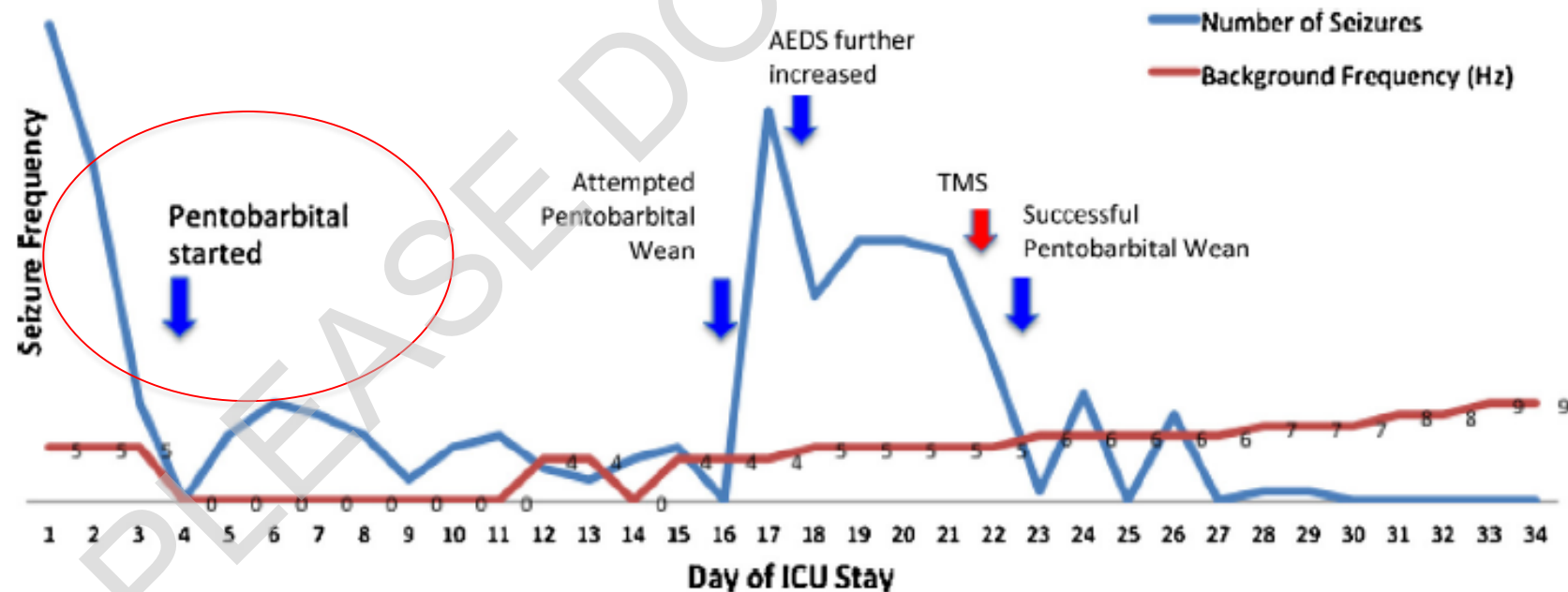


Short Communication

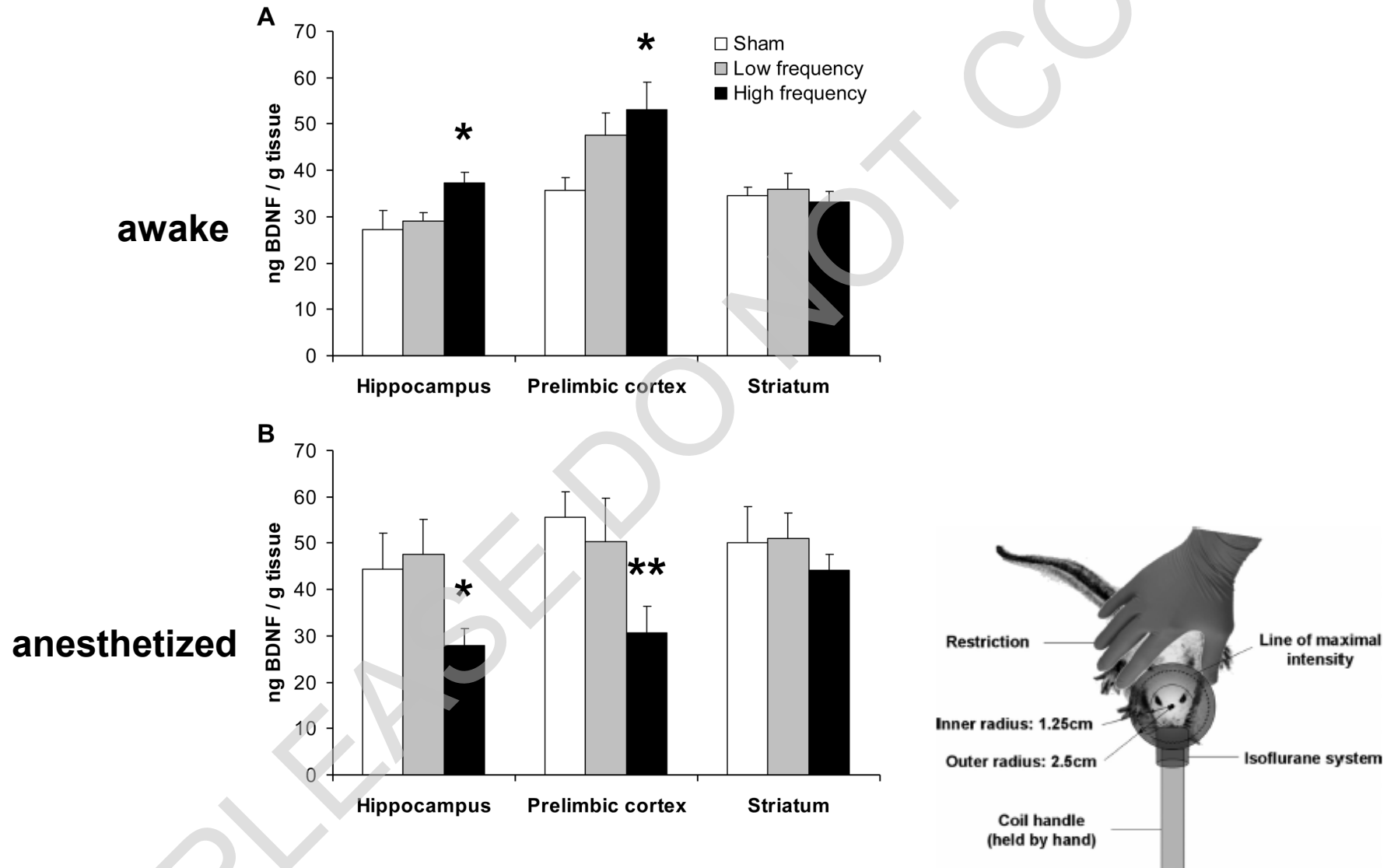
Transcranial magnetic stimulation for refractory focal status epilepticus in the intensive care unit

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

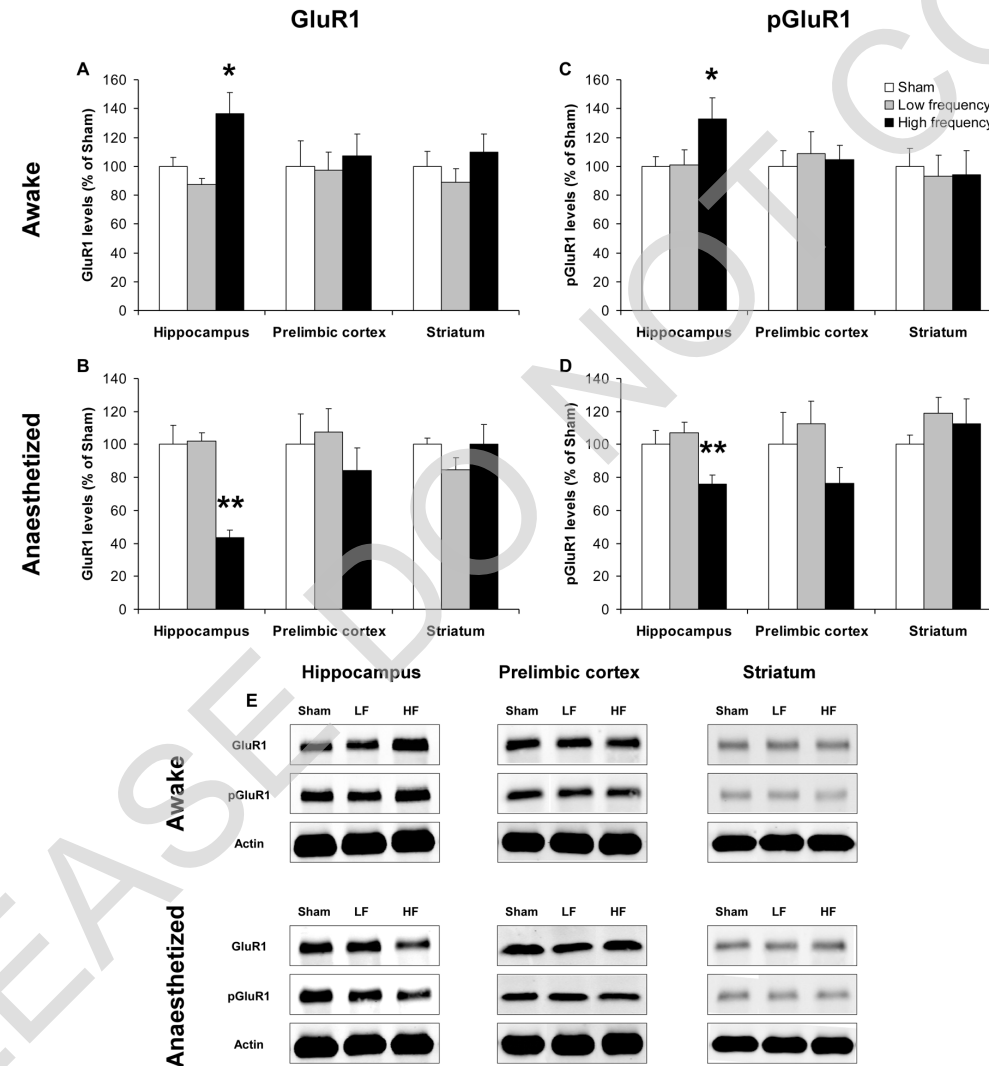
A. Liu et al. / Seizure xxx (2013) xxx–xxx



BDNF expression after rTMS

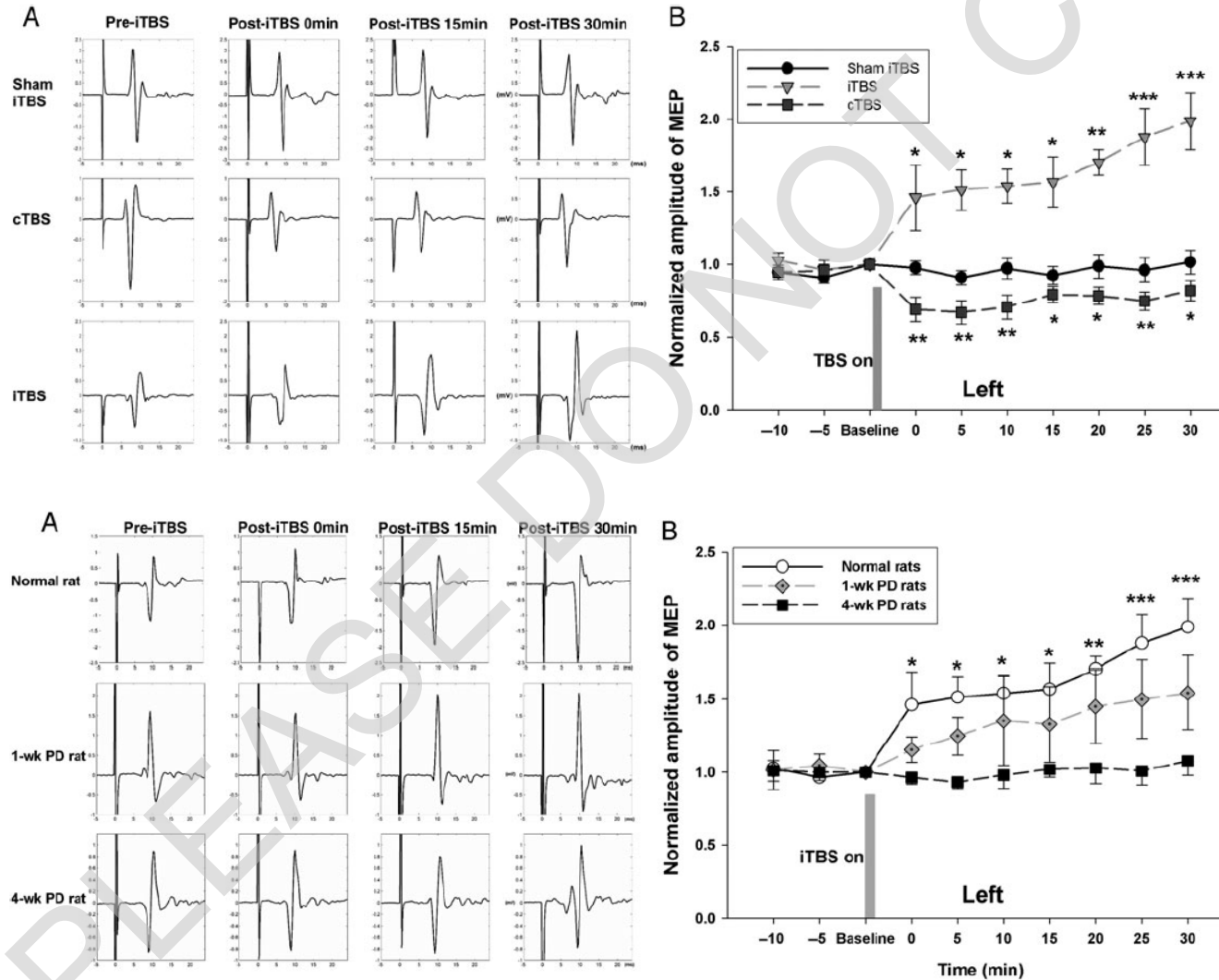


GluR1 expression and phosphorylation after rTMS



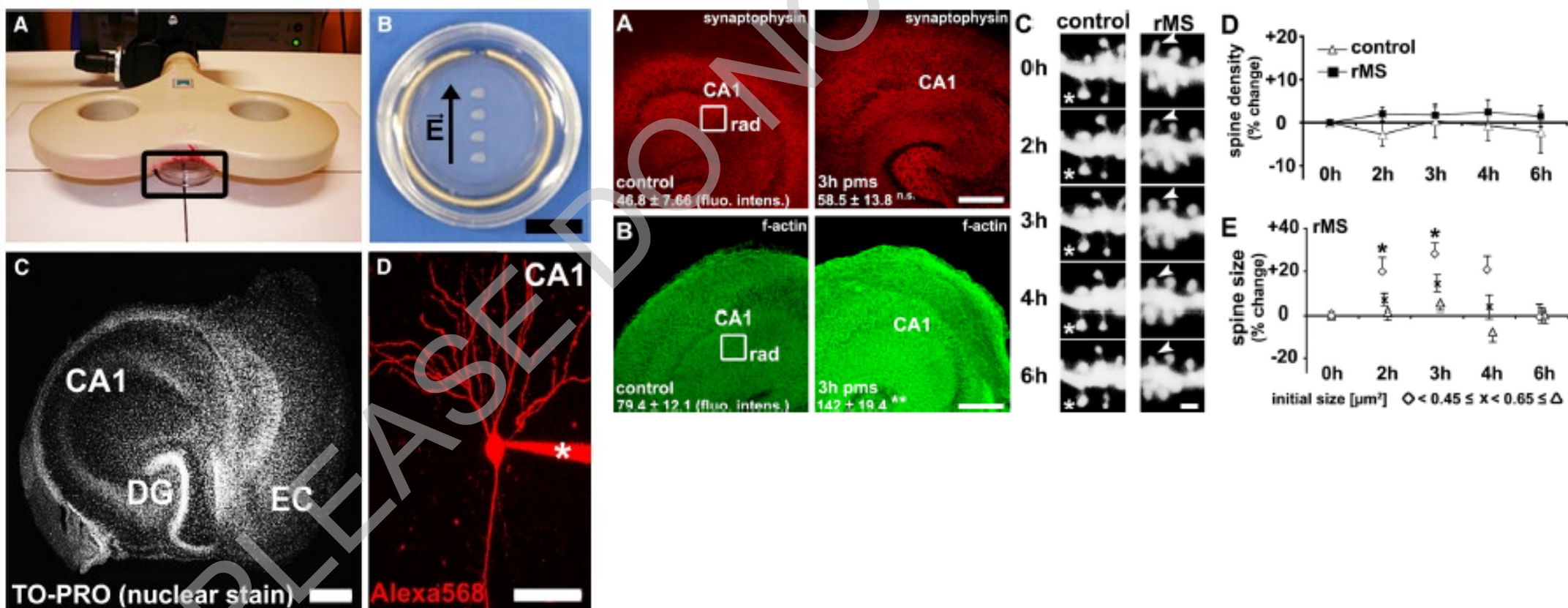
Functional Dopaminergic Neurons in Substantia Nigra are Required for Transcranial Magnetic Stimulation-Induced Motor Plasticity

Tsung-Hsun Hsieh^{1,2,4,†}, Ying-Zu Huang^{6,†}, Alexander Rotenberg⁷, Alvaro Pascual-Leone⁸, Yung-Hsiao Chiang^{1,2,9}, Jia-Yi Wang³ and Jia-Jin J. Chen^{4,5}

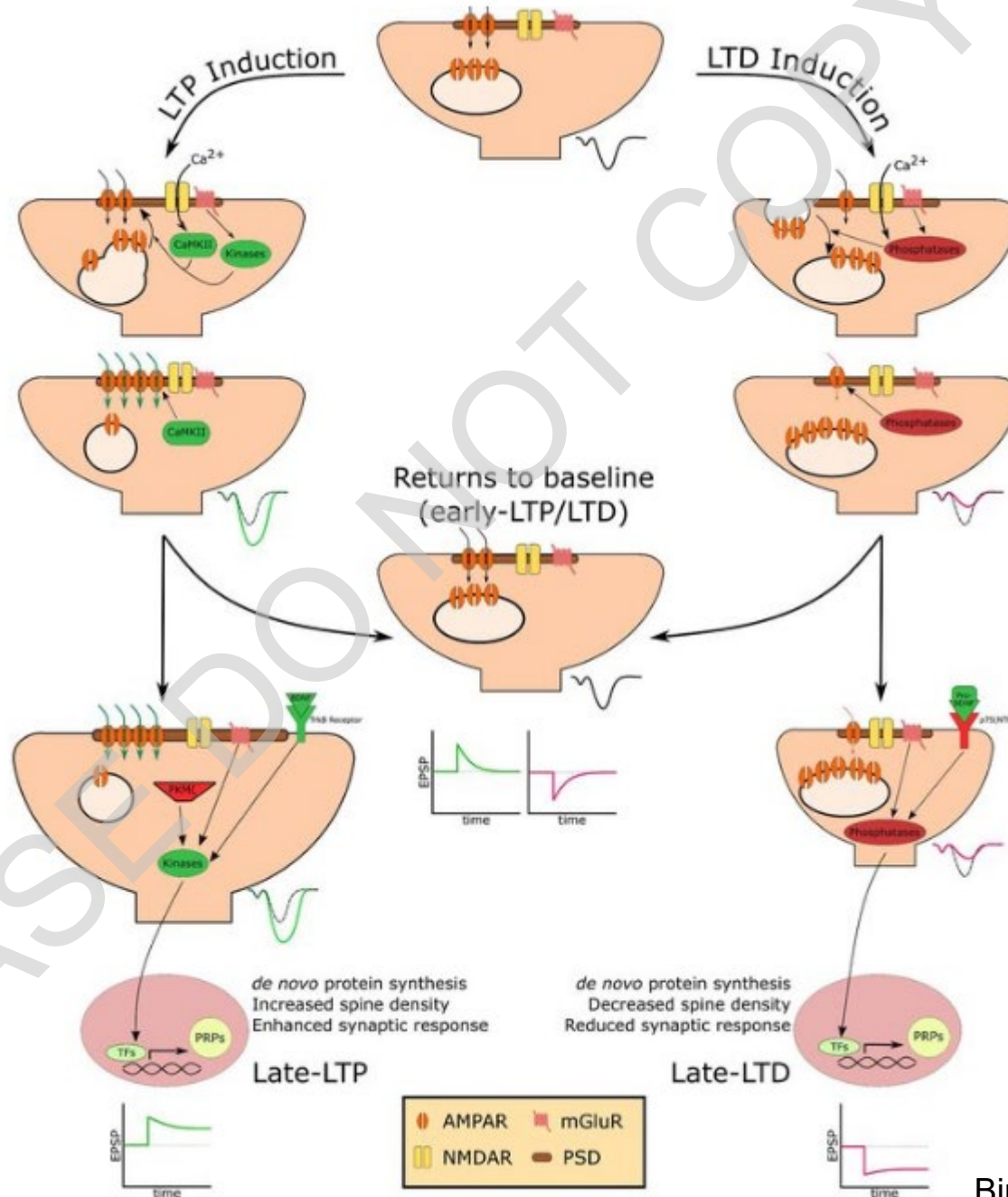


Repetitive Magnetic Stimulation Induces Functional and Structural Plasticity of Excitatory Postsynapses in Mouse Organotypic Hippocampal Slice Cultures

Andreas Vlachos,^{1*} Florian Müller-Dahlhaus,^{1,2*} Johannes Roszkopp,^{1,2} Maximilian Lenz,¹ Ulf Ziemann,^{2,3†} and Thomas Deller^{1†}



In vitro studies may provide insight into how rTMS modifies synapses

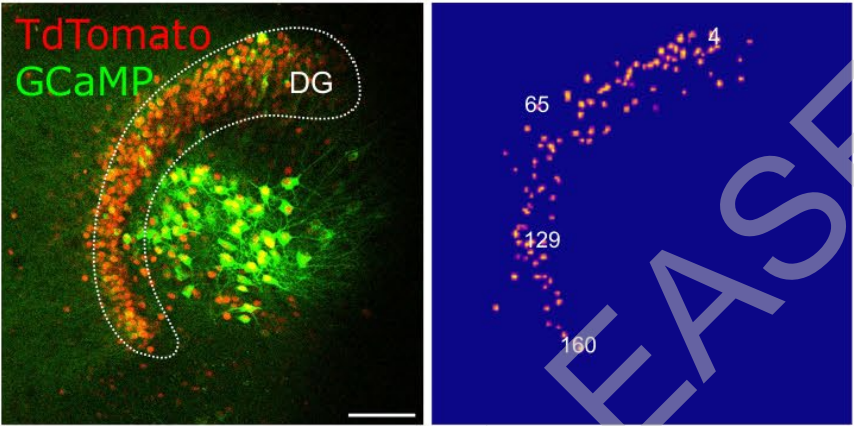


Repetitive magnetic stimulation with iTBS600 induces persistent structural and functional plasticity in mouse organotypic slice cultures

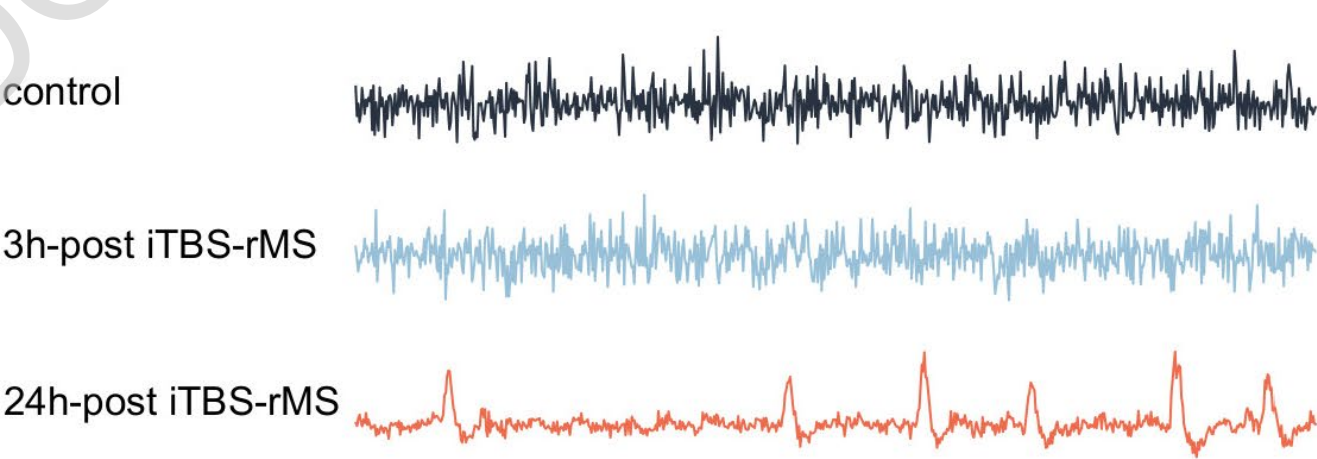
Abbreviated title: iTBS600 induces structural and functional plasticity

Han Lu^{1,2,3,*}, Shreyash Garg^{1,4}, Maximilian Lenz^{1,5}, Andreas Vlachos^{1,2,6,*}

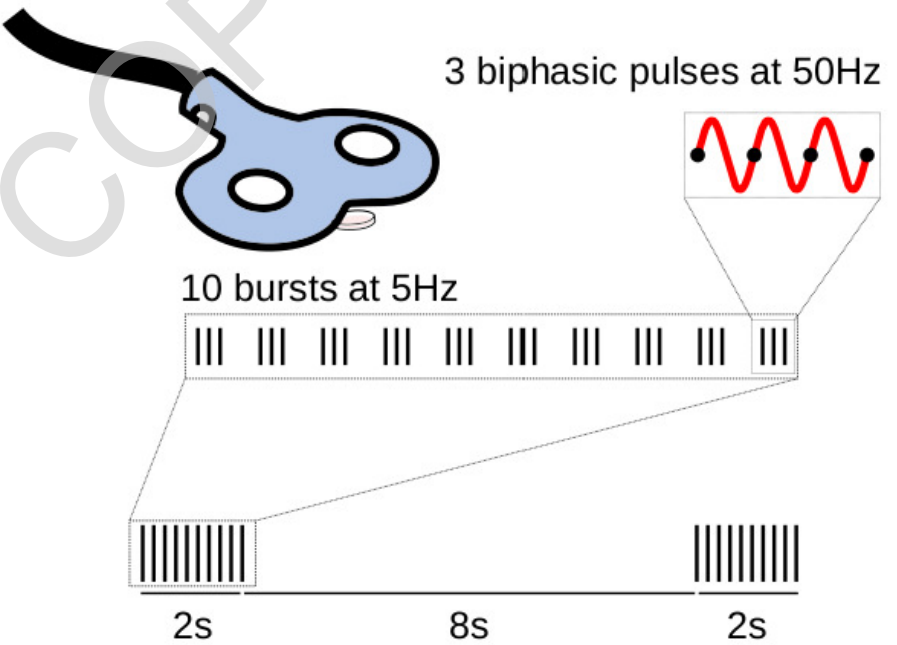
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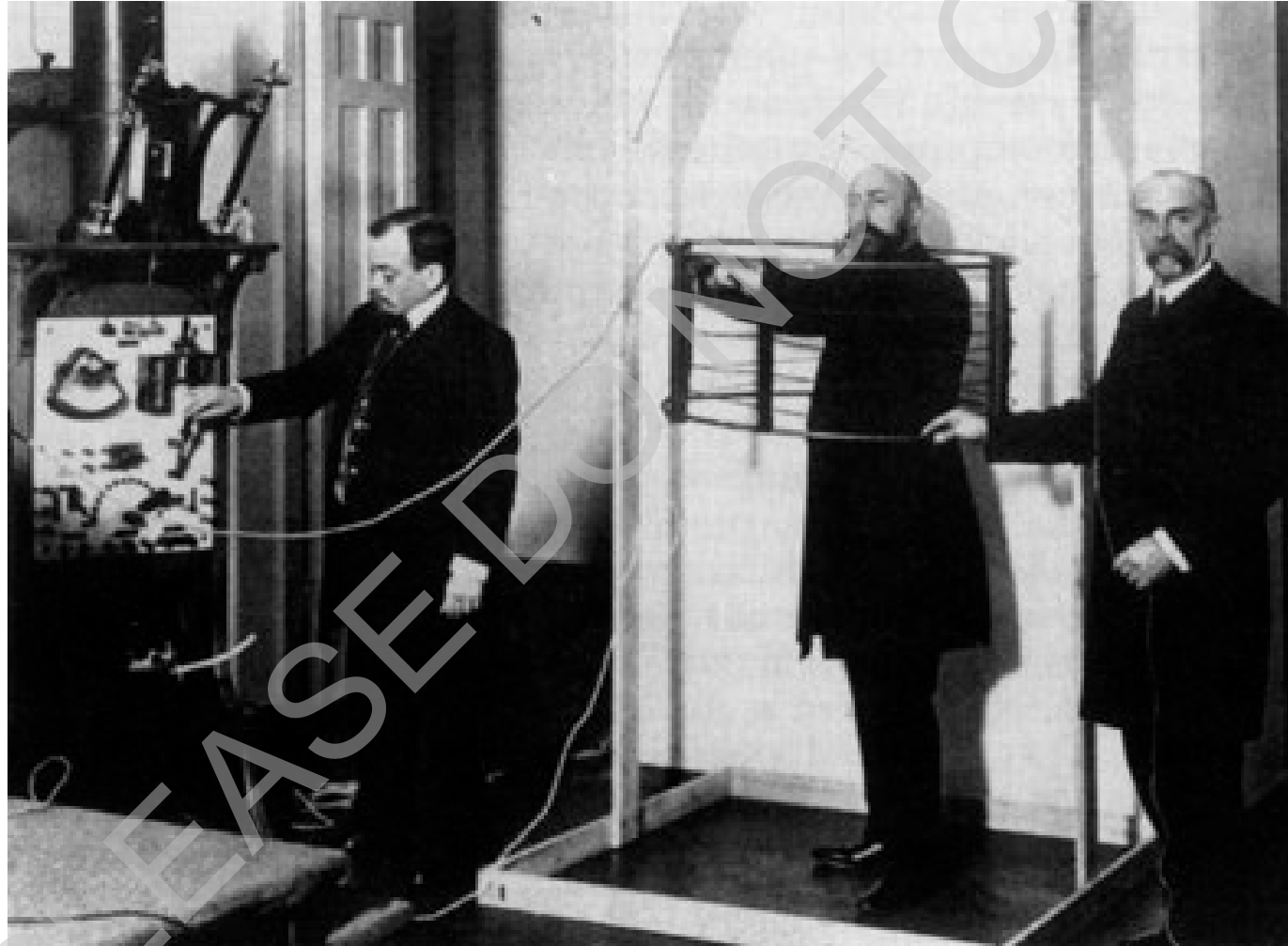
B



A FDA-approved 600-pulse iTBS-rMS



Who's doing the heavy lifting?



Thanks!!!

