


NEUROVATIONS
A PATIENT CARE & INNOVATION COMPANY

DTM
"Differential Target Multiplexed"
Spinal Cord Stimulation
... and how does it work?

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September 2020



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A Patient Care and Innovation Company

NEUROVATIONS

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Patient Care & Innovation Since 1992

| Year | Event |
|---------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1989-90 | Inaugural Napa Pain Conference Dr. Grigely starts one of the first university pain management clinics in the US at UC Davis. |
| 1991-94 | Napa Pain Institute Dr. Grigely is invited to first cohort of pain management by the Board of Anesthesiology. |
| 1997-98 | Clinical Research Leveraging Mayo Clinic training Dr. Grigely becomes Principal Investigator in early stage trials with active involvement in clinical and translational patient care. |
| 2005 | Neurovations! Research and education combine to become Neurovations a patient care and innovation company. |
| 2010-11 | N3 Laboratories Neurovations: The Science debate focused on science and innovation of neurovations. Napa Pain Institute serves as continued medical education venue in various conferences and events. N3 Laboratories is established. |
| 2013-14 | Spine and Pain Center of Kauai The Kauai Clinic is established in part to handle an underserved chronic pain population. |
| 2016 | Redwood Pain Institute Redwood Pain Institute opens in partnership with St. Joseph's Health. |
| 2018-19 | Neurovations Center for Hope The Neurovations Center for Hope begins research and development phase with 3 patients. |

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Clinics which do clinical research

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An innovation company which also provides

medical services

NEUROVATIONS

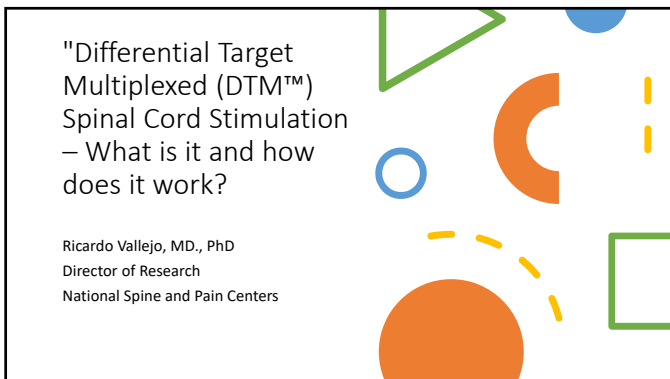
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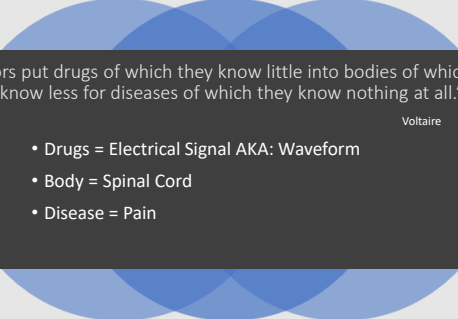




Disclosures

- **Speaker's Bureau:** Avanos & Medtronic
- **Advisory Board:** Medtronic
- **CEO SGX Medical (No commercial interest)**
- **Product Royalties:** N/A
- **Equity:** SGX Medical
- **Company employee:** National Spine and Pain Centers

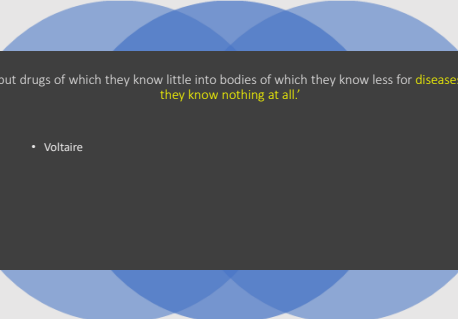
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'Doctors put drugs of which they know little into bodies of which they know less for diseases of which they know nothing at all.'

Voltaire

- Drugs = Electrical Signal AKA: Waveform
- Body = Spinal Cord
- Disease = Pain



'Doctors put drugs of which they know little into bodies of which they know less for diseases of which they know nothing at all.'

- Voltaire

Pain is the Disease

- How pain transition from acute to chronic
- Role of Neuroinflammation

'Doctors put drugs of which they know little into bodies of which they know less for diseases of which they know nothing at all.'

- Voltaire

Glial cells greatly outnumber neurons in the spinal cord

Outnumber Neurons 12:1

Journal of Anatomy

Glia to neuron ratio in the posterior aspect of the human spinal cord at thoracic segments relevant to spinal cord stimulation

Amparo Ruiz-Sanz¹, Jorge M. Oñativia-Vallés^{1,2}, Arantxa Blasco-Serra⁴, Carlos Romero-Torres^{2,3}, David L. Ceballos^{1,4}, General Espinosa-Oliveros¹, Alfonso A. Valero-Cabré^{1,5}, Ramon Benayán^{1,6,7} and Ricardo Vallejo^{1,8,9}

- SCS electrical pulses reach glial cells in addition to neurons

'Doctors put drugs of which they know little into bodies of which they know less for diseases of which they know nothing at all.'

- Voltaire

What are the effects of the Electrical Signal on the neural tissue?

We can do the same!!!

Fc = 20 Hz to 10 kHz

Target

Burst pulses = 4?, 57, 67, 7?

I = Subthreshold
Suprathreshold

PW = 200 to 1,000 μ secs.

Phases = Active recharge balance
Passive recharge balance

Fig. 3. Membrane potential of a neuron during electrical stimulation. (A) Membrane potential (mV) vs. time (ms) showing subthreshold and suprathreshold responses. (B) Membrane potential (mV) vs. time (ms) showing active and passive recharge balance phases. (C) Plot of membrane potential (mV) vs. time (ms) showing burst pulses. (D) Plot of membrane potential (mV) vs. time (ms) showing current pulse patterns. (E) Plot of membrane potential (mV) vs. time (ms) showing different stimulation frequencies. (F) Plot of membrane potential (mV) vs. time (ms) showing current pulse patterns. (G) Plot of membrane potential (mV) vs. time (ms) showing glutamate release from stimulated astrocytes.

Glial Cells Respond to Electrical Stimuli...

GLIA: Membrane depolarization, neurotransmitter release

A Neuron

B Glia

A Different Stimulation Frequencies

A Current Pulse Pattern

B Glutamate Release Current

Glutamate is released from stimulated astrocytes (Agnese et al. (2010), J. Neural Eng.)

Roitbak & Farnedjian, 1981, Neuroscience

M1

Molecular Mechanisms of SCS for pain: Transcriptomics, Proteomics and Cell Functionality

- Previous MoA studies focused on neurons and their AP
- MoA should account for biological processes affected by the electric fields (beyond gate theory)
- Chronic pain results as an unbalance of key **Neuro-Glial Interactions**
- **ELECTRICAL FIELD, APPLIED IN THE RIGHT WAY, MAY BE USED TO HELP TO BALANCE IT**

From National Human Genome Research Institute (genome.gov)

MECHANICAL HYPERSENSITIVITY

Fig 2 Experimental design and timeline

Fig 3 Scheme of phosphoproteomic protein purification and analysis

Transcriptomics – Rodent Models in SCS

| Model | FCR value (up/down) | Relevant GO (up/down) | Relevant significant genes and fold-changes (in parentheses) |
|-------|---------------------|-------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1 | up | Inflammatory response, muscle waste | SNK-1, CN-1, CYP-1, CYP-2, CYP-3, CYP-4, CYP-5, CYP-6, CYP-7, CYP-8, CYP-9, CYP-10, CYP-11, CYP-12, CYP-13, CYP-14, CYP-15, CYP-16, CYP-17, CYP-18, CYP-19, CYP-20, CYP-21, CYP-22, CYP-23, CYP-24, CYP-25, CYP-26, CYP-27, CYP-28, CYP-29, CYP-30, CYP-31, CYP-32, CYP-33, CYP-34, CYP-35, CYP-36, CYP-37, CYP-38, CYP-39, CYP-40, CYP-41, CYP-42, CYP-43, CYP-44, CYP-45, CYP-46, CYP-47, CYP-48, CYP-49, CYP-50, CYP-51, CYP-52, CYP-53, CYP-54, CYP-55, CYP-56, CYP-57, CYP-58, CYP-59, CYP-60, CYP-61, CYP-62, CYP-63, CYP-64, CYP-65, CYP-66, CYP-67, CYP-68, CYP-69, CYP-70, CYP-71, CYP-72, CYP-73, CYP-74, CYP-75, CYP-76, CYP-77, CYP-78, CYP-79, CYP-80, CYP-81, CYP-82, CYP-83, CYP-84, CYP-85, CYP-86, CYP-87, CYP-88, CYP-89, CYP-90, CYP-91, CYP-92, CYP-93, CYP-94, CYP-95, CYP-96, CYP-97, CYP-98, CYP-99, CYP-100 |
| 2 | down | Ion channel regulation, synaptic plasticity, Generation of neurons, synaptic transmission | SNK-1, CN-1, CYP-1, CYP-2, CYP-3, CYP-4, CYP-5, CYP-6, CYP-7, CYP-8, CYP-9, CYP-10, CYP-11, CYP-12, CYP-13, CYP-14, CYP-15, CYP-16, CYP-17, CYP-18, CYP-19, CYP-20, CYP-21, CYP-22, CYP-23, CYP-24, CYP-25, CYP-26, CYP-27, CYP-28, CYP-29, CYP-30, CYP-31, CYP-32, CYP-33, CYP-34, CYP-35, CYP-36, CYP-37, CYP-38, CYP-39, CYP-40, CYP-41, CYP-42, CYP-43, CYP-44, CYP-45, CYP-46, CYP-47, CYP-48, CYP-49, CYP-50, CYP-51, CYP-52, CYP-53, CYP-54, CYP-55, CYP-56, CYP-57, CYP-58, CYP-59, CYP-60, CYP-61, CYP-62, CYP-63, CYP-64, CYP-65, CYP-66, CYP-67, CYP-68, CYP-69, CYP-70, CYP-71, CYP-72, CYP-73, CYP-74, CYP-75, CYP-76, CYP-77, CYP-78, CYP-79, CYP-80, CYP-81, CYP-82, CYP-83, CYP-84, CYP-85, CYP-86, CYP-87, CYP-88, CYP-89, CYP-90, CYP-91, CYP-92, CYP-93, CYP-94, CYP-95, CYP-96, CYP-97, CYP-98, CYP-99, CYP-100 |

CCI (29 days), 50 Hz, 200 μ s PW, 80%MT, 7 sessions of 2h over 3d Ipsilateral SC caudal to stim (L4-L6) (Stephens et al. 2018, Mol. Pain)

SNI (7 days), 50 Hz, 20 μ s PW, 70%MT for 72h cont. Ipsilateral SC below stim (L1-L2) (Vallejo et al. 2016, Neuromodulation)

Despite differences in models and designs, both studies concluded that:

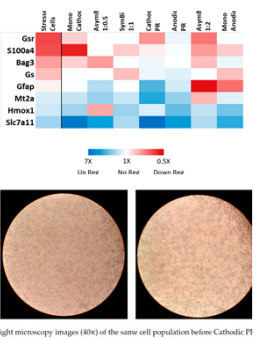
- SCS at low frequency involves modulation of gene expression associated with immune and inflammatory response and synaptic signaling.
- Glial cells are involved.

Slide 19

M1 Last bullet rephrased slightly
M, 6/25/2019

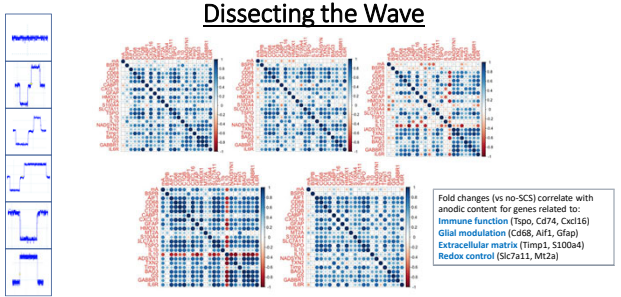
Invitro

- Immune function → Gfap
- Synaptic transmission → Slc7a11 & Glul
- Neuroprotection → S100a4
- Oxidative stress processes → Mt2a, Gsr, Hmox1
- Cell adaptive responses to stressful stimulation → Bag3.



Light microscopy images (40x) of the same cell population before Cathodic PI (right).

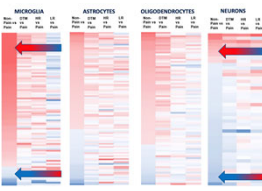
Dissecting the Wave



Correlation diagrams illustrating relationships between gene expression, SCS Current (mA), and Behavioral Score in % of Baseline (BSFB).
 A: Sham, B: No-SCS (SM), C: biphasic symmetric SCS, D: monophasic cathodic SCS, E: monophasic anodic SCS, F: asymmetric biphasic SCS 1:2, G: asymmetric biphasic 1:8.5. The blue dots represent a positive correlation and red dots represent a negative correlation. The size and darkness of the dot is proportional to the value of the Pearson correlation coefficient.

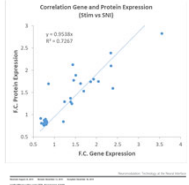
Differential Target Multiplex best modulates Cell Specific Glial and Neuronal Cell Gene Expression back towards the non-pain state.

| | Microglia | Astrocytes | Oligodendrocytes | Neurons |
|------------------------|------------|------------|------------------|--------------|
| # of genes in data set | 101 | 188 | 154 | 72 |
| Correlation with DTM | Strong (+) | Strong (+) | Strong (+) | Strong (+) |
| Correlation HR | Strong (+) | Weak (+) | Weak (+) | Moderate (+) |
| Correlation LR | Weak (-) | NS | NS | Weak (-) |

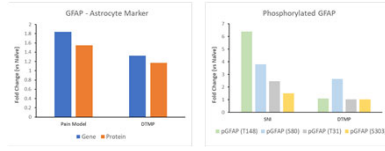


Correlated Gene and Protein Expression

• Gene expression modulated by SCS correlates proportionately to protein expression changes (Stim vs. SNI, R² = 0.73)



Example: GFAP gene and protein expression increased by pain model and modulated toward naive levels by DTM programming. Phosphorylated GFAP proteins are also increased by pain and modulated downward by DTM programming.



Proteomic Modulation in the Dorsal Spinal Cord Following Spinal Cord Stimulation Therapy in an In Vivo Neuropathic Pain Model
Bassu A, Foley R, Chatterjee S, Lee W, Patel S, Cocco SP, Roehrig B, Kelly MP, Lempert K, PhD, Rowan G. *Mol Cell Neurosci*.

The pain model upregulated 1,252 and down-regulated 896 phosphoproteins by at least 2.5-fold relative to naive levels.

Upregulated Proteins involved in transport, signaling, glutamate binding mediated activation, and ECM regulation

Downregulated involved in endocytosis, membrane trafficking, protein interaction at synapses, signaling, and activation of NMDA receptors

DTMP reversed expression of 52% of these by at least 2.5-fold in the direction of naive expression

40% of these had expression values within 25% of the naive levels.

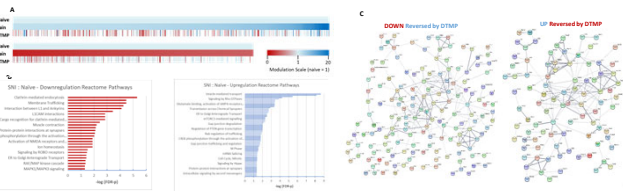


Fig 4. Heat maps showing modulation of phosphorylated proteins by DTMP relative to changes by the pain model (Stim) and naive expression (naive) (naive vs. Stim, in yellow). (B) Bar graphs showing significantly enriched pathways (FDR $p < 0.05$) containing proteins downregulated by upregulation in the pain model (stim vs naive) or upregulated by downregulation in the pain model (naive vs stim) that were reversed by DTMP within 25% of the naive expression.

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THE SCIENCES

Mother Nature's Medicine Cabinet

Scientists scour the earth in search of miracle drugs

By Kate Wong on April 9, 2001

- Aspirin extracted from: *Salix Alba*, *Spirea spp.*, and *Betula*
- Quinine (*Cinchona colisaya*)
- Opiates *Papaveracea somniferum*
- Dogoxin (Foxglove plant)
- Pacitaxe! (Bark of pacific Yew)
- Vincristine & Vinblastine (Madagascar periwinkle)
- Atropine, scopolamine (*Belladonna*)



Developments in Neuroscience over the last few decades has shown us that neuroinflammation and glial cells are pivotal in the development and maintenance of neuropathic pain

Different components within the waveform have differential effects in pain related biological processes

Cell-specific modulation may be obtained by multiplexed signals

Modulation of neuron-glia interactions can be achieved


Molecular technology may help us unravel the effects of the different components of the waveform on neural tissues

Differential Target Multiplex is supported by strong preclinical data



Questions?

Thank you for attending!

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Eric Grigsby MD, MBA
CEO and Founder of Neurovations

Join the Conversation:
Thursday
September 24, 2020
5:30 PDT/8:30 EDT

Register at
[Neurovations.com/webinars/](https://neurovations.com/webinars/)



David Caraway MD, PhD
Chief Medical Officer, Neuro