Orthopedic surgery modulates neuropeptides and BDNF expression at the spinal and hippocampal levels

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Agenda

1) Introduction – status quo
2) Aims of the study
3) Materials/Methods
4) Results
5) Discussion
6) Summary – Own opinion
Agenda

1) Introduction – status quo
   • Background
   • Pain
   • Tibial fracture
   • Neurotrophins
   • Problem to be solved

2) Aims of the study

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6) Summary – Own opinion
Background - pain

• Many forms of pain
• Actual circumstances, expectation, stress, emotions....
• Pain critical component of recovery
  • Hindering recovery after surgery
  • Longer periods of rehabilitation and immobilisation

• Complex pathway
  • Periphery -> central nervous system (CNS)
Background – pain - anatomy

Noxious stimulus

Touch

Mechanoreceptors

Primary afferent neurones ($A_\beta$ fibres)

Primary afferent neurones ($A_\delta$ and C fibres)

Cognitive activities

Sensory cortex

Limbic system

Thalamus

Secondary afferent neurones

Descending pathway

Dorsal horn

Dorsal root ganglion

Spino-thalamic tract

Ventral horn
Background

• Neuropathic pain
  • Caused by neuronal damage
  • Abnormal sensations – paresthesia
  • Normal non-painful stimuli – allodynia

• Current treatment:
  • Opioids
  • Anticonvulsants
Background

- Postoperative pain after surgery
  - into chronic pain
  - Reduction of life quality
  - 50% of hip-fracture repair patients
  - Acute confusional state (delirium)
- Recent studies
  - Hippocampal abnormalities in animal models
  - Reduction in elderly patients with chronic pain
  - Changes in regional brain volume related to postoperative cognitive dysfunction (POCD)

Background

• Tibial fracture mouse model – intermedullary pinning

• well established neuropathic pain model
Background

• After fracture
  • Excessive substance P signalling
  • and regional inflammatory response
    • Release of systemic proinflammatory cytokines
      • TNF alpha
      • IL 1 beta
  • Mouse model (tibial fracture)
    • Similar proinflammatory changes
    • Activation of nuclear factor κB signalling in macrophages
    • Blood-brain barrier permeability changes
    • Hippocampal neuroinflammation
    • Subsequent cognitive impairment

Neurotrophins

• Family of proteins that induce
  • Survival, development, function of neurons

• NGF

• Neurotrophin 3&4

• Brain derived neurotrophic factor (BDNF)
  • Wide range of central functions and neuronal plasticity
  • Cell survival
  • Growth and differentiation neurons and synapses
  • Migration
  • Learning and memory
  • Active in hippocampus, cortex,...
Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor

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Human endothelial cells secrete neurotropic factors to direct axonal growth of peripheral nerves

Jonathan M. Grasman & David L. Kaplan
BDNF

Profile of neurotrophic factors in ApoSec (Secretome of apoptotic leukocytes) and animal treated with ApoSec
Aim of the study

• Characterization of the effects of tibial fracture with intramedullary pinning in the primary somatosensory system

• Analyse markers in dorsal root ganglia, spinal cord, brain regions (hippocampus)
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Methods

• Adult male, C57BL/6 wildtype mice
  • Tibial fracture surgery with intramedullary pinning was performed
  • Transection of sciatic nerve
• 2 hours up to 2 weeks
• Behavior tests
  • Frey filaments
  • Safety pin – mechanical hyperalgesia
  • Acetone – cold allodynia
Methods

- Immunohistochemistry
- In situ-hybridization
  - Plasmid DNA containing RNA probes specific for mouse BDNF
- RT-qPCR
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Cold allodynia triggered by unilateral tibial fracture.
ATF3

• Activating transcription factor 3
• mammalian activation transcription factor/cAMP responsive element-binding (CREB) protein family
• ATF3↑
  • Physiological stress in various tissues
  • Marker for regeneration
    following injury of dorsal root ganglion neurons

Activation of ATF3 in DRGs after unilateral tibial fracture.

Ming-Dong Zhang et al. PNAS 2016;113:43:E6686-E6695
Activation of ATF3 in motor neurons after unilateral tibial fracture.

Ming-Dong Zhang et al. PNAS 2016;113:43:E6686-E6695
Activation of ATF3 in Schwann cells after unilateral tibial fracture.
Galanin-LI

- Neuropeptide
- Widely expressed
  - Brain, spinal cord, and gut of mammals
- Modulation and inhibition of action potential neurons
- DRG cells remove galanin – impaired ability to extend neurites in culture
- Adult mutant mice showed 35% less capacity of regenerating the sciatic nerve after injury
- Emerged as an injury marker

Bartai, T (2000); Berger A, et al. (2005); White HS, et al. (2009)
NPY

- Neuropeptide Y
- Various physiological and homeostatic functions
  - In CNS and PNS
- Synthesized in GABAnergic inhibitory neurons
- Acts as neurotransmitter
- High concentrations in hypothalamus and hippocampus
- Play an important role in cell neurogenesis in various brain parts
- known – strongly upregulated in seizure

Heilig M, Widerlöv E (1995); Decressac M et al. (2012); Tatemoto (2004)
Increased expression of galanin, NPY, and BDNF in DRG neurons after unilateral tibial fracture.

Ming-Dong Zhang et al. PNAS 2016;113:43:E6686-E6695
C-Fos

- Protooncogen
- Part of the transcription factor AP-1
- Indirect marker for neuronal activity
  - Because often expressed when neurons fire action potentials

Modulation of c-Fos expression in the spinal cord and hippocampal formation after unilateral tibial fracture.

Ming-Dong Zhang et al. PNAS 2016;113:43:E6686-E6695
BDNF and neuropeptide expression in the brain after unilateral tibial fracture.

Dyn: dynorphin  
CCK: cholecystokinin  
ENK: enkephalin
BDNF and neuropeptide expression in the brain after unilateral tibial fracture.

- DCX (Doublecortin)
  - Microtubule associated protein

Ming-Dong Zhang et al. PNAS 2016;113:43:E6686-E6695
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Discussion

• Tibial fracture mice model
  - three different pain behavior levels
    • Only able to detect cold allodynia
    • Short period study?

• Other groups – various cognitive tests and associated dysfunction with BDNF signaling
  • Reported decreased BDNF protein levels
  • Distinct differences
    • Species, injury model, analysis method, and time course
    • -> may explain conflicting results
Discussion

• Transferable in humans?
• Support long-term cognitive deficits from surgery?
• Limitation of rodent models
• Sex differences
  • Previous studies showed distinctions
• Future studies are needed
  • Clarify role of BDNF
    • In pain signaling and memory function
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Summary

• Comparison
  • Orthopedic surgery model
  • Classical nerve injury model (sciatic nerve transection)
• Changes in pain behaviour up to 2 weeks
• Analysis of pain-related and other markers
  • Somatosensory system
  • Brain (Hippocampus)
My opinion

• Sham group?
• Influence of stress/ anaesthesia/ anaesthesia time surgery time?
• Translation into humans?
• Neuropathic pain models relevant?
• Impact on SCI patients?
THANK YOU FOR LISTENING!

ANY QUESTIONS?
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