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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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)

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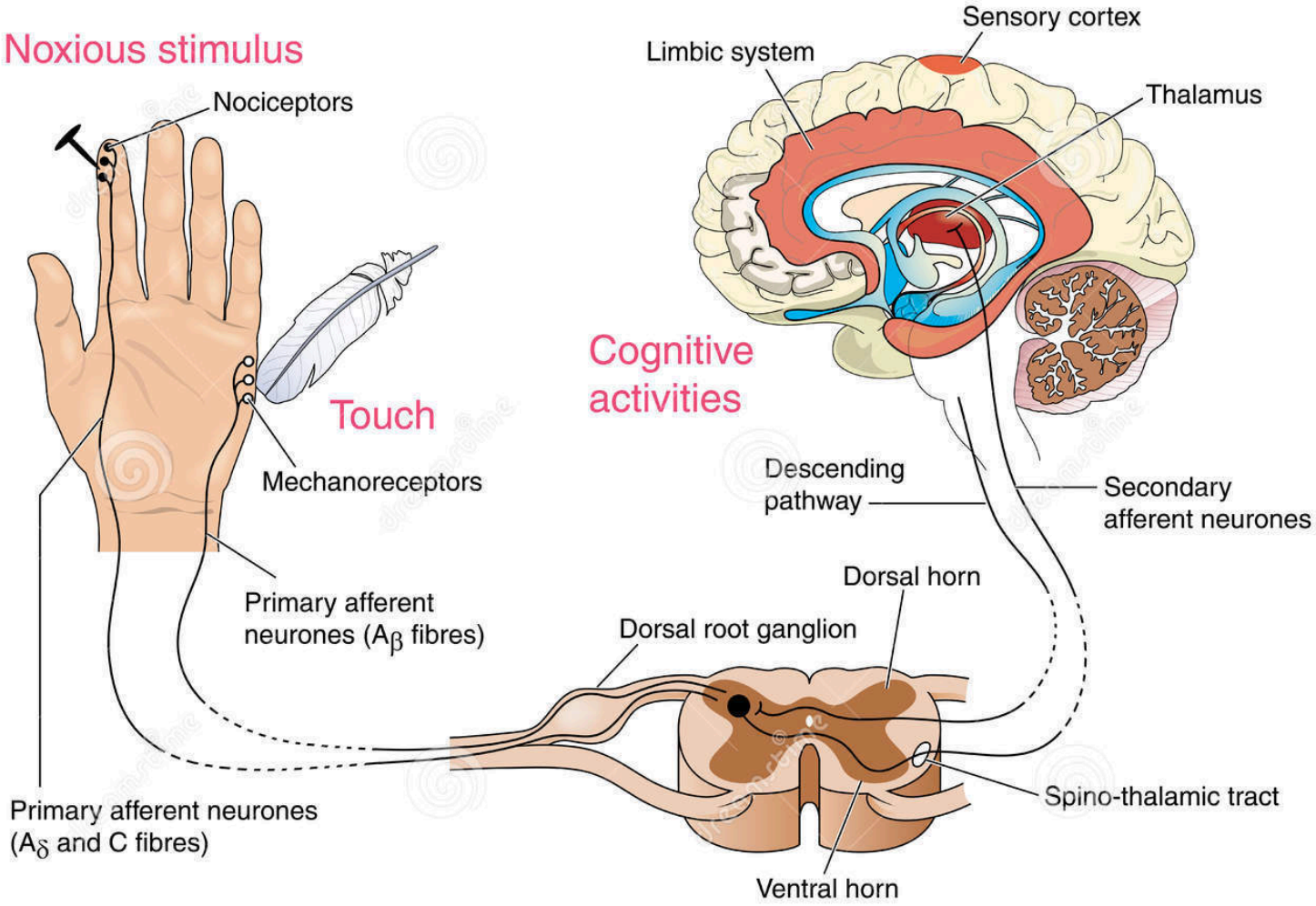
Background – pain - anatomy



Background

- **Neuropathic pain**
  - Caused by neuronal damage
  - Abnormal sensations – paresthesia
  - Normal non-painful stimuli – allodynia
  - Current treatment:
    - Opioids
    - Anticonvulsants

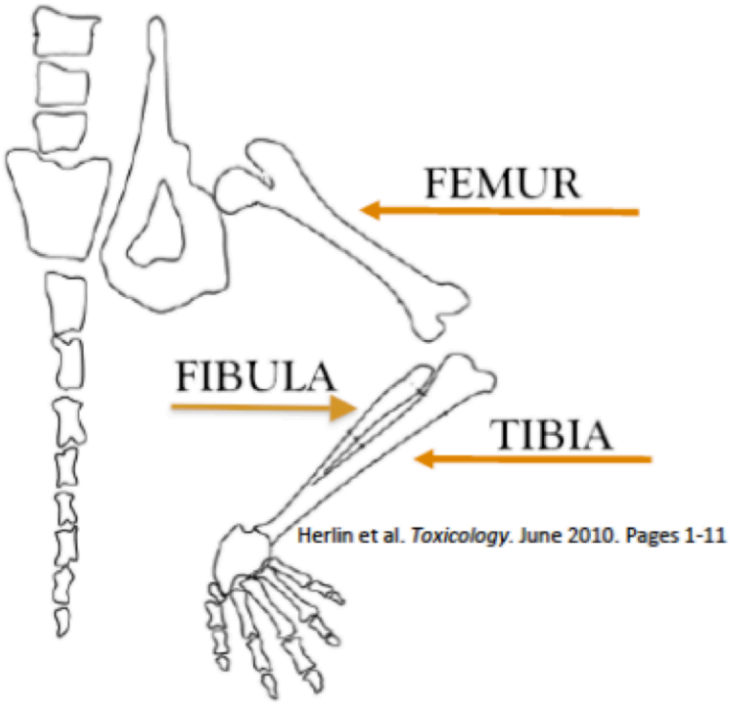
Background

- **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)

Ren WJ, et al. (2011); Zimmermann ME, et al. (2009); Mutso AA, et al. (2012); Kline RP, et al. (2012)

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

Sabsovich I, et al. (2008); McCaffrey G, et al. (2014); Sabsovich I, et al. (2008); Li WW, et al. (2009) Terrando N, et al. (2010), Terrando N, et al. (2011), Lu SM, et al. (2015)

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,...

Binder DK, Scharfmann HE (2004),Zheng et al. (2012), Altmann P, et al. (2014),

BDNF

FEBS 23442

FEBS Letters 470 (2000) 113–117

Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor

Takeshi Nakahashi, Hironobu Fujimura, C. Anthony Altar, Jess Li, Jun-ichi Kambayashi, Narendra N. Tandon, Bing Sun*

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

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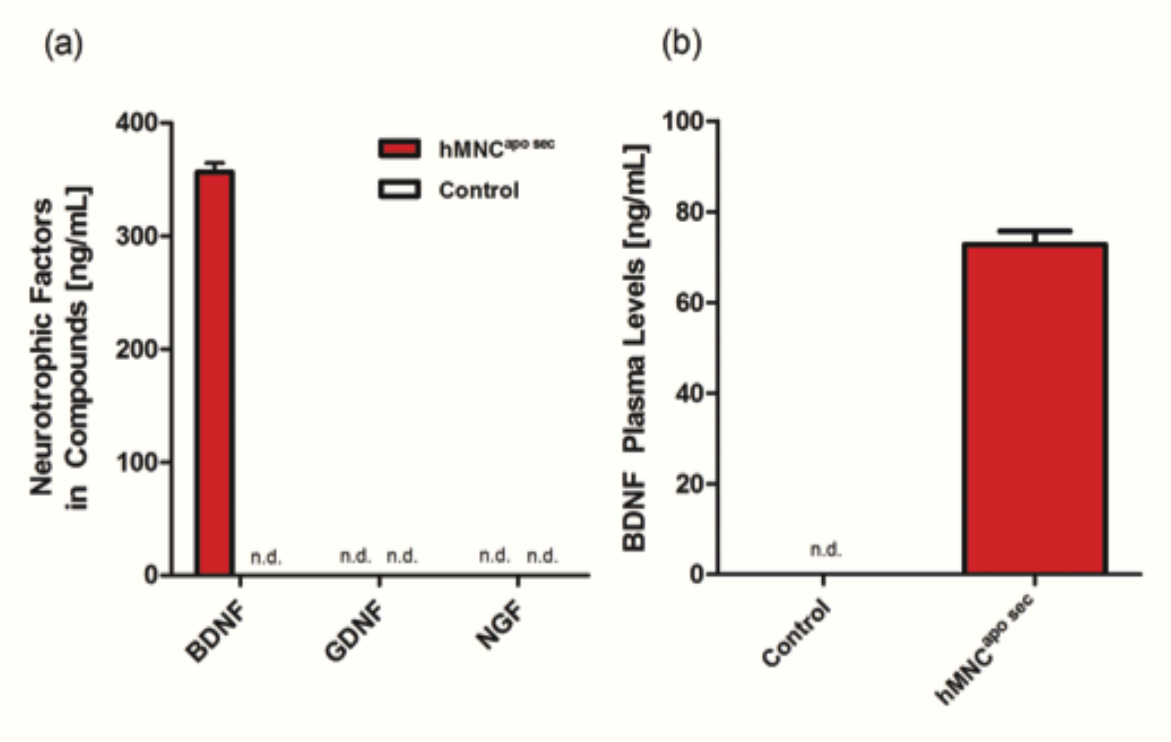
Jonathan M. Grasman & David L. Kaplan

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 specifice for mouse BDNF
- RT-qPCR

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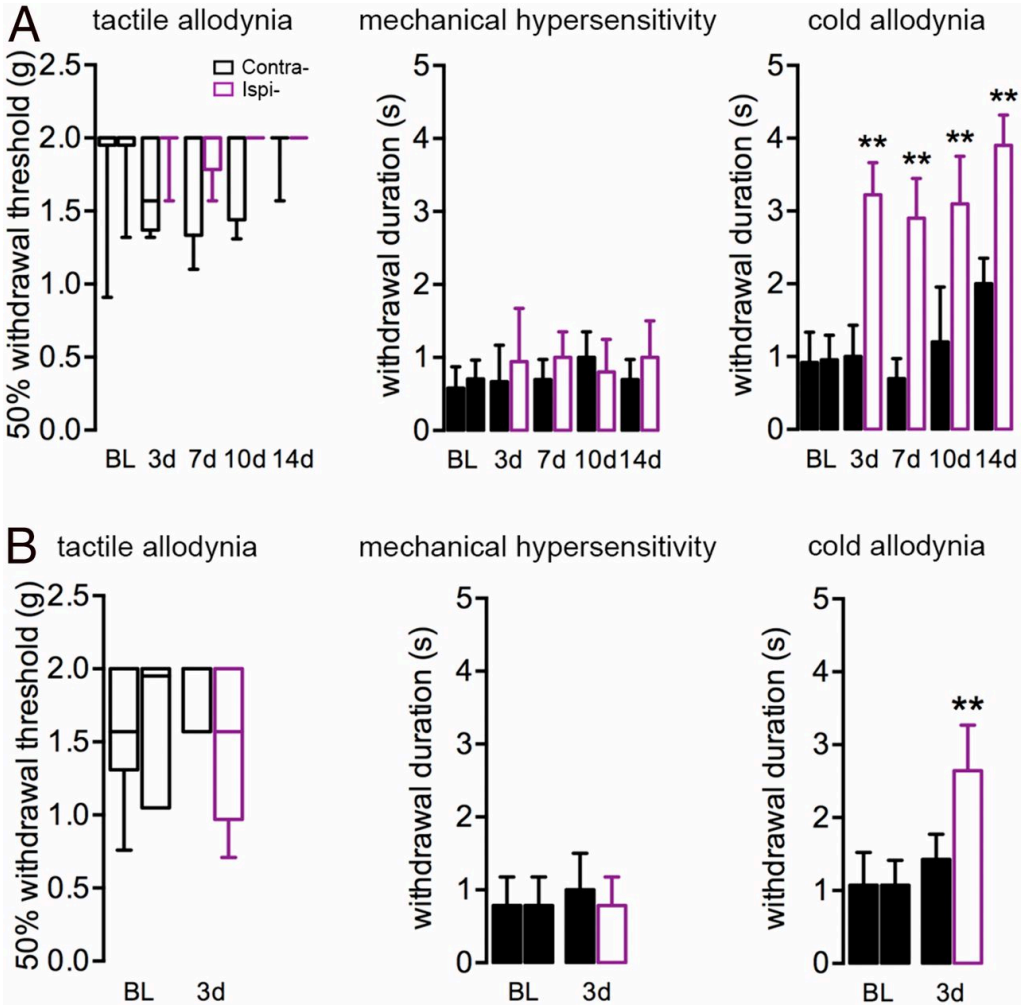
Cold allodynia triggered by unilateral tibial fracture.



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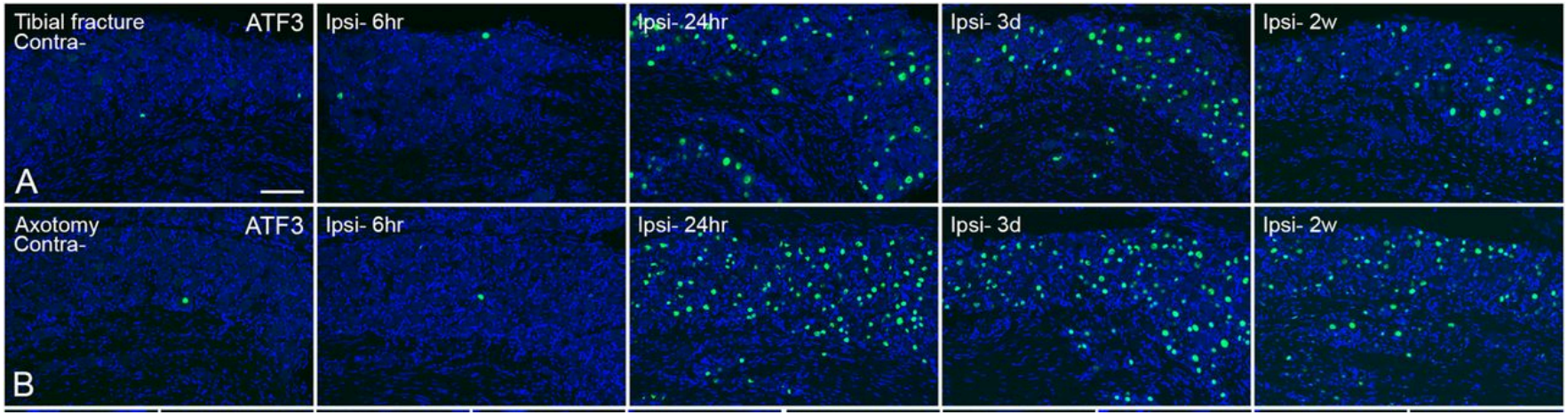
ATF3

- 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

Chen BP, et al. (1996), Linda H, et al. (2011)

Activation of ATF3 in DRGs after unilateral tibial fracture.



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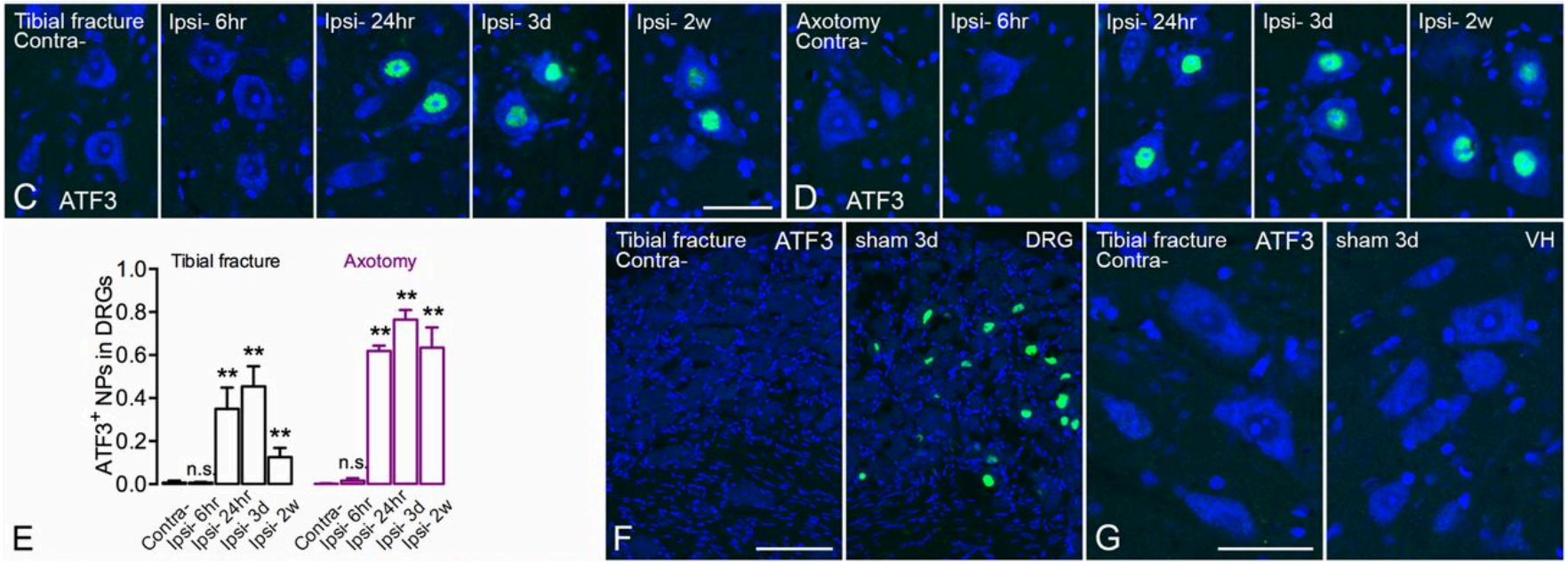
Activation of ATF3 in motor neurons after unilateral tibial fracture.



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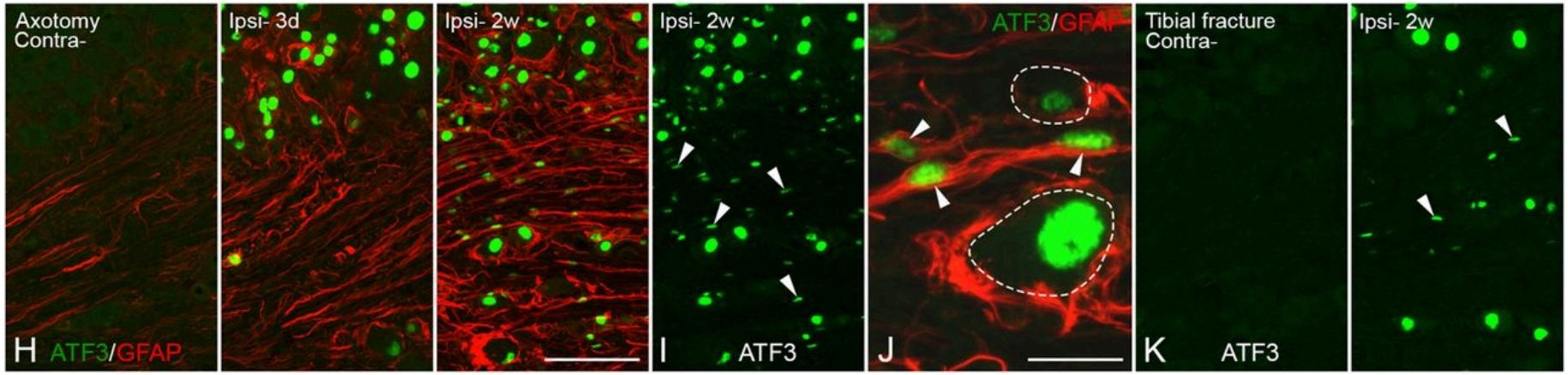
Activation of ATF3 in Schwann cells after unilateral tibial fracture.



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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

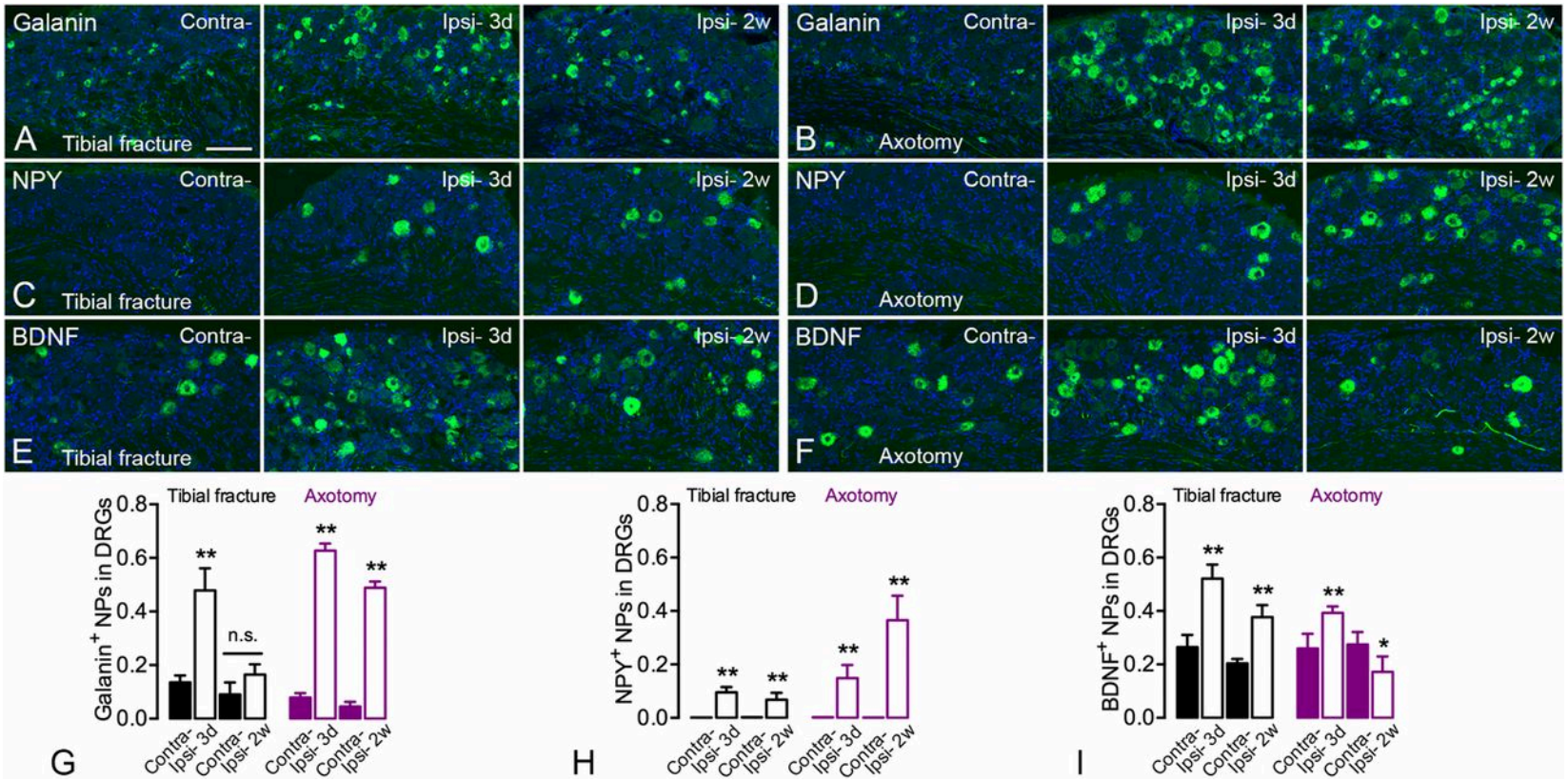
Bartai, T (2000); Berger A, et al. (2005); White HS, et al. (2009)

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.



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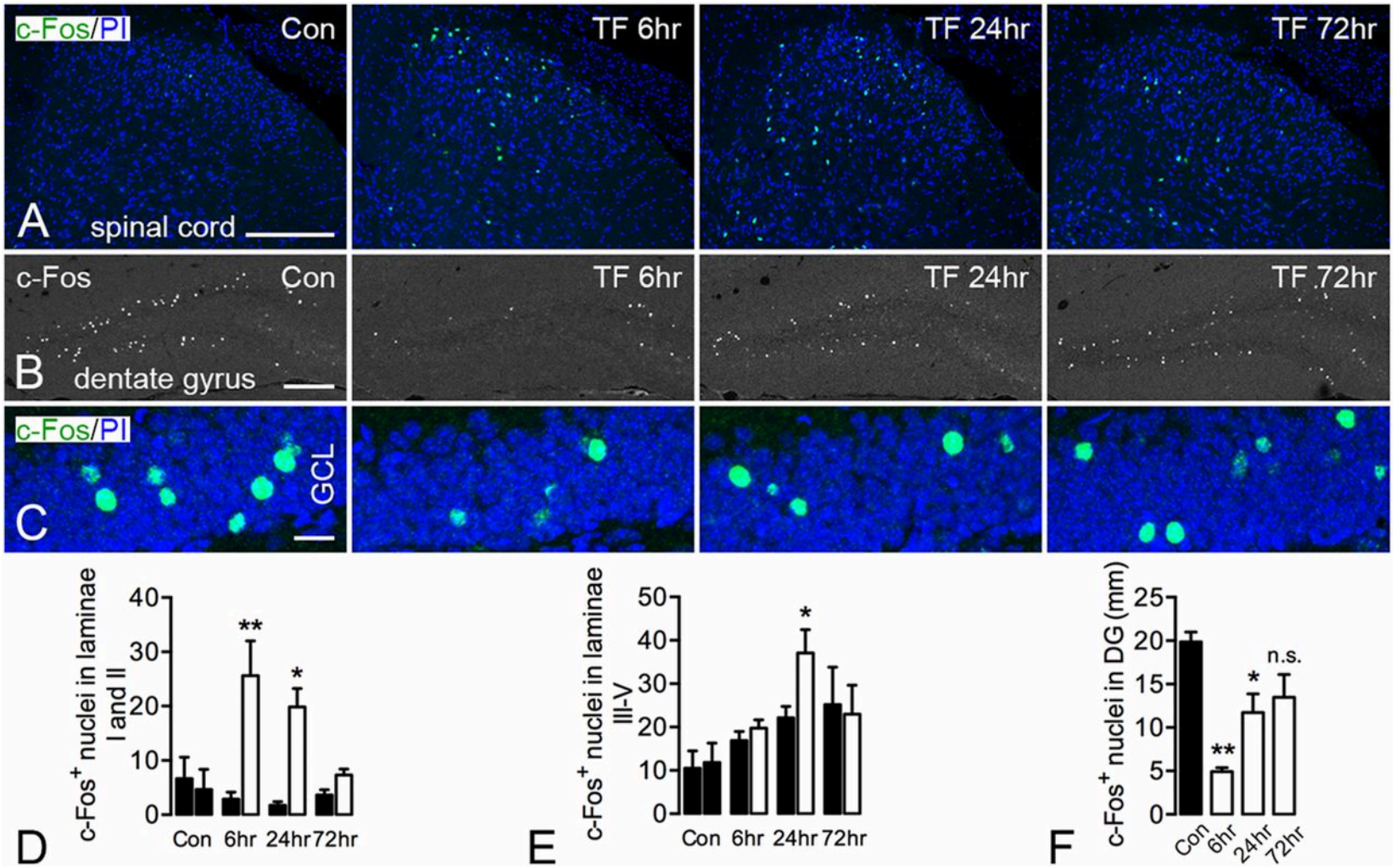
C-Fos

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

Dragunow M, Faull R (1989); Mide-Langosch (2005); VanElzakker M, et al. (2008)

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



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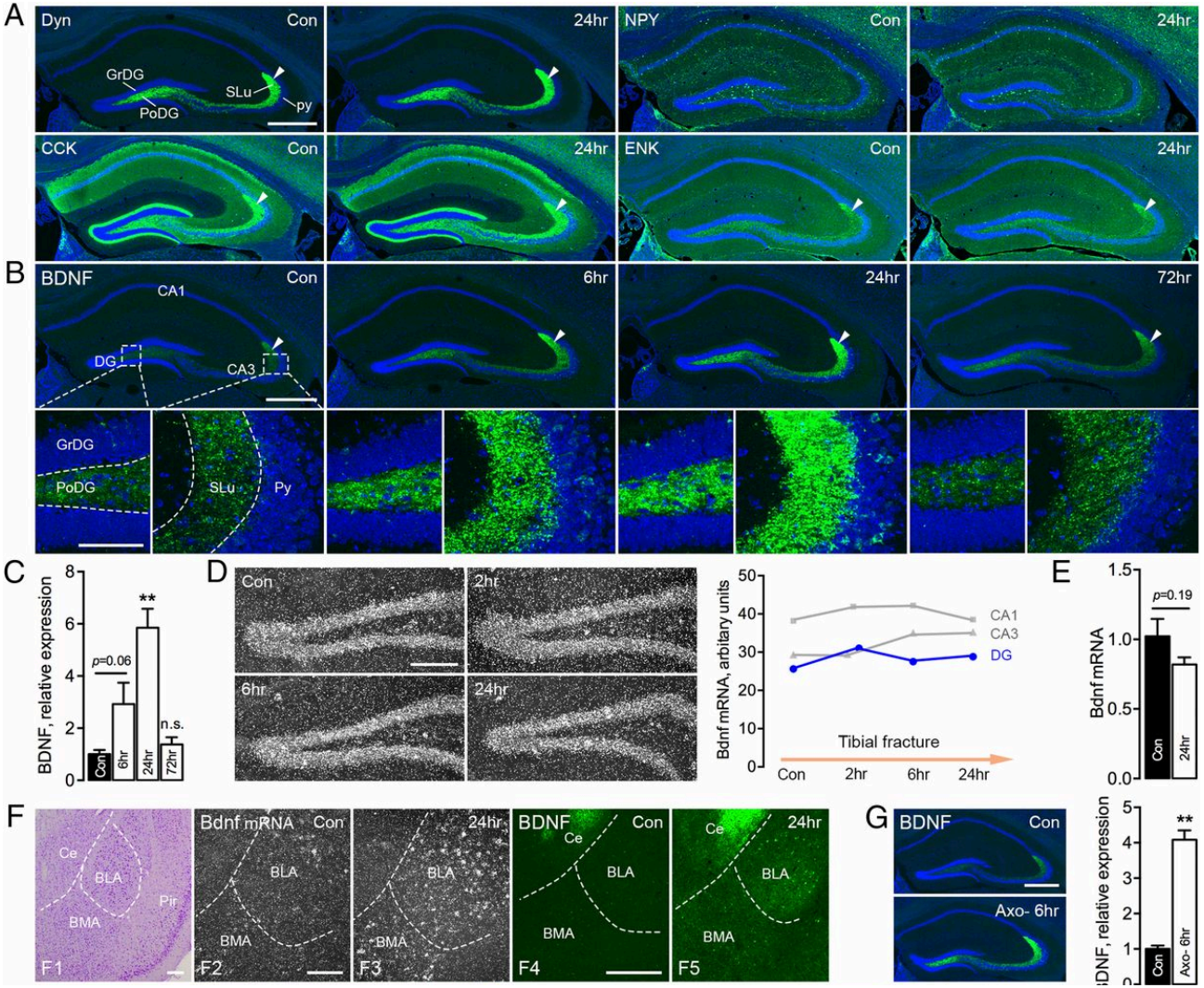
BDNF and neuropeptide expression in the brain after unilateral tibial fracture.

Dyn: dynorphin
CCK: cholecystokinin
ENK: enkephalin



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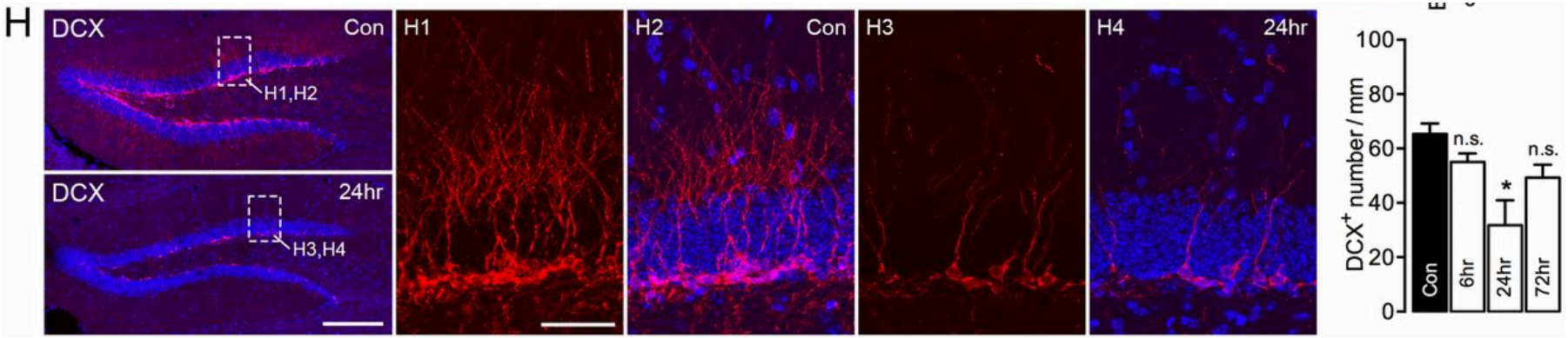

- DCX (Doublecortin)
  - Microtuble associated protein

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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/ anasthesia time surgery time?
- Translation into humans?
- Neuropathic pain models relevant?
- Impact on SCI patients?

THANK YOU FOR LISTENING!

ANY QUESTIONS?

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