# Johns Hopkins Engineering

## Methods in Neurobiology

Experimental Approach to PN Regeneration

# Models of PN regeneration

| In vitro models |
| --- |
| Immortalized neuronal or glia cell lines |
| Primary cultures (neurons and glia) |
| 3D or organotypic cultures |

| In vivo models |
| --- |
| Rats and mice, including genetic modified organisms |
| Rabbits, cats, dogs and primates (less common) |

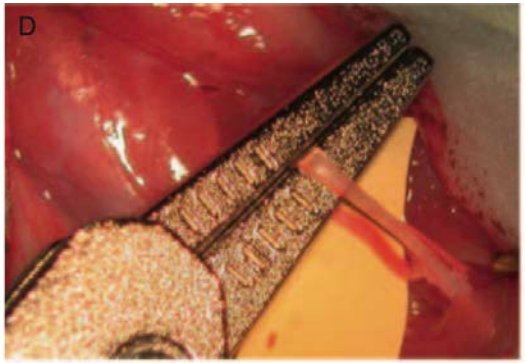
# Examples of GEM mice used to study regeneration

| References | Type of transgene | Type of study | Results |
|---|---|---|---|
| (Hirota *et al.* 1996) | Double transgenic expressing IL-6 and IL-6 receptor | Hypoglossal nerve ligation | Transgenic mice showed improved regeneration. These results suggest that IL-6 signal may play an important role in nerve regeneration |
| (Gondré *et al.* 1998) | ΔSCIP transgene | Sciatic nerve crush injury | The transgenic mice showed markedly accelerated regeneration and hypertrophy of both myelin and axons |
| (Inserra *et al.* 2000) | IL-6-null mice | Sciatic nerve crush injury and end-to-end neurorrhaphy | The absence of IL-6 does not impair peripheral nerve recovery after injury. The histomorphometric findings were consistent with the functional results, suggesting that IL-6 does not have a significant effect on nerve regeneration |
| (Kim *et al.* 2003) | Transgenic mice expressing Nogo-C in peripheral Schwann cells | Sciatic nerve crush injury | The transgenic mice regenerate axons less rapidly than do wild-type (WT) mice. This is associated with a decreased recovery rate for motor function after sciatic nerve injury. Thus, expression of the Nogo-66 domain by otherwise permissive myelinating cells is sufficient to hinder axonal reextension after trauma |
| (Rong *et al.* 2004) | Transgenic mice expressing DN RAGE in mononuclear phagocytes and/or peripheral neurons | Sciatic nerve crush injury | After lesion, transgenic mice displayed decreased functional and morphological recovery, and myelinated fiber density. In double transgenic mice, regeneration was even further impaired, suggesting the critical interplay between RAGE-modulated inflammation and neurite outgrowth in nerve repair |
| (Triolo *et al.* 2006) | GFAP-null mice | Sciatic nerve crush injury | Without lesion, peripheral nerves develop and function normally in GFAP-null mice; no significant differences in axonal sorting, Schwann-cell axon relationship, and myelination were observed. Axonal regeneration after damage was delayed. Mutant Schwann cells maintained the ability to dedifferentiate but showed defective proliferation |

# Paradigm to experimental regeneration

TABLE III
CLASSIFICATION OF NERVE LESIONS ACCORDING TO SUNDERLAND (LEFT COLUMN)

| | | |
|---|---|---|
| Interrruption of axon conduction of the action potential | Axonotmesis | MILD nerve lesion (it does not require surgical repair) |
| Loss of axon continuity | | |
| Loss of fiber continuity | | |
| Loss of perineurium continuity | Neurotmesis | SEVERE nerve lesion (it requires surgical repair) |
| Loss of epineurium continuity | | |



# Nerve injury and repair

Table 1. Classification systems of peripheral nerve injury.

| Recovery indication | Recovery speed | Overall recovery | Sunderland injury classification | Seddon injury classification |
|---|---|---|---|---|
| Favorable | Fast (< 1 month) | Complete | I | Neurapraxia |
| Favorable | Slow (~ 1 mm/day) | Complete | II* | Axonotmesis |
| Favorable | Slow (~ 1 mm/day) | Partial or incomplete | III | |
| Unfavorable | None | None | IV | Neurotmesis |
| Unfavorable | None | None | V | |
| Mixed | Variable | Incomplete | VI (Mackinnon) | All |

| Factors influencing successful nerve repair in the PNS |
|---|
| Speed of regeneration |
| Distance from end target |
| Understanding nerve topology |
| Delayed reconstruction due to trauma |
| Aging |

# Strategy to improve PN regeneration

- Drugs that improve regeneration (Erythropoietin, Fausidil);
- Nerve grafts;
- Nerve transfers;
- Nerve conduits.

**Table 2**
Potential therapeutic options that primarily target the cAMP/PKA pathway.

| Intervention | Mechanism(s) of action | Observed effect(s) |
|---|---|---|
| Rolipram | – Phosphodiesterase 5 inhibition (prevents breakdown of cAMP) | – Prevented the decline of cAMP following injury<br>– Increased myelination<br>– Increased numbers of motor and sensory neurons regenerating across the repair site |
| Testosterone | – Attenuates GFAP expression<br>– Increases BDNF expression<br>– Up-regulation of RAGs including βII-tubulin and GAP-43<br>– Suppresses HSP expression | – Increased the rate of axonal regeneration |
| Fasudil | – Inhibition of ROK<br>– Prevents collapse of growth cones, promoting the regenerative process | – Increased number of nerve fibers, density and width<br>– Increased numbers of large diameter myelinated axons<br>– Dose-dependent improvement in sensory neurite outgrowth |
| Chondroitinase ABC | – Degrades chondroitin sulfate proteoglycans (inhibitory myelin proteins)<br>– Inactivation of RHOA<br>– Improves the ability of axons to access SC basal laminae in the distal nerve stump | – Increased number of motor and sensory neurons that regenerated their axons across the repair site |
| Ibuprofen | – Inhibits RhoA through peroxisome proliferator-activated receptor gamma | – Improved functional recovery<br>– Increased area and thickness of myelinated axons |

# References

| Slide | Reference |
|---|---|
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| 5 | Wood MD, Mackinnon SE. 2015 Pathways regulating modality-specific axonal regeneration in peripheral nerve. *Exp Neurol*. 265:171-175. |
| 6 | K. Ming Chan, Tessa Gordon, Douglas W. Zochodne, Hollie A. Power, H.A. 2014 Improving peripheral nerve regeneration: From molecular mechanisms to potential therapeutic targets Experimental Neurology, 261, 826-835. |