Part 2, Nervous System Development and Diseases

2.4. Repair and regeneration in the nervous system

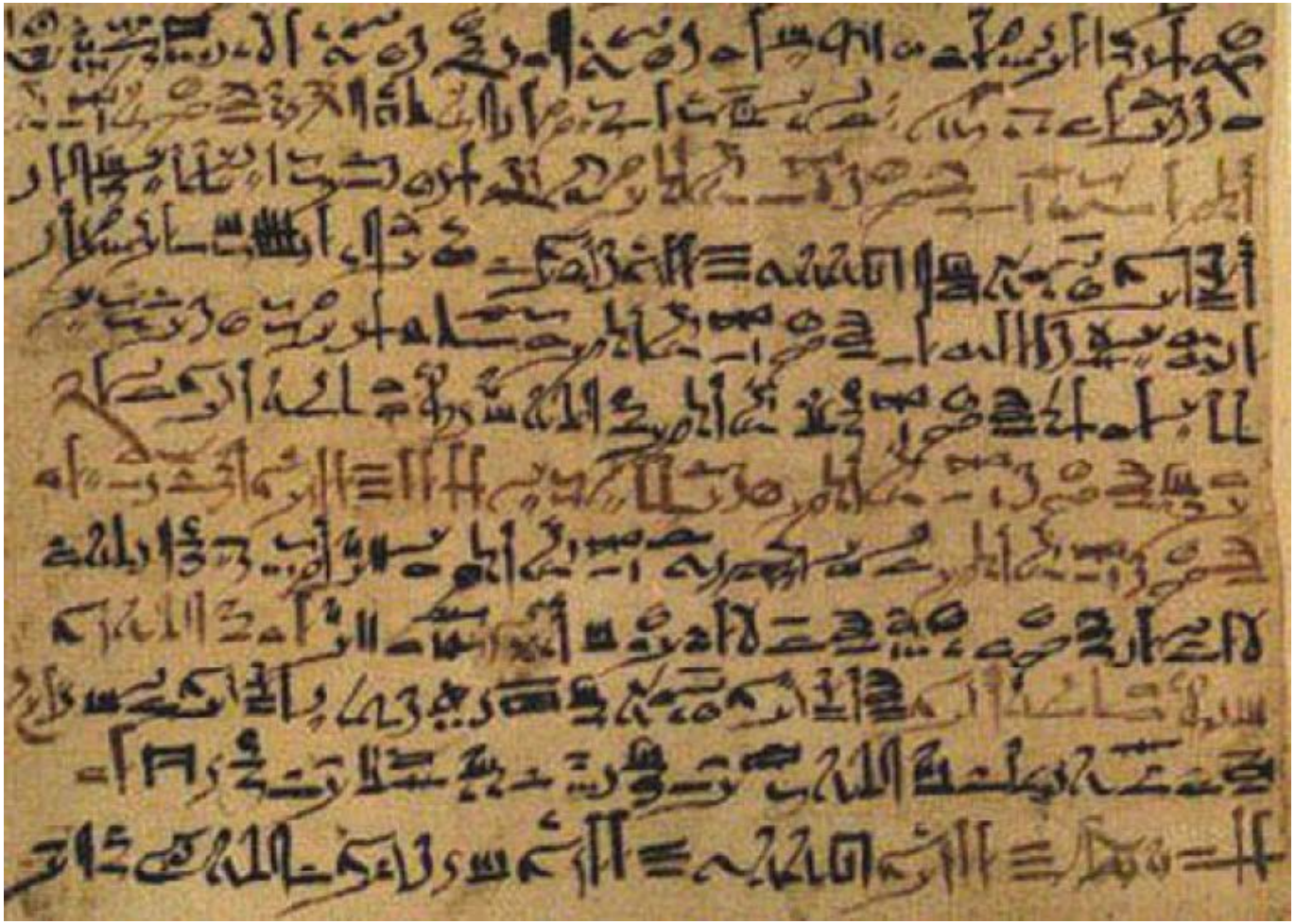
Limited ability of brain to alter, renew or repair

- Unlike many other organs-notably the skin, lungs, intestine, and liver-that continuously generate new cells, human brains do not produce large numbers of new neurons once the initial complement is established.

- Three barriers restrain central nervous system regeneration.

1. The consequences of local injury to brain tissue often lead to neuronal death.
2. Several other cell classes, particularly glial cells, actively inhibit axon growth.
3. Although neural stem cells are retained in the adult brain, most are constrained in their ability to divide, migrate, and differentiate.

An ancient Egyptian papyrus acknowledges the difficulty of repairing the brain and spinal cord



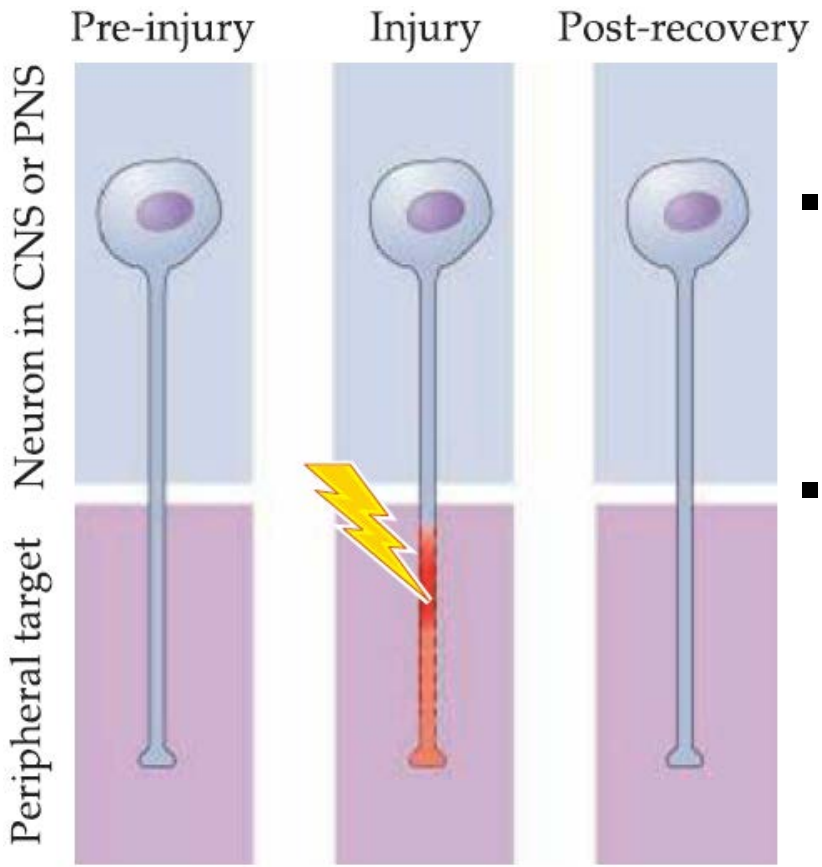
When you examine a man with a dislocation of a vertebra of his neck, and you find him unable to move his arms, and his legs… Then you have to say: a disease one cannot treat. (From Case and Tessier-Lavigne, 2005.)

- Nevertheless, some nervous system repair does occur after injury.
  - In the periphery, axons can regrow through vacated peripheral nerve sheaths and eventually reinnervate sensory specializations in the skin or synaptic sites on muscles.
  - The modest recovery seen after brain injury is usually attributed to reorganization of function using remaining, intact circuits rather than repair of damaged brain tissue.
    - Few existing neurons can grow a new axon if the original axon is severed or injured.
    - Neurons can not replace dendrites lost due to local tissue damage or degenerative disease.

Three types of neuronal repair

1. *Peripheral nerve regeneration:*

- When peripheral axons are severed, the neuron, whether in a peripheral ganglion or in the central nervous system, regenerates the distal portion of the axon.

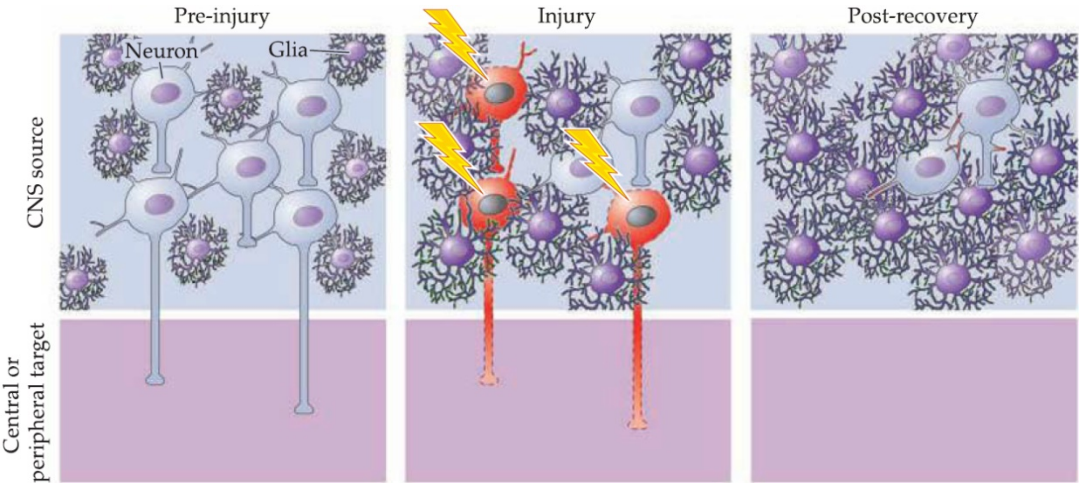


- This requires a reactivation of the developmental processes for axon growth and guidance, as well as those for initial synapse formation.
- Such repair may also require activity-dependent competitive mechanisms to insure proper quantitative matching of newly regrown afferents to temporarily denervated targets.
- This first type of repair is seen primarily when sensory or motor nerves are damaged in the periphery, leaving the nerve cell bodies in the relevant sensory and autonomic ganglia or in the spinal cord intact.

Three types of neuronal repair

2. *Restoration of damaged central nerve cells:*

- Prior to injury, glial cells (processes in dark green) are quiescent.
- Immediately following the injury, the glial cells grow, axons and dendrites degenerate, and connections are lost.
- Following recovery, some modest axon and dendrite growth may be seen, but the hypertrophic glial cells remain to form a "scar'" at the site of the tissue damage.
  - To achieve this, several developmental mechanisms must be re-engaged, including appropriate regulation of cell polarity to distinguish dendrites and axons; adhesion signals to direct process extension; and trophic signaling to support growth.

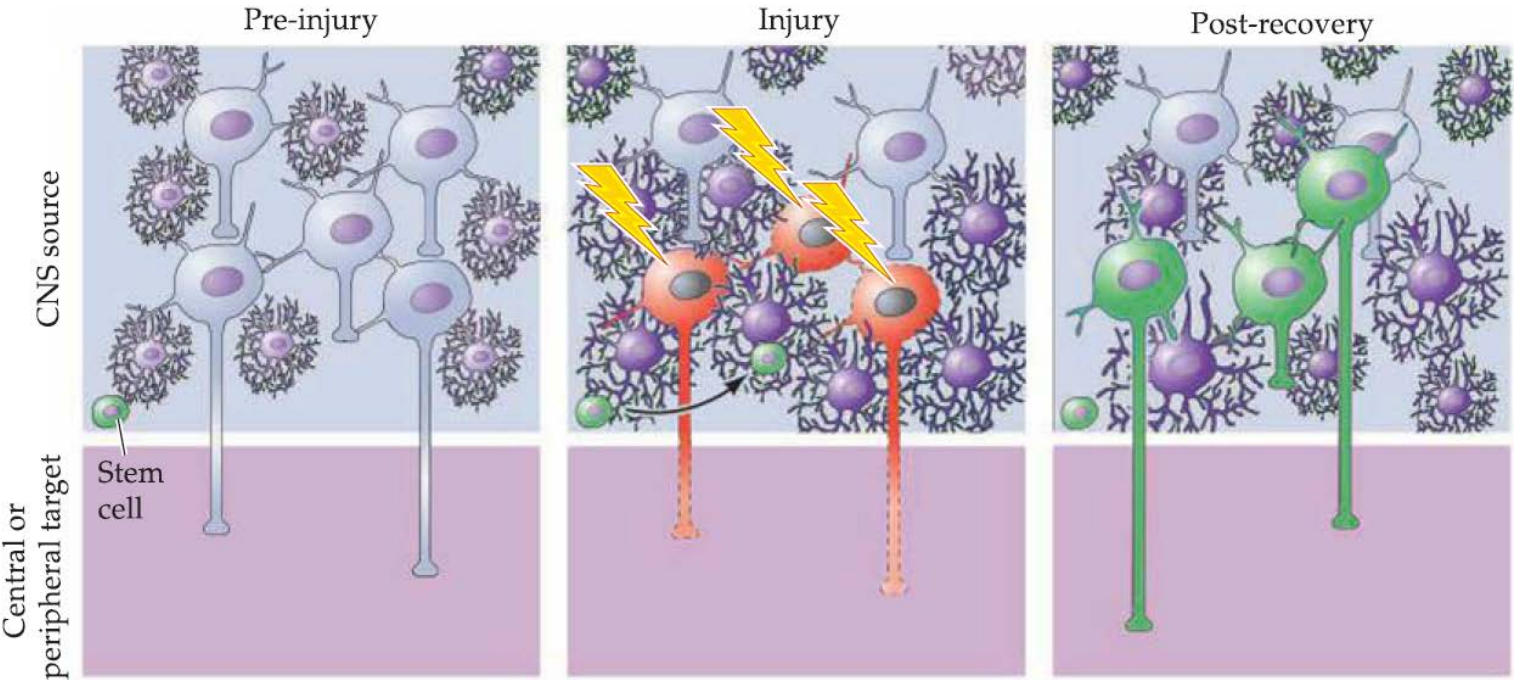


- However, it fails in the injured central nervous system, most likely because of local overgrowth of glial cells and their production of signals that inhibit neuron growth.

Three types of neuronal repair

3. Wholesale genesis of new neurons:

- Neuronal replacement depends on the maintenance of a neural stem cell (green).
- Following Injury, this stem cell proliferates and gives rise to new neuroblasts that then differentiate and integrate into the damaged tissue.
- These new neurons (green) make connections with existing cells.
  - Such adult neuronal genesis occurs rarely, and its mechanisms are controversial.



3. Wholesale genesis of new neurons:

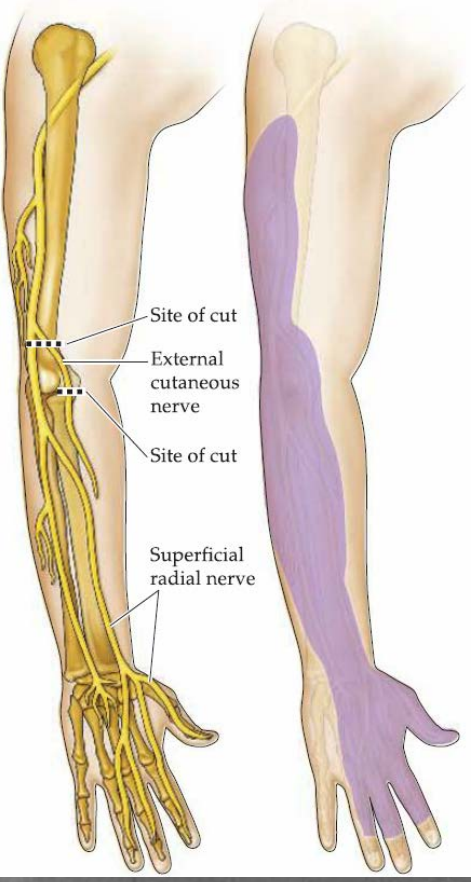
- For such repair to occur, several criteria must be met:

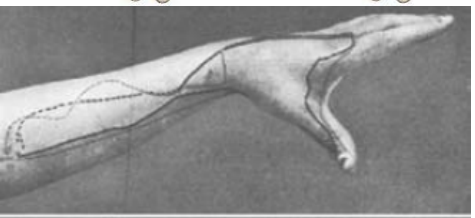
1) Nervous tissue must retain a population of **multipotent neural stem cells** able to give rise to all of the cell types of the brain region that has been damaged .
2) These neural stem cells must be present in a distinct region-a "***niche***" -that retains an appropriate environment for the genesis and differentiation of new nerve cells and glia.
3) The regenerating tissue must preserve the capacity to recapitulate the migration, process outgrowth, and synapse formation necessary to reconstitute local functional networks of connections as well as long distance connections.

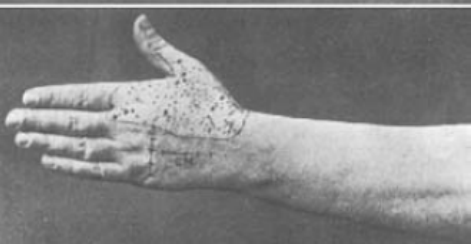
Peripheral nerve regeneration

- In the early 1900s, the British neurologist **Henry Head** provided a particularly dramatic account of repair in the peripheral nervous system:
  - It had become clear that damage to a peripheral nerve resulted in a gradual but usually incomplete restoration of sensory and motor function.
  - The speed and precision of this recovery could be facilitated by the surgical reapposition of the two ends of the severed nerve.
  - He performed a precise nerve transection and reapposition experiment on himself, documenting the results as a personal narrative:
    - *On April 25, 1903, the radial (ramus cutaneus radialis) and external cutaneous nerves were divided (cut) in the neighborhood of my elbow, and after small portions had been excised, the ends were united with silk sutures. Before the operation the sensory condition of the arm and back of the hand had been minutely examined and the distances at which two points of the compass could be discriminated had been everywhere measured.* (Head, Rivers, and Sherren, 1905, ***Brain*** 28: 99-115).

Henry Head's peripheral nerve regeneration experiment





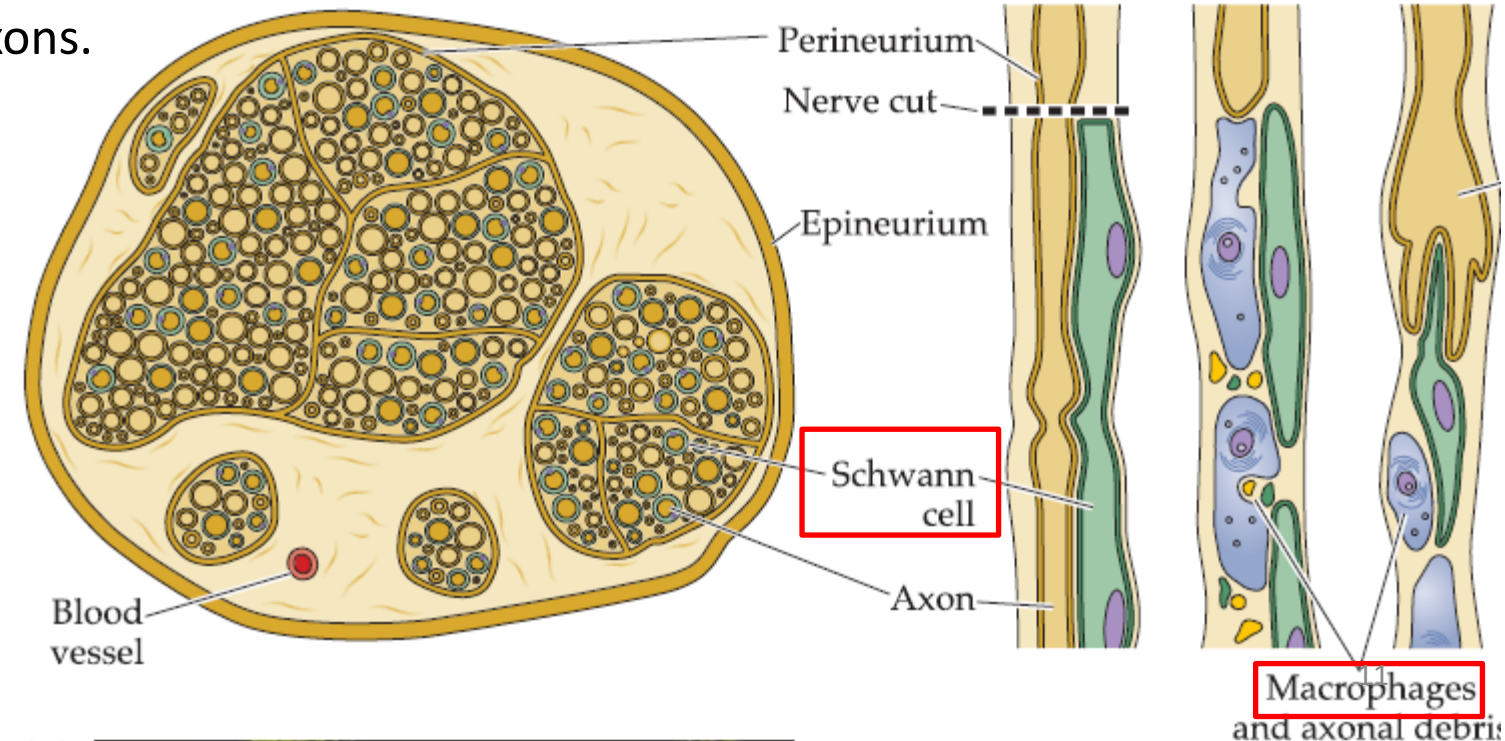


- Location of the radial nerve, which was severed and the proximal and distal ends surgically re-apposed in Head's experiment on his own arm.
- Territory normally innervated by the radial nerve.

- Outlines of regions, dotted line and various marks on Head's arm and hand showing his recovery from the peripheral nerve cut.
  - The first indication of recovery was a difference in the return of general sensitivity to pressure and touch that was not well localized (a sensitivity he called "*protopathic*''), beginning at approximately 6 weeks and lasting about 13 weeks.
  - Head also experienced a set of sensations (sensitivity to light touch, temperature discrimination, pinprick, and two-point discrimination, as well as fine motor control which he referred to as "*epicritic*" abilities) that recovered more slowly and with less restoration to his recollection of his original sensory state: >2 years

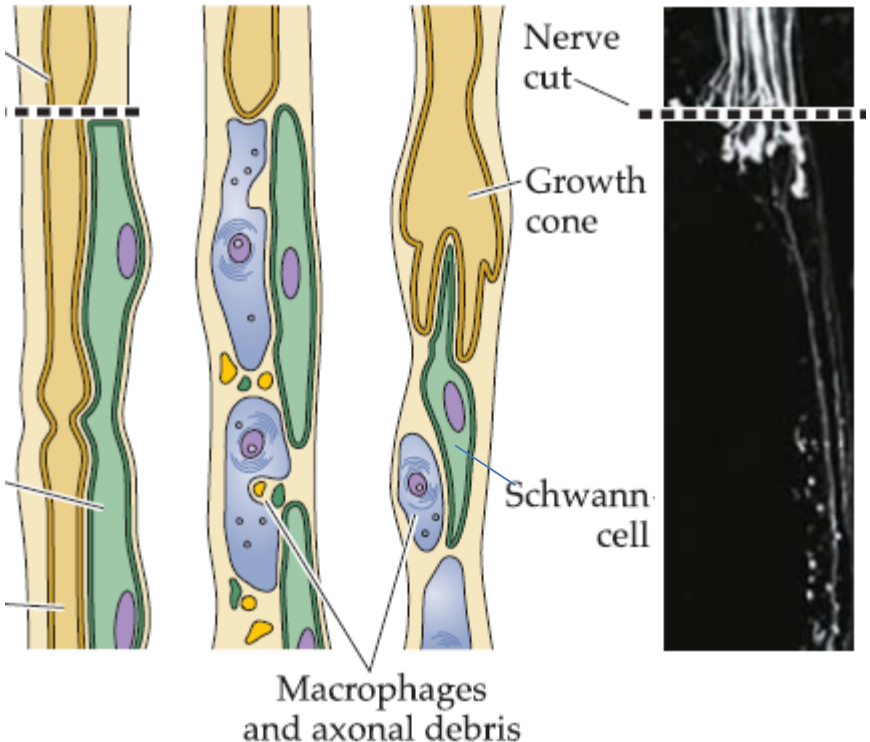
Cellular and molecular basis of peripheral nerve repair

- The mechanisms that repair neural damage are similar to those used to promote initial axon growth and synapse formation during development.
- The environment for adult peripheral nerve repair is far different than that in the embryo.
- The major cellular elements that contribute to peripheral axon regrowth and the reinnervation of targets:
  - **Schwann cells**: the glial cells that myelinate peripheral axons.
  - **Macrophages**: immune system cells that clear the degenerating remains of severed axons.

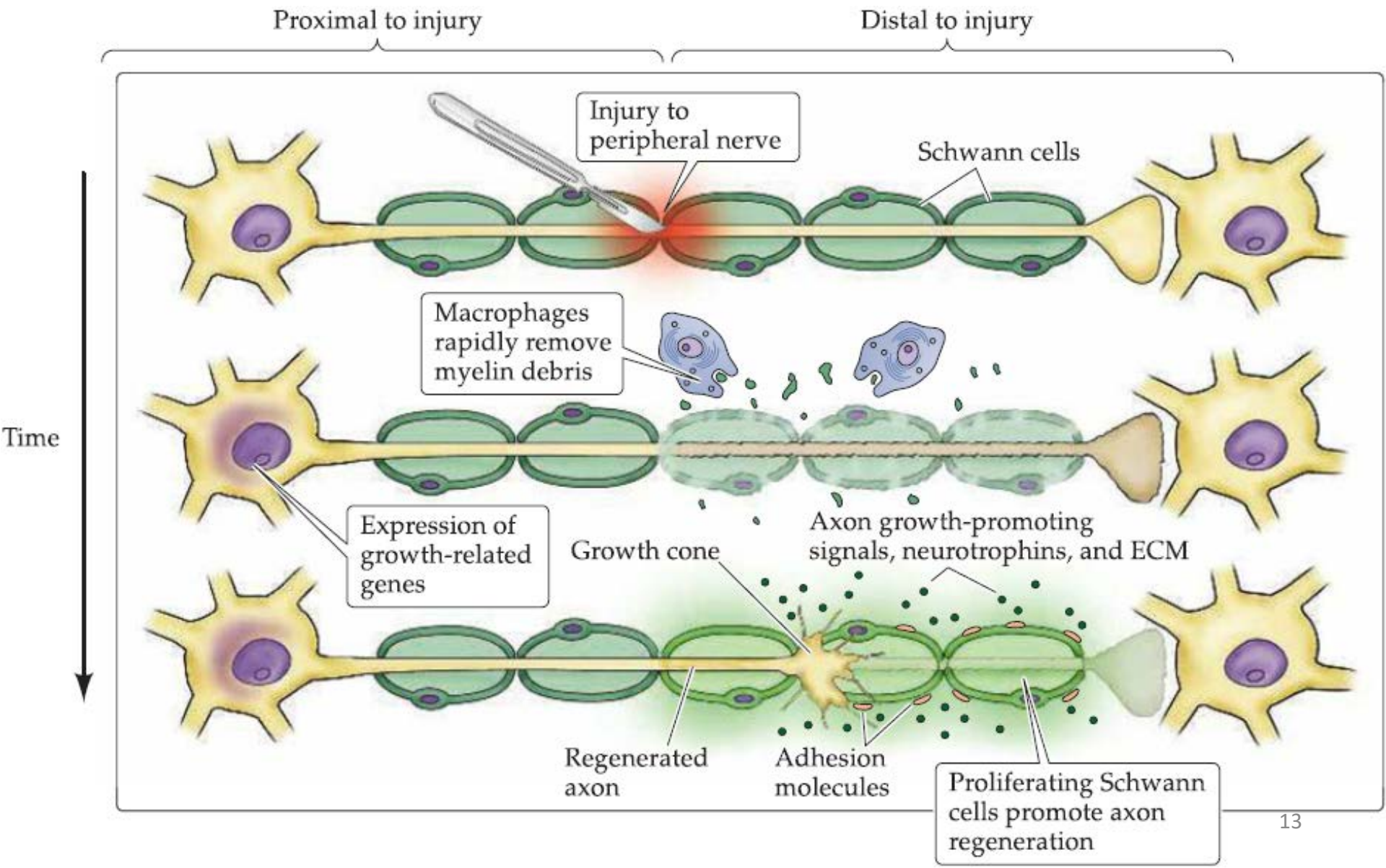


Regeneration in peripheral nerves

- Once a peripheral axon is cut, the distal portion degenerates and its remains are cleared by macrophages.
- After the debris is mostly cleared, the proximal axon stump transforms into a growth cone, and this growth cone interacts with the adjacent Schwann cells which stimulate and guide regeneration.
- The regenerating axons express integrins that mediate recognition of the matrix; subsequent intracellular signaling facilitates growth.



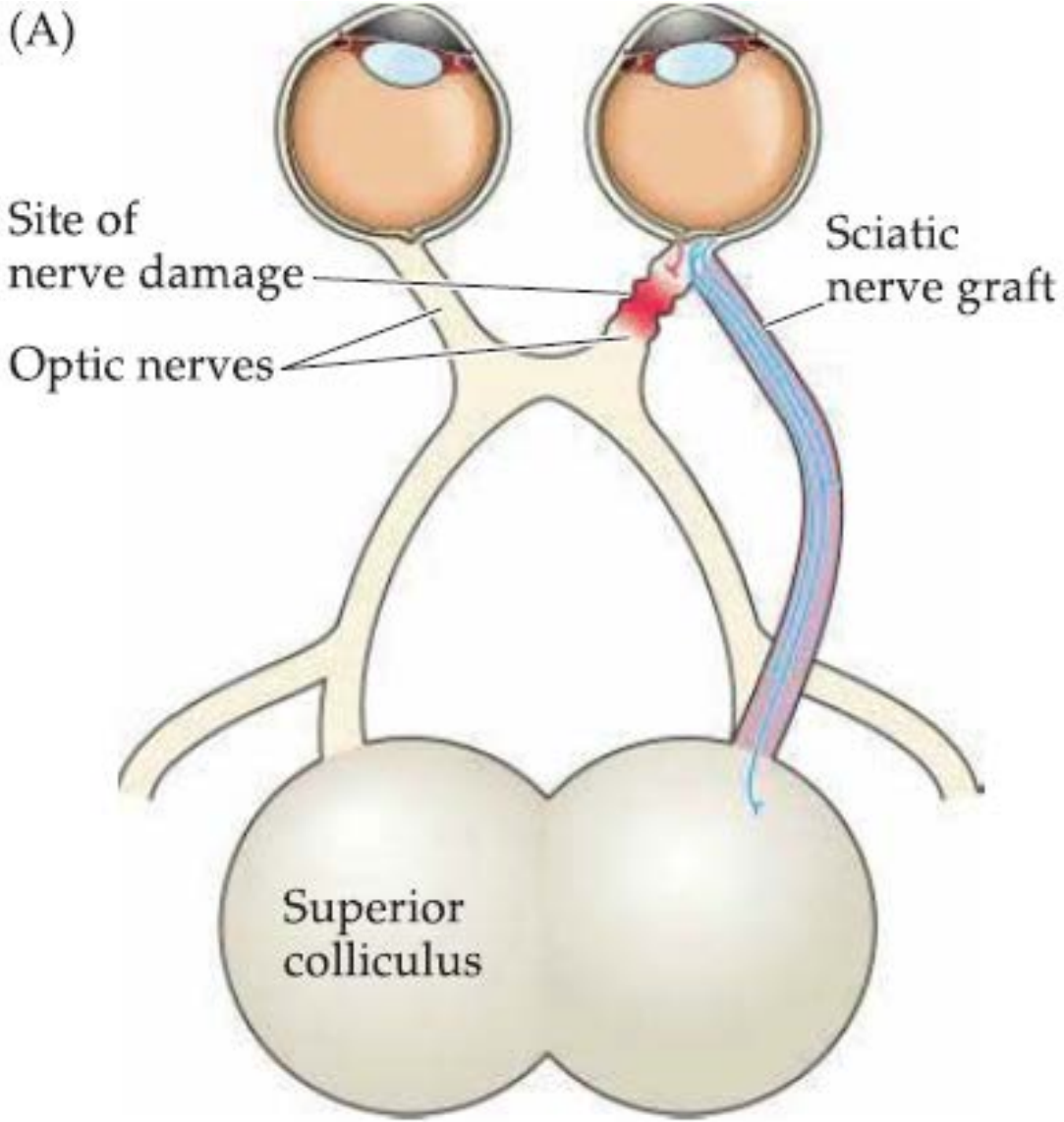
Molecular and cellular responses that promote peripheral nerve regeneration



Molecular and cellular responses that promote peripheral nerve regeneration

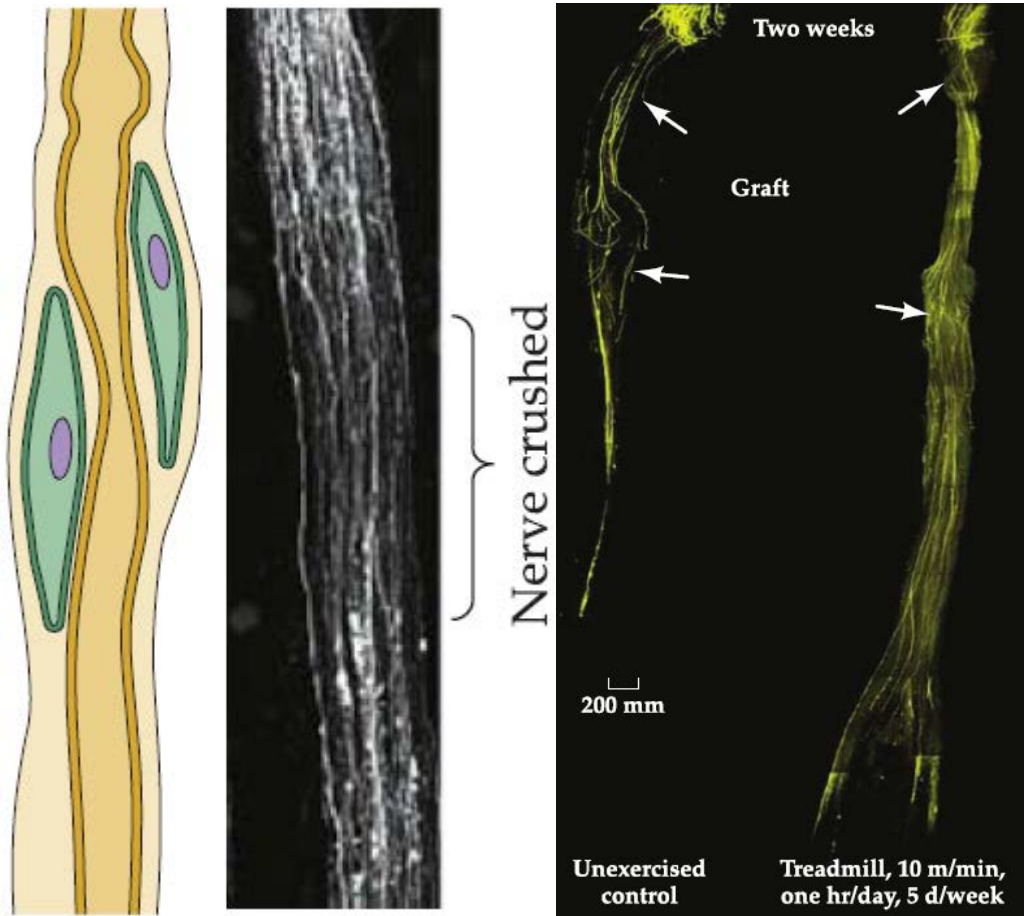
Schwann cells	Regenerating axons	Regenerating neurons
Secreting extracellular matrix molecules such as laminin, fibronectin, and collagens that provide a substrate for growth cone extension		Expression of growth-related genes such as GAP43
Increasing the amount of cell surface adhesion molecules such as N-CAM, L1 and N-cadherin on their surfaces	Expressing complementary cell surface adhesion molecules	Molecular mechanisms re-activated
Increasing expression and secretion of a number of neurotrophins such as BDNF	Elevated Trk and p75 neurotrophin receptors	Cytoskeleton restored to a "growth" state

Growth-promoting properties of peripheral nerve sheaths and Schwann cells facilitating growth of damaged axons in CNS



Ways to improve peripheral nerve regeneration

- Regeneration is more efficient after crushing *versus* cutting a nerve: the damaged distal segments provide a helpful guide to the regenerating proximal axons.
- Attach both proximal and distal ends to a nearby intact nerve.

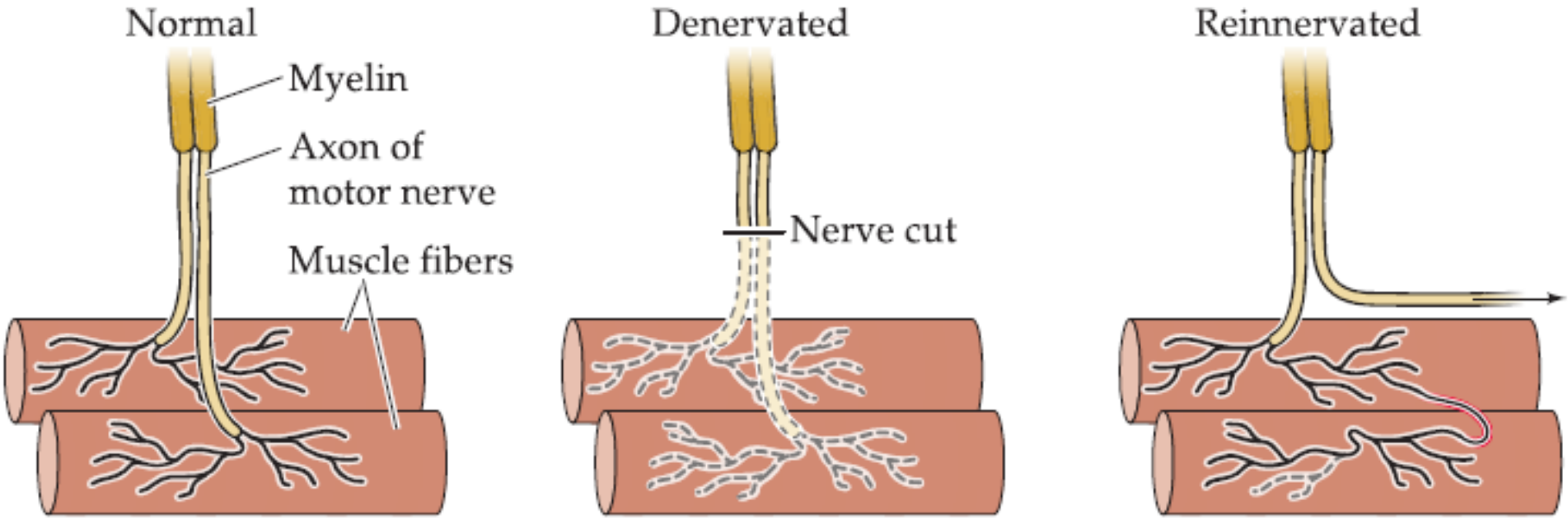


- Develop biomaterials that can substitute for the lost length of peripheral nerve.
- Take a length of nerve from a donor and attach the proximal and distal ends to this heterologous graft.
- Modest exercise training in injured animals whose peripheral nerves have been repaired by nerve grafts significantly improves the extent of nerve growth.

Regeneration of peripheral synapses

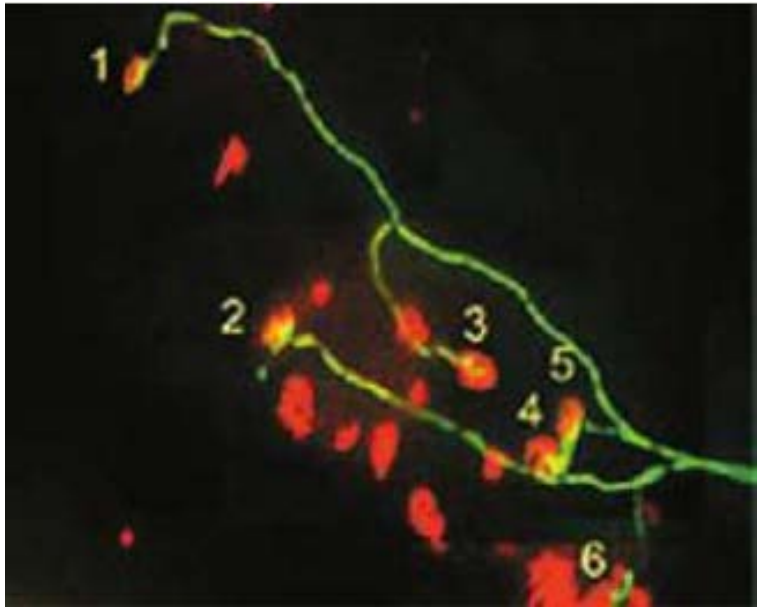
- Extension of injured peripheral axons is only the first step, the next essential event in successful recovery of function is reinnervation of appropriate target tissues and reestablishment of synaptic connections.

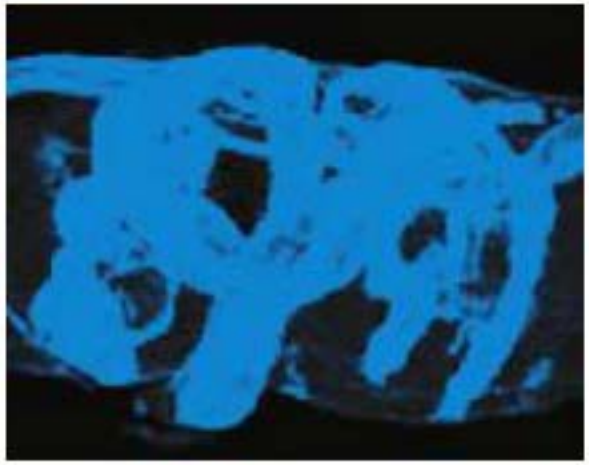
- The most thoroughly characterized system is the **neuromuscular junction**.



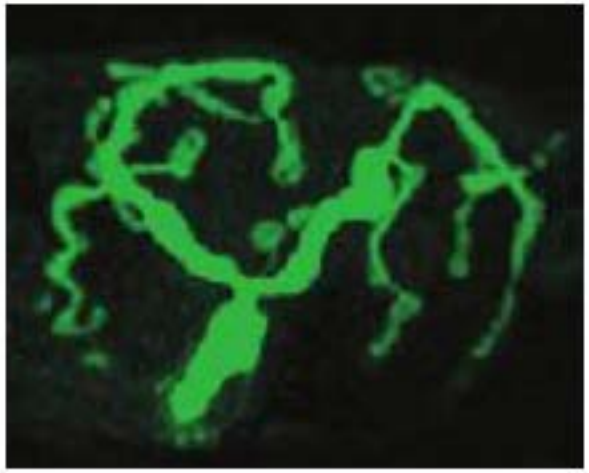
Reinnervation of muscles following peripharal motor nerve damage

- Why in the neuromuscular junction?
  - The relative ease of identifying and visualizing synaptic sites on neuromuscular junction.
  - The ability to define major molecular constituents—synaptic extracellular matrix, postsynaptic receptors and related proteins.

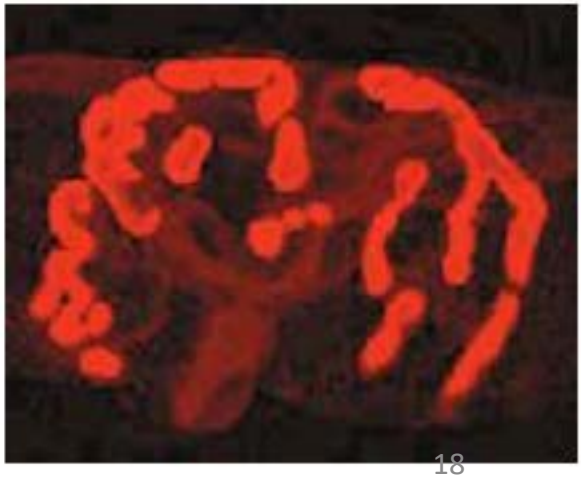




Schwann cells



Axons



Acetylcholine receptors

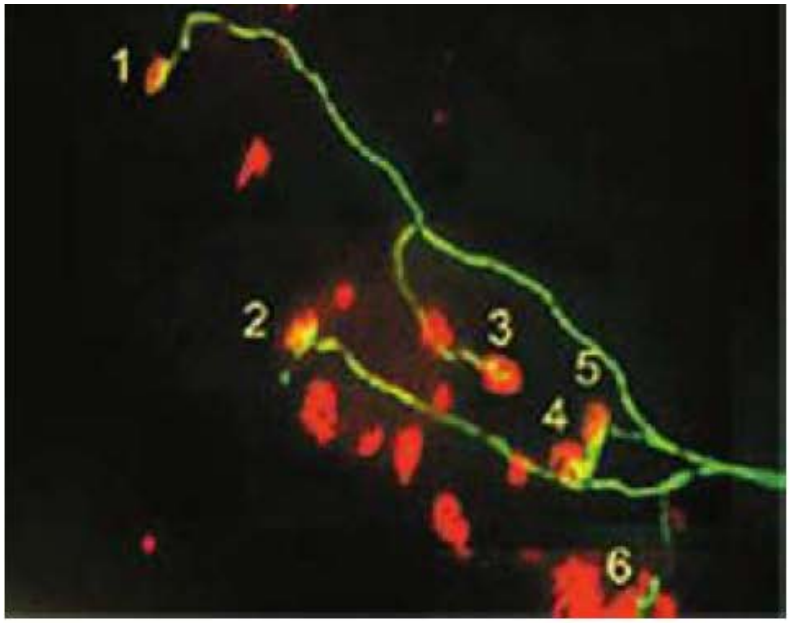
Molecular events during regeneration of peripheral synapses

- When skeletal muscle fibers are denervated, the original neuromuscular synaptic sites remain intact for weeks.
- Many secreted signaling molecules are either increased (NGF and BDNF) or decreased (NT3 and NT4) in both the muscle cells and the Schwann cells near the denervated end plate sites.
- The clustering of acetylcholine receptors that defines the postsynaptic membrane specialization remains.
- The secreted factor neuregulin and its receptors, involved in the initiation of receptor clustering, remain expressed at the denervated synapse.

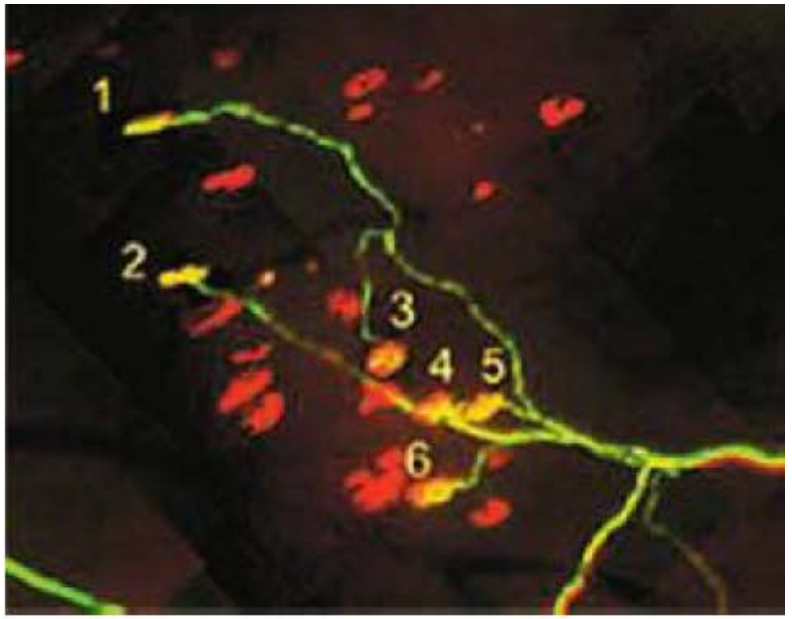
Acetylcholine receptors

- The extracellular matrix components are maintained when mature muscle fibers are denervated. They include specialized forms of laminin (synaptic, or S laminin).

Faithfulness and imprecision in peripheral nerve regeneration



Pre-injury



25 days later

- Imprecision is due not only to inappropriate target matching, but to the return of polyneuronal innervation at neuromuscular synapses during regeneration and reinnervation.
- Much of this innervation is eventually eliminated, presumably via the same activity-dependent mechanisms that operate during the early postnatal period.

Damage to the central nervous system

1. The brain or spinal cord can be injured acutely by external physical trauma (blunt forces to the head or body).

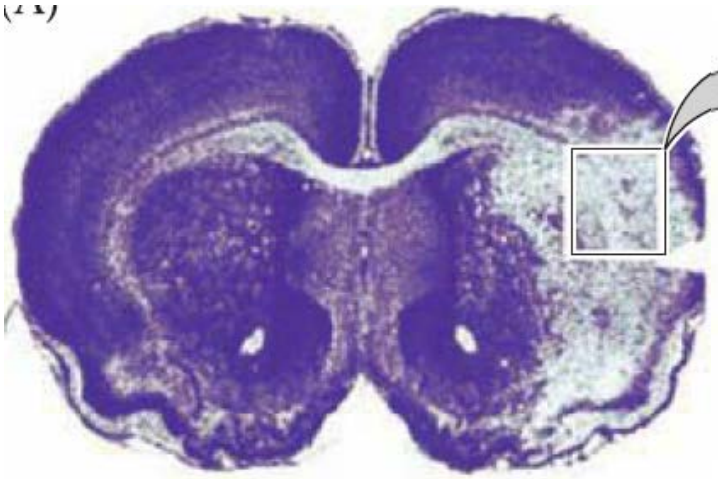
2. Hypoxia, a lack of oxygen usually created by locally diminished blood flow (ischemia) due to a vascular occlusion (e.g. stroke) or by a global deprivation of oxygen (e.g., drowning or cardiac arrest).

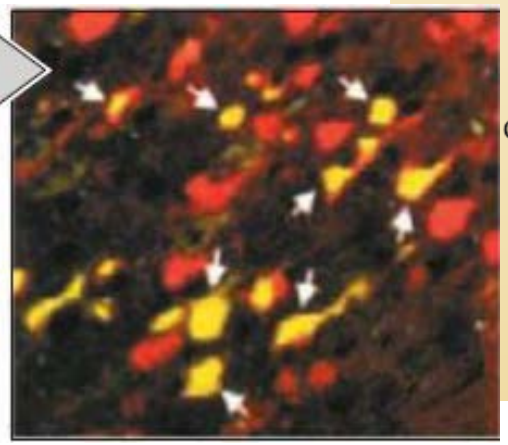
3. Neurodegenerative diseases such as Alzheimer's.

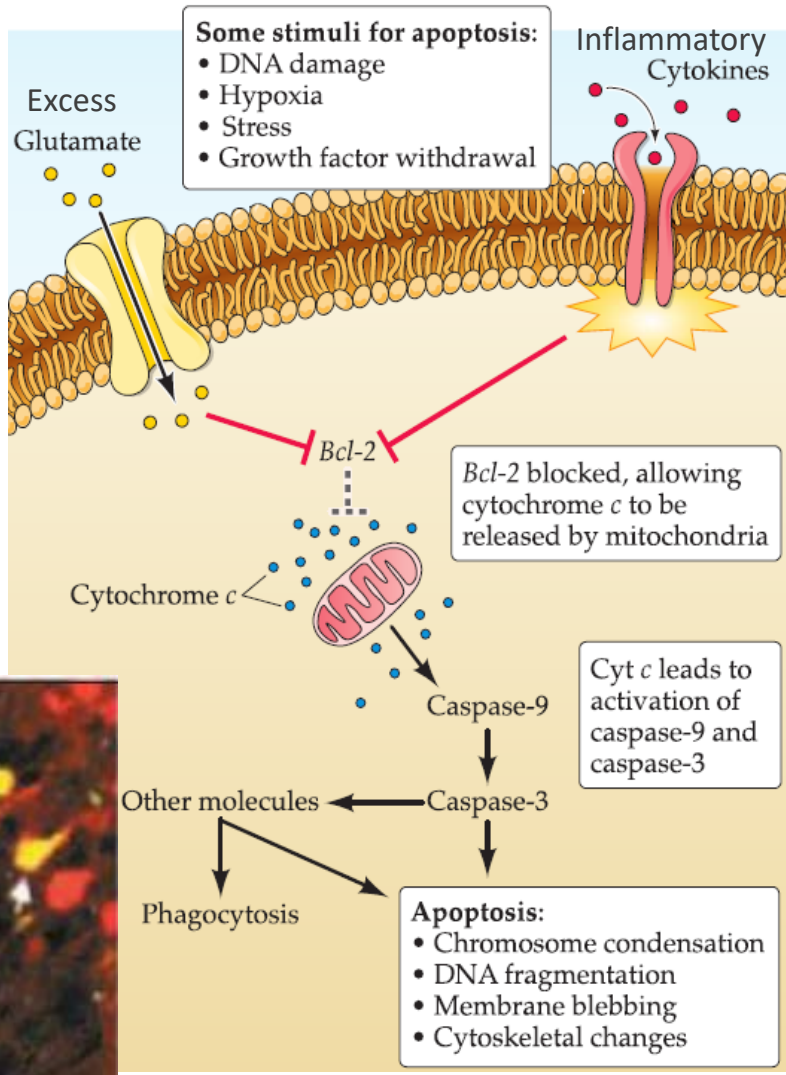
   - All three types of damage result in some neuronal death, either immediately or over time.
   - When the insult is less severe, some neurons can survive and some local axonal or dendritic growth occurs.

Successful peripheral regeneration *vs* limited regeneration in CNS

1. Damage to brain tissue tends to engage the mechanisms that lead to <u>necrotic and apoptotic cell death</u>.





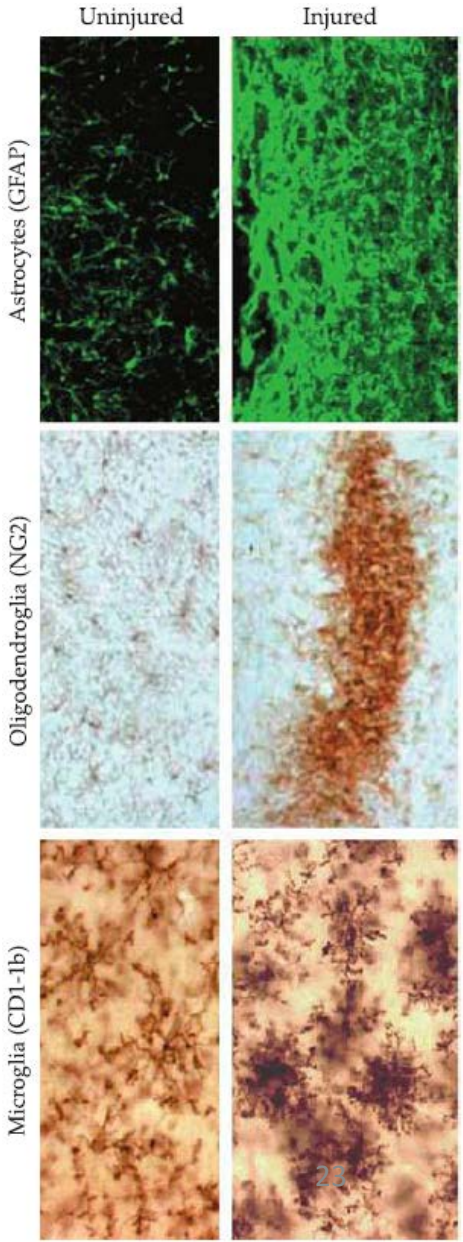


Successful peripheral regeneration *vs* limited regeneration in CNS

2. The cellular changes at the site of injury do not recapititulate developmental signaling that supports growth. Instead, a combination of glial growth and proliferation along with microglial activity inhibits growth.

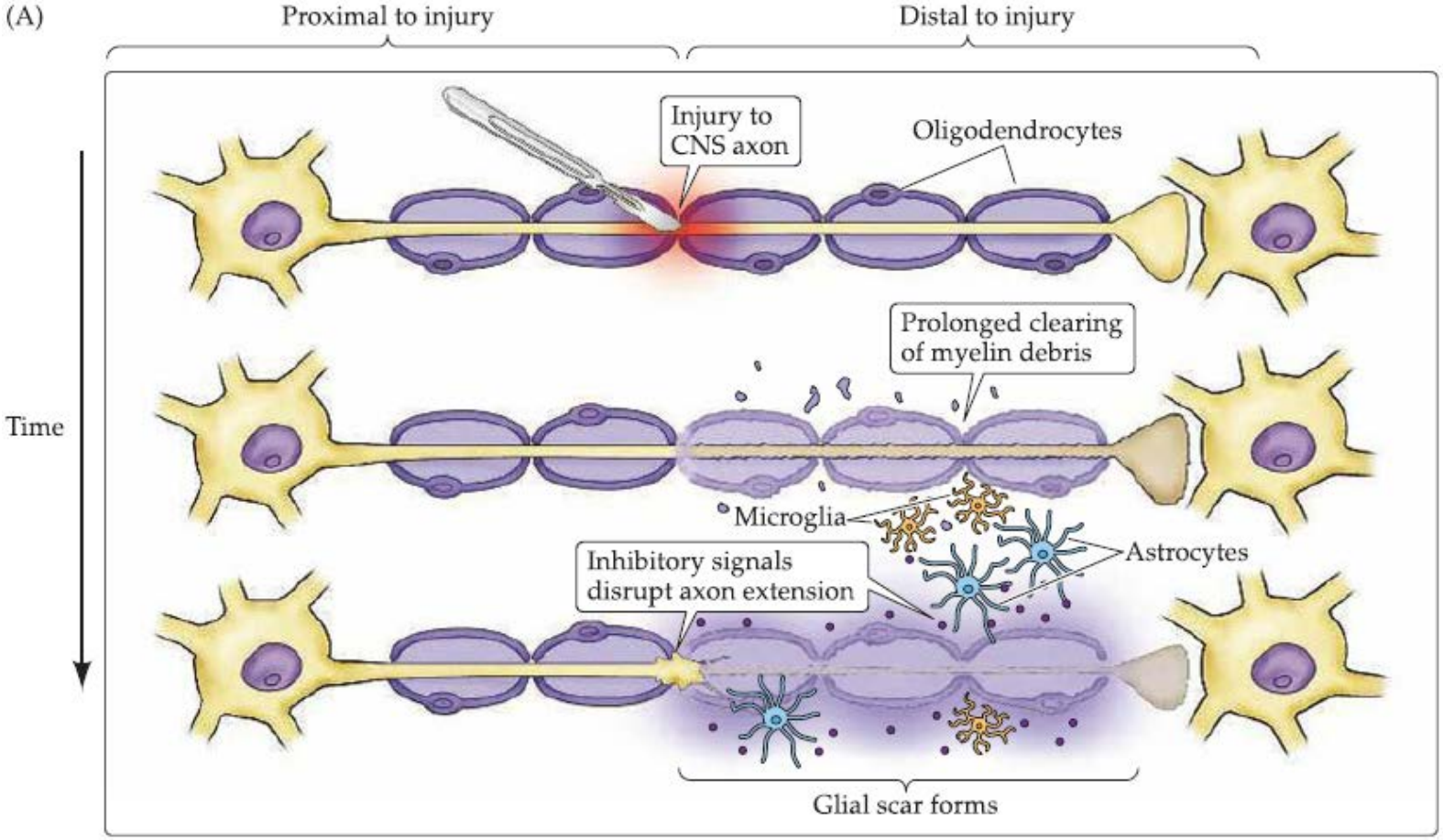
   - Brain injury elicits responses from all three glial classes—astrocytes, oligodendroglia and microglia—that actively oppose neuronal regrowth.

   - Most brain lesions cause local proliferation of otherwise quiescent glial precursors, as well as extensive growth of processes from existing glial cells within or around the site of injury.



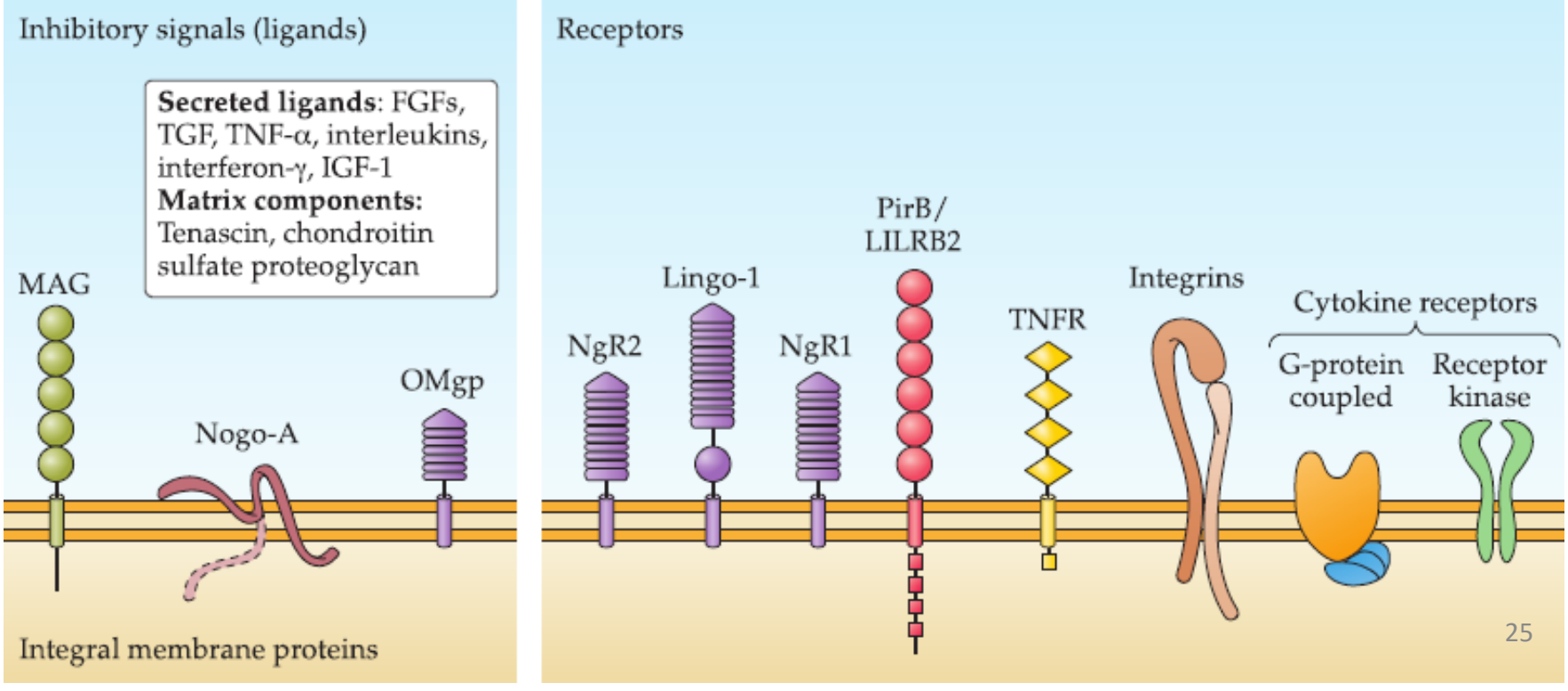
Cellular response to injury in CNS

- **Glial scarring**:
- Increased secretion of signals: TGF, FGF, TNF-α, interleukins, interferon-γ and IGF-1, which can either promote cell death and phagocytosis or provide protective signals for remaining nerve cells.
- Astrocytes within the glial scar produce several molecules that inhibit axon growth: semaphorin 3A, ephrins, Slits.



Cellular response to injury in CNS

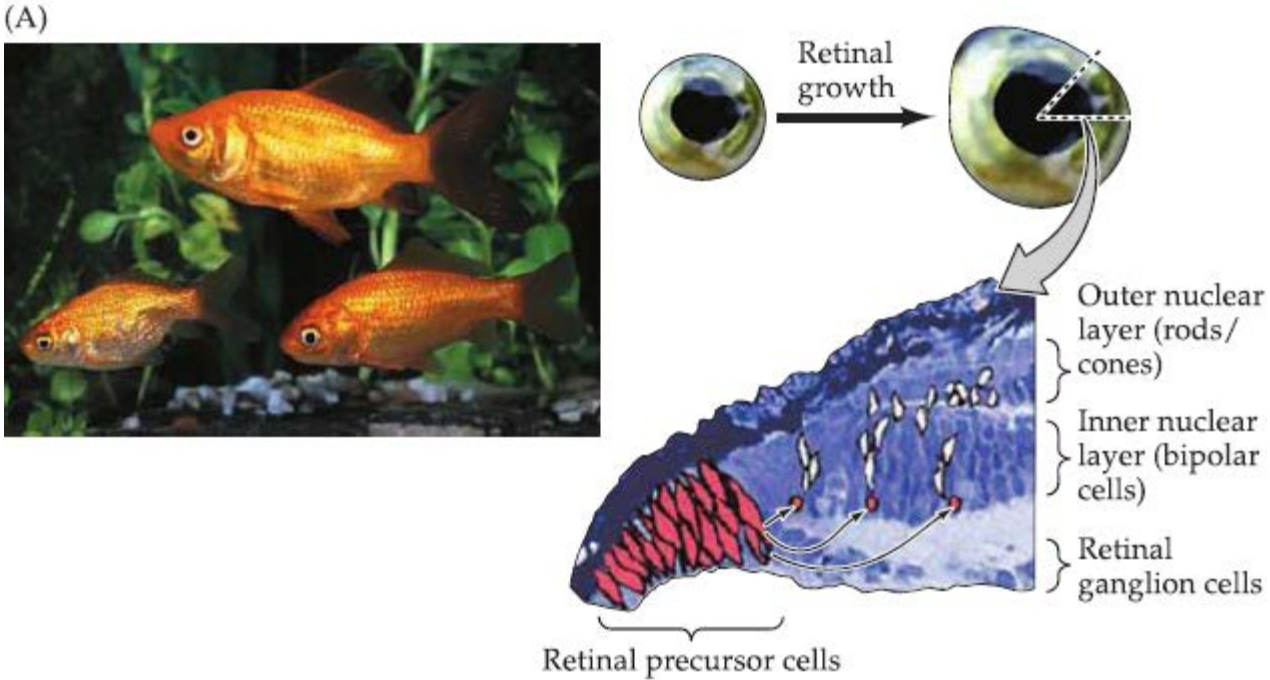
- Matrix components that inhibit axon growth (e.g. chondroitin sulfate proteoglycan) are enriched in the extracellular spaces within the glial scar.
- Brain myelin, which is produced by oligodendroglia, inhibits axon growth, including the diminished ability of axons to grow on substrates enriched in myelin-associated proteins such as myelin-associated glycoprotein (MAG), Nogo-A.



Adult neurogenesis in non-mammalian vertebrates

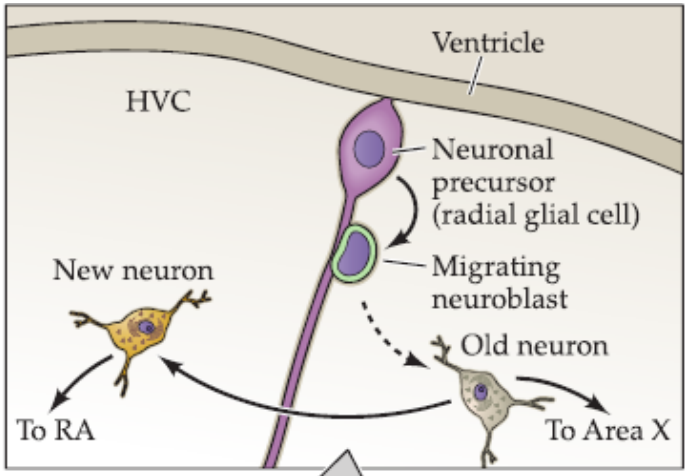
❖ Goldfish

- Goldfish grow throughout their entire adult lives; the growth of the fish's body is matched by the growth of its eyes and brain.
- The retina grows by adding new neurons generated from a population of stem cells distributed in a ring at the very margin of the retina.
- These stem cells give rise to all retinal cell types except the rods (which are regenerated from precursors found in the existing differentiated region of the retina).

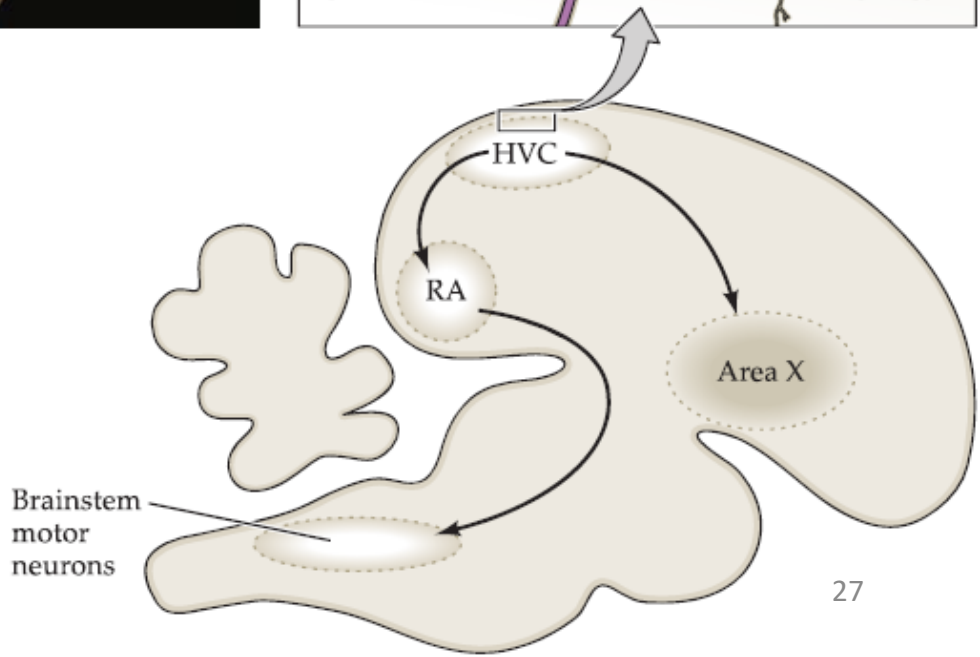


Adult neurogenesis in non-mammalian vertebrates

- **Songbird**
  - Male songbirds such as the canary lose and replace significant numbers of neurons in forebrain nuclei (HVC, RA, area X) that control the production and perception of song.



  - In HVC, a population of radial stem cells is maintained. The cell bodies are adjacent to the ventricular space, and their processes extend into the neuropil of the nucleus.

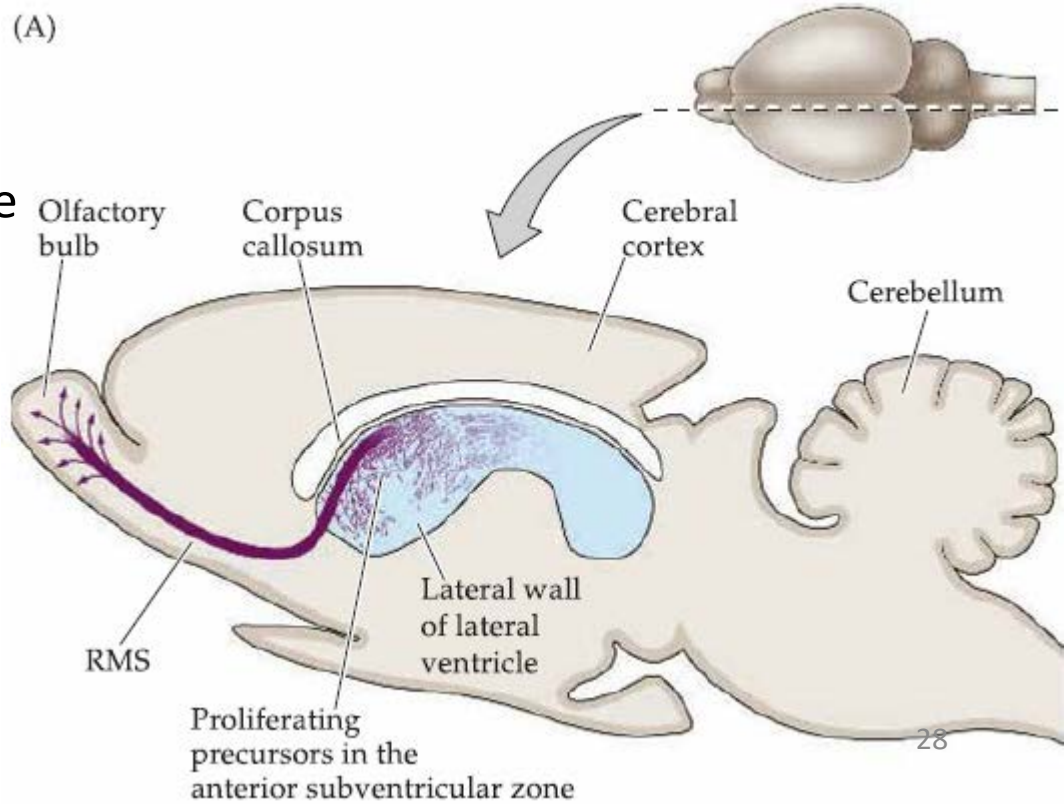


  - Neuroblasts migrate from the ventricular zone along the radial processes of the precursor cells and then integrate into circuits with existing neurons.

Neurogenesis in the adult mammalian brain

- New nerve cells in the central nervous system are generated reliably in just two regions, the olfactory bulb and the hippocampus.

- **Neurogenesis in the olfactory bulb:**
  - Neural precursors are located in the epithelial lining of the anterior lateral ventricles in the forebrain (a region called the **anterior subventricular zone**, or **SVZ**).
  - Postmitotic neuroblasts derived from the precursors migrate to the olfactory bulb via a distinctive pathway known as the **rostral migratory stream** or **RMS**.
  - Neuroblasts that migrate to the bulb via the RMS become either olfactory bulb granule cells or periglomerular cells; both cell types function as interneurons in the bulb.



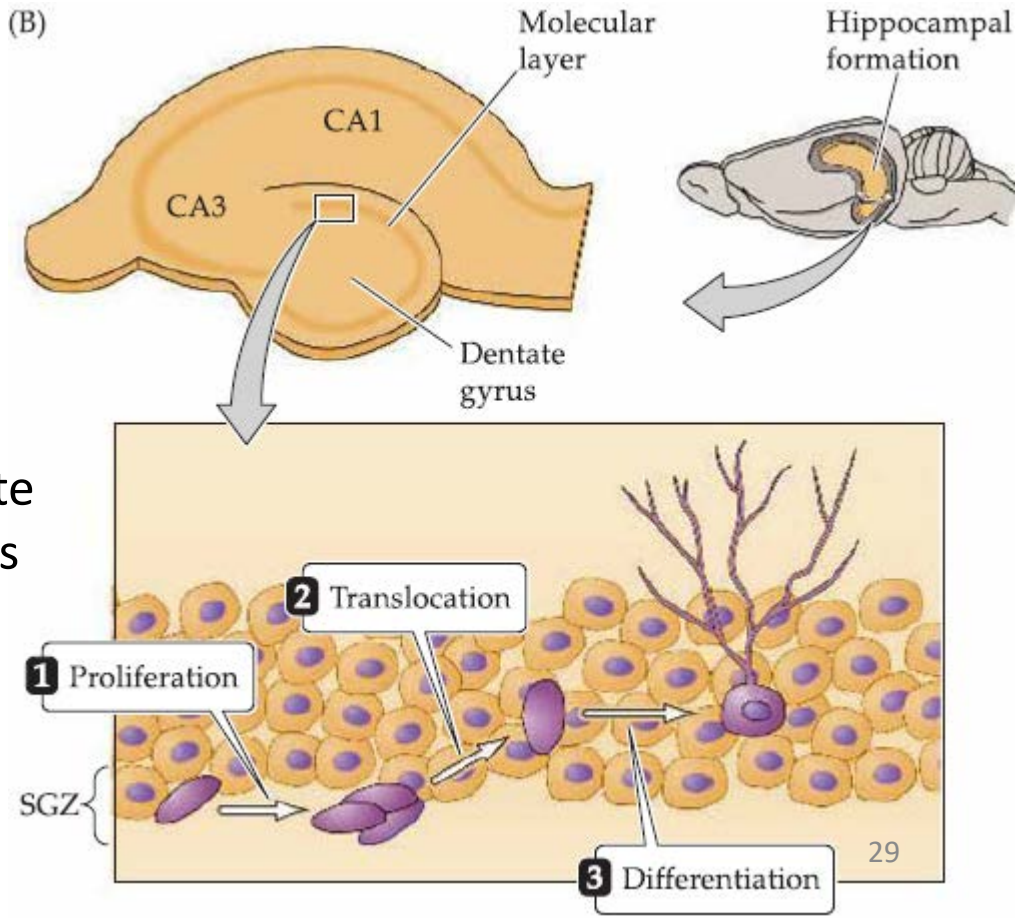
Neurogenesis in the adult mammalian brain

❖ **Neurogenesis in the hippocampus:**

- In the mature hippocampus, a population of neural precursors is resident in the basal aspect of the granule cell layer of the dentate gyrus (the **subgranular zone**, or **SGZ**).

- These precursors give rise to postmitotic neuroblasts that translocate from the basal aspect of the granule cell layer to more apical levels.

- Some of these neuroblasts elaborate dendrites and a local axonal process and apparently become interneurons within the dentate gyrus.

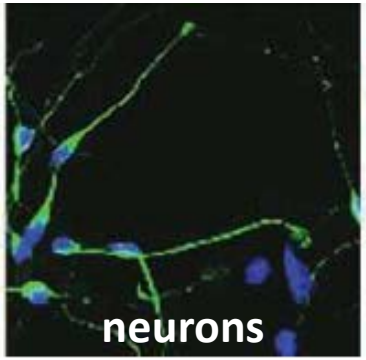


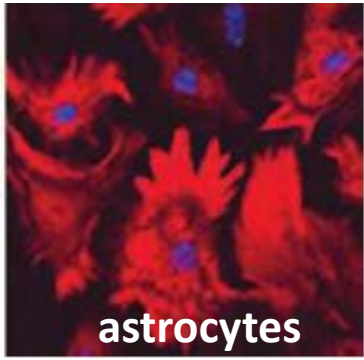
Neurogenesis in the adult mammalian brain

- At least some new nerve cells become integrated into functional synaptic circuits; however, most new neurons generated in the adult brain die before being integrated into existing circuitry.

- There may be a premium placed on stability in the mammalian brain, thus limiting opportunities for new neurons to join existing circuits.

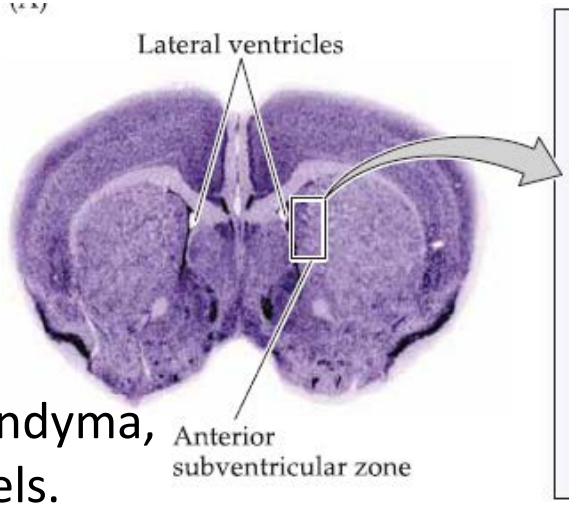
- When isolated and maintained in culture, stem cells can give rise to additional stem cells or to neurons and glia, depending on the experimental conditions：

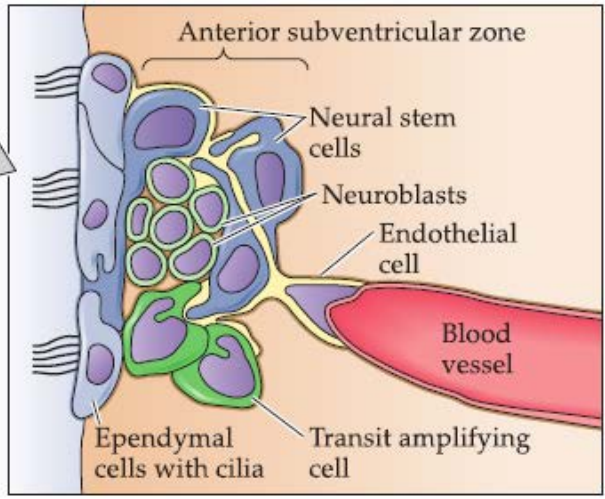




Cellular and molecular mechanisms of adult neurogenesis

- The forebrain's anterior subventricular zone constitutes a stem cell ***niche***:
  - The ciliated ependymal cells form a tight epithelial boundary separating cerebrospinal fluid from brain tissue.
  - Neural stem cells are either immediately adjacent to ependyma, or in proximity to blood vessels.



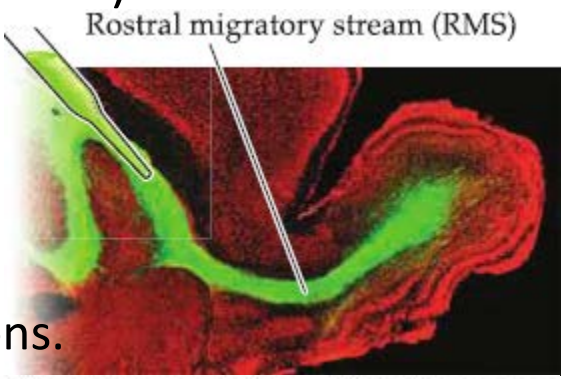


  - Transit amplifying cells are also found in this domain, and their progeny--the neuroblasts--are often clustered close by.

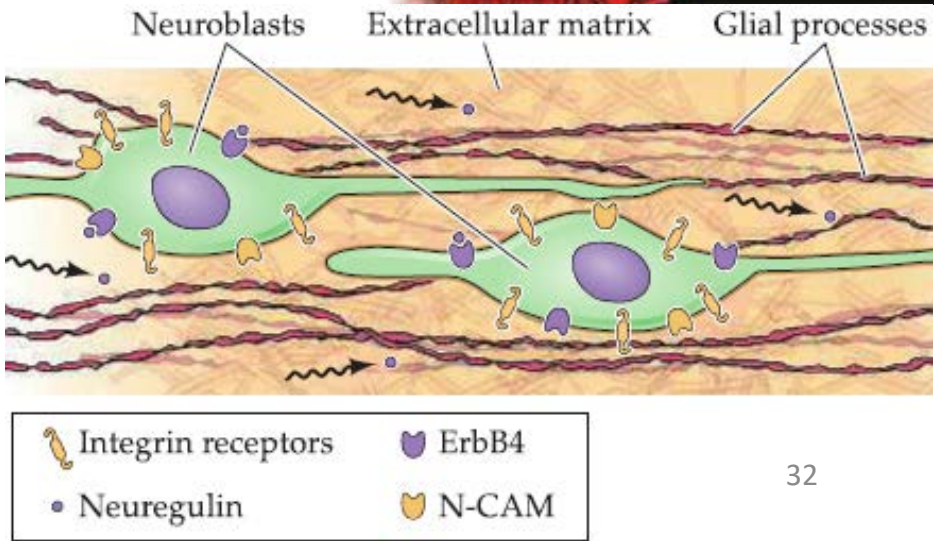
- **Transit amplifying cell**: intermediate precursor cell class
  - These cells retain the ability to divide; however, their cell cycles are much faster than those of stem cells, and they divide asymmetrically.
  - After each cell division, a transit amplifying cell gives rise to a postmitotic neuroblast or glioblast, plus another transit amplifying cell that reenters the cell cycle for an additional round of asymmetric division.
  - Transit amplifying cells are limited in their number of divisions, and eventually their potential for generating postmitotic blast cells is exhausted.

Cellular and molecular mechanisms of adult neurogenesis

- Neuroblast and glioblast cells are no longer competent to divide, and they move away from the SGZ or SVZ into regions of the olfactory bulb or hippocampus where mature neurons or glia are found.
- From SVZ to olfactory bulb, a specific migratory route, defined by a distinct subset of glial cells, facilitates migration of newly generated neurons. This route is referred to as the **rostral migratory stream** (**RMS**).



  - Glial processes form conduits for migrating neurons.
  - The extracellular matrix (ECM) associated with these processes influences migration, mediated by integrin receptors for ECM component found on migrating neurons.
  - Secreted neuregulin also influences motility of the migrating neurons in the RMS, via the ErbB4 neuregulin receptor.
  - Polysialyated N-CAM on the surfaces of newly generated neurons facilitates migration through the RMS.



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