1) Based on methods and models learned in this course until now, propose a detailed description of a strategy or a model useful for the study of regeneration. 2) Include examples (with a brief description) of possible and useful applications related to the field of regeneration of the proposed strategy/model.

Background

Macrophages play a critical role in the process of peripheral nerve regeneration. Following a transection of a peripheral nerve, macrophages in the bridge established between the two stumps, sense the hypoxia happening to the cells in the bridge and secrete vascular endothelial growth factor (VEGF) to promote angiogenesis which induces Schwann cell (SCs) migration [1]. On the distal stump macrophages remove the cellular axonal and myelin debris, facilitating axonal regrowth. SCs promote, also macrophages recruitment through the protein coding gene CCL2 to stimulate neurite outgrowth. Electrical stimulation (EStim) has been successfully applied in healing/scarring and regeneration processes for bone, bone marrow, cartilage, skin, and muscle. For partial or complete recovery, studies have reported up-regulation of inflammatory macrophages and VEGF [2][3] [4][5].

Aim We are going to describe a method to determine how EStim influences macrophage activation and phenotype in peripheral neuronal regeneration. The method can be reused for different transgenic mice or in addition of other therapies.

Overall protocol

Divide the animal model population into two groups (it could be rats if the surgical procedures seem easier to perform):

- For the first group, crush the right sciatic nerve using a fine hemostatic forceps following a simple and easy reproducible method[1] and mark the crush with powdered carbon site demarcating the area to sample tissue.
- For the other group, perform a focal demyelination lesion of the same nerve by injecting 1% lysophosphatidic choline (LPC). Similarly, to first group, area of interest will be delimited by fluorogold (FG) which will be co-injected with LPC.

Within each group, select animals of different age as regeneration ability declines with age to evaluate impact of electrical stimulation on age-dependent regeneration process.

Immediately after nerve crush (time is important for regeneration) or few days in case of LPC injection (prescribed time for demyelinating the neuron), attach an electrode between the skin and muscle, proximal to the damaged sciatic nerve and wrap the cathode wire around the exposed nerve under anesthesia. Then send electrical pulses for one-hour, mimicking firing patterns of motor neurons.

During the regenerative process, macrophages can modify their metabolic functions from a pro-inflammatory state (M1) to heal/growth state (M2). You will then analyze the impact of EStim on macrophage number and phenotype. To identify the number of macrophages and their functional phenotype (M1 vs. M2) use ED-1 as macrophage marker, iNOS and TN-α for M1 macrophage marker and Arginase-1 (Arg1) for M2 macrophage marker (dual staining ED-1/iNOS, ED-1/TN-α and ED-1/Arg1). Then follow these steps:

- Examine photomicrographs of the regions, and check for immunofluorescence signals.
- Perform temporal analysis of the crush zone, or demyelinated zone for each group of mice at different time point: 5 days (5d), 8d, 10d after the EStim event and reapply after 10d a new EStim for an hour, and rerun the analysis checking for macrophages M1 or M2 and comparing %iNOS, %TN-α and %Arg1 for each group [6].
- Investigate how delayed EStim application (delay between trauma and electrical stimulation), impacts the regenerative process.
- To understand CCL2's impact on M1 and M2 polarization, use this time dual immunofluorescence, GFAP/CCL2 to detect activated dedifferentiated Schwann cells and CCL2 chemokine expression. Run a temporal assessment of CCL2 expression and determine whether this expression is localized to SCs and count the ED-1 cells detected, i.e., number of macrophages in the crushed and demyelinated zones.

Applications

Every year cross the world, traumatic nerve lesion affects thousands of people and consequences are life-altering and often devastating. Treatments relying on nerve grafts, transfers or conduits present many disadvantages, may require multiple surgeries and therefore are not satisfactory. We propose an EStim methodology to manipulate chemokine signaling and politization state of the macrophages which could be used in conjunction with many therapies currently being actively tested [7]. This strategy could potentially accelerate the impact of these therapies and promote quicker nerve regeneration.

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