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6. AUTHOR(S)
Damien Kuffler

7. PERFORMING ORGANIZATION NAMES(S) AND ADDRESS(ES)
Institute of Neurobiology
University of Puerto Rico
201 Blvd. del Valle
San Juan, P.R. 00901

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13. ABSTRACT *(Maximum 200 words)*

Understanding the interactions between neurons and their denervated targets is critical to developing techniques to improve reinnervation of the denervated following nerve injury, and for attempting to facilitate regeneration in the central nervous system (CNS) where it does not occur. Experiments were designed to determine whether target-derived factors direct the outgrowth or regenerating axons and their own reinnervation. We have shown that neurite outgrowth from both sensory and motor neurons is directed up concentration gradients of target-derived factors both in vitro and in vivo. Using these gradients and the cells that secrete the factors we have succeeded in promoting regeneration across peripheral nerve gaps of up to 2 cm. Experiment are underway to bridge gaps of up to 8 cm. The target-derived factors that promote and direct axon regeneration, as well as determine the morphology of the processes are being characterized (more than 6 have been demonstrated). The presence of a peripheral nervous system regeneration inhibiting factor has been demonstrated and methods developed to block its influence. Experiments are underway to determine

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Name of Institution: Institute of Neurobiology, Medical Sciences Campus, Univ. of Puerto Rico

Authors of Report: Damien Kuffler, Ph.D.

List of Manuscripts in Preparation
Kuffler, D.P. Inhibition of neurite outgrowth in the peripheral nervous system.

Betancourt, A., & D.P. Kuffler. Blocking peripheral nervous system neurite outgrowth inhibition with neurotrophic factors.

Betancourt, C. Sekirnjak, A, Rivalta, A., Q. Zhao, & D.P. Kuffler. Promoting axon regeneration across a 3 cm gap via seeded peripheral nerve cells in a silicon tube.

Hill, E., G. Latalladi, & D.P. Kuffler. Diffusible concentration gradients of target-derived factors direct motor axon regeneration in vitro.

Zheng, M. & D.P. Kuffler. Regenerating axons in vivo growth up concentration gradients of target-derived factors.

Scientific Personnel Supported:
Fukang Xie
Nivia Pérez

Report of Inventions by Title: None

Statement of Problems Studied:
1. Do concentration gradients of peripheral nerve-released factors direct the growth cone sensory and motor neuron growth cones in vitro?
2. Do concentration gradients of peripheral nerve-released factors direct the growth motor axons in vivo?

3. Can novel methods be developed to facilitate regeneration across long gaps in peripheral nerves?
4. What factors are required to protect adult human neurons from neurotoxicity following spinal trauma?
5. Characterization of peripheral nerve-released factors that promote neurite outgrowth and modify neurite morphology.
6. Are there regeneration inhibiting factors in the peripheral nervous system?
7. What role does innervation have on the maintenance of skeletal muscle fiber subsynaptic nuclei clustering?
8. What influences do skeletal muscle fiber-released factors exert on motoneuron process outgrowth?
9. What are the membrane properties of adult sensory and motor neurons?

Summary of Most Important Results

1. Do concentration gradients of peripheral nerve-released factors direct the growth cone sensory and motor neuron growth cones in vitro?

We have demonstrated diffusible that concentration gradients of peripheral nerve-derived factors reliably direct the outgrowth of sensory (28/28) and motor neuron (47/47) growth cones in vitro. This influence is exerted over gradients up to 200 μm long.

2. Do concentration gradients of peripheral nerve-released factors direct the growth motor axons in vivo?

Concentration gradients of peripheral nerve-derived factors reliably direct the outgrowth of motor axons in vivo. This influence is exerted over gradients up to 6.5 mm.

3. Can novel methods be developed to facilitate regeneration across long gaps in peripheral nerves?

The central and distal nerve stumps of a peripheral nerve have been inserted into silicon tubes filled with bioresorbable matrices into which are injected dissociated cells of a peripheral nerve. Using these bridging tubes peripheral axons can now be made to successfully regenerate across gaps of more than 2 cm.

4. What factors are required to protect adult human neurons from neurotoxicity following spinal trauma?

Using methods developed in this laboratory, adult human sensory neurons have been isolated and maintained in culture for more than 8 weeks where they extend neurites and remain electrically excitable. The best survival of such isolated adult neurons by other laboratories is 24 hours. Normally such neurons die due to the neurotoxic environment created during the isolation procedures or spinal trauma. The methods used to isolate the neurons are being analyzed to determine which components of the protocols are responsible for protecting the neurons from neurotoxicity.

5. Characterization of peripheral nerve-released factors that promote neurite outgrowth and modify neurite morphology.

We have demonstrated that medium conditioned by a length of peripheral nerve contains a number of factors (a minimum of 6), with vastly differing molecular weights, that promote neurite outgrowth from adult neurons and modify the morphology of the neurites. Although the responsible

factors have not been isolated, the molecular weights of factors in some fractions indicate that some of these factors are not part of the known neurotrophin family. These results also demonstrate multiple factors must be present and act simultaneously on adult neurons to promote neurite outgrowth and extensive elongation of the neurites, as well as to induce neurites with a physiological appropriate morphology.

6. Are there regeneration inhibiting factors in the peripheral nervous system?

We have demonstrated that satellite cells associated with adult rat sensory neurons inhibit neurite outgrowth. However, this inhibitory influence can be reduced by specific neurotrophic factors and completely blocked by a combination of peripheral nerve-released factors. The influence of the neurite outgrowth inhibitory factor appears to be most effective when the neurons are on contact with the satellite cells, however, a the factor also appears to act in a diffusible manner on neurons not in contact with satellite cells. Preliminary results indicate that the factor is a heparan sulfate proteoglycan.

7. What role does innervation have on the maintenance of skeletal muscle fiber subsynaptic nuclei clustering?

Subsynaptic nuclei clustering in adult skeletal muscle fibers appears to result from nerve released factors that become bound to the muscle fiber extracellular matrix and potentially interact with integrin receptors to induce and maintain the nuclei clustering.

8. What influences do skeletal muscle fiber-released factors exert on motoneuron process outgrowth?

Skeletal muscle fibers release diffusible factors that promote and direct neurite out from cultured adult motoneurons.

9. What are the membrane properties of adult sensory and motor neurons?

The biophysical properties of adult sensory and motor neurons have been characterized using electrophysiological approaches. It was shown that several types of sensory neurons can be identifies by their morphology and that each population has different membrane properties. This technique should allow for the identification of different populations of neurons based on morphology alone.

List of All Publications and Technical Reports Published

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list of all participating personnel (include any degrees gained while on the project):
Maria S. Sosa (Faculty, Institute of Neurobiology)
Jorge Lastra Power (Resident, Division of Neurosurgery)
Ivan Sosa (Medical Student / Resident, Division of Neurosurgery)
Jaimi Inserni (Staff, Division of Neurosurgery)
Alijandro Betancourt (Resident, Division of Neurosurgery)
Javier Amadeo (Resident, Division of Neurosurgery)
Juan A. Vigo Prieto (Staff, Pediatric Neurosurgery)
Onix Reyes (Medical Student / Resident, Division of Orthopedic Surgery)
Zhao Qing (Staff, Division of Neurosurgery, Malmø, Sweden)
Garcia-Castiñeiras (Professor, Dept. of Ophthalmology)
Serguei Skatchkov (Postdoctoral Fellow)
Ladislav Vyklicky (Visiting Professor, University of Prague, Czech Republic)
Richard Orkand (Professor, Institute of Neurobiology)

Graciella Latalladi (Research Associate)
Christian Broesamle (Masters Student) (received MS degree)
Min Zheng (Research Associate)
Fukang Xie (Postdoctoral Fellow)
Evan Hill (Graduate Student)
Chris Sekirnjak (Transitional Student)
Nivia Pérez (Technician)
Laurie Bonilla (Technician)
Francisco Quiles (Undergraduate Student)
Ajneb Rivalta (Undergraduate Student)
Ana Morales (Undergraduate Student)
Iris Bonilla (Undergraduate Student)