Immunohistochemical methods in neurobiology

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HUMAN CENTRAL NERVOUS SYSTEM

- Consists of $10^{11}$ neurons
- Neurons generated by division from pluripotent stem cells
- First problem: connectivity
- Second problem: phenotypic differentiation
At the simplest level neurons can be defined by the neurotransmitter they use to signal.

More than 20 putative neurotransmitters are known, including amino acids, catecholamines, neuropeptides and acetylcholine.

Each of these neurotransmitters is supported by a variety of transmembrane pumps and enzymes.
THE AUTONOMIC NERVOUS SYSTEM

- Controls blood pressure and visceral functions.
- Parts of it are largely peripheral and hence accessible.
- Two neuron chain consisting of a preganglionic neuron in the central nervous system and a postganglionic neuron in the periphery.
SYMPATHETIC NERVOUS SYSTEM

Preganglionic neurons in the spinal cord

Postganglionic neuron in peripheral ganglion

Adrenal medulla

Target tissues
ORGANISATION OF SYMPATHETIC NERVOUS SYSTEM

- Discrete subpopulations of postganglionic neurons innervate different end organs.
- Discrete subpopulations of preganglionic neurons innervate functionally different postganglionic neurons.
- The peripheral sympathetic nervous system consists of parallel, but different neural pathways.
PARALLEL PATHWAYS IN THE SYMPATHETIC NERVOUS SYSTEM

PREGANGLIONIC NEURONS

POSTGANGLIONIC NEURONS

SECRETOMOTOR  HEART  VASODILATOR  IRIS  BROWN FAT  PILOMOTOR  ADRENAL MEDULLA
Functional specificity

- Postganglionic neurons project to (innervate) functionally very heterogeneous target tissues (organs)
  - Iris, piloerector muscles, salivary and lachrymal glands, blood vessels...
Functional specificity

- Do neurons projecting to (innervating) tissues which have functionally similar or identical properties have common morphological, physiological and neurochemical properties?
Functional specificity

- Do neurons projecting to (innervating) tissues which have functionally similar or identical properties have common morphological, physiological and neurochemical properties?

- Can we identify functionally specific neurons on the basis of their anatomical properties?
Principle methods in anatomical approach to studying neuronal connectivity

- Retrograde neuronal tracing
- and
- Multiple immunohistochemistry for various neuroactive substances including neuropeptides!
Fast Blue into the salivary gland
Fluoro Gold into the skin
Rat Stellate Ganglion
Calbindin and NPY Immunoreactivity
Rat Stellate Ganglion
Fast Blue into skin of leg Calbindin and NPY Immunoreactivity
Rat Stellate Ganglion
Fast Blue into skin of leg, Calbindin and NPY Immunoreactivity
Functional specificity

- Postganglionic neurons project to (innervate) functionally very heterogeneous target tissues (organs)
## Substances co-localised in rat pre- and postgaglionic neurons

<table>
<thead>
<tr>
<th>Ganglionic Neurons</th>
<th>Preganglionic Neurons</th>
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<tbody>
<tr>
<td>Ach</td>
<td>Galanin</td>
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<td>Ach</td>
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<td>Ach</td>
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<td>Ach</td>
<td>VIP/CGRP</td>
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<td>NA</td>
<td>Calbindin</td>
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<td>Secretoneurin</td>
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<tr>
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<td>SP</td>
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<td>VIP/PHI</td>
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CHEMICAL CODING OF POSTGANGLIONIC NEURONS TO SUBMANDIBULAR SALIVARY GLAND

IML

Vasoconstrictor neuron NA/NPY

Secretory neuron NA/-

Blood vessel

Acini
Chemical coding theory

- Neurons that share identical functions share identical combination of neurotransmitters, enzymes, receptors...
- These combinations are unique
Ganglionic neurons:
- Calbindin/NOS
- Calbindin/NOS/Calretinin
- NOS only
- CGRP only
- Calretinin/NOS
- CGRP/NOS

Preganglionic neurons:
- Not Calbindin
- Not NPY
- Calbindin
- Not NPY
- VIP
- Not CGRP
- Not Calbindin
- Not NPY
- Calbindin
- NPY
- Calbindin
- NPY
- Calbindin
- NPY
- VIP
- Not CGRP

Organs:
- Piloerector muscle
- Salivary gland
- Brown Fat
- Iris
- Heart
- Skeletal muscle vasodilator
Identification of neural pathways using anatomical approach

- Neurochemically distinct subpopulations of both pre- and post-ganglionic neurons are connected to form functional pathways
Identification of neural pathways using anatomical approach

- How specific are connections between preganglionic terminals and postganglionic somata and dendrites?
Rat Stellate Ganglion
Fast Blue into skin of leg, Calbindin and NPY Immunoreactivity
How specific is pre- to postganglionic neuron connection?

- Iris projecting sympathetic pathway
- Preganglionic neurons in IML (intermediolateral column) of T1-T4
- Postganglionic neurons in the SCG (superior cervical ganglion)
How is peptidergic transmission related to the synaptic machinery?

- Interaction between SP and NK1 receptor in the enteric nervous system
Innervation of the Heart
Srce do sada?


Zašto ‘bol’ iz srca?

Ciljevi projekta:

1. Karakterizacija spinalnih i vagalnih afferentnih neurona (broj, distribucija u ganglijima i jezgrama moždanog debla, te neurokemijske karakteristike) koji se projiciraju u dijelove ventrikularnog i atrijalnog miokarda.
Zašto 'bol' iz srca?

Ciljevi projekta:

2. Karakterizacija živčanih profila (gustoća, specifična lokalizacija i neurokemijske karakteristike) u ventrikularnom i atrijalnom miokardu prikazanih anterogradnim neuralnim transportom boja aplikiranim u leđnu moždinu/dorzalne ganglije/moždano deblo.
Innervation patterns in the young, mature and aged ventricular myocardia.

α = 0.05. Mean SEM. asterisks!!
n = 10 (young); n = 3 (mature); n = 4 (aged).

Zašto ‘staro’ srce?