Major Problems

1) Mech. regulation during differentiation
   1) DNA $\xrightarrow{1}$ mRNA $\xrightarrow{2}$ protein
   * 2) Codon Functions

2) Bcf Sequence mRNA & DNA
   a) Freq. & dist. codon usage.

3) Neurobiology.
   1) Effect, cell on another.
   2) Neuronal codes.

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Assay: Rate of Neuron Firing as function of rate of transport of fluorescent material

1) Aplysia

* 2) Squid axon

3) Crayfish Sensitization stretch receptor

* 4) Nematode

? 5) Tissue culture

a) Tsaki

6) Surface of brain

1) Methylene blue

2) Fluorescein butyrate

3) 2 Lipids labeled cpd?

Fluorescence Measurement:

1) Follow by eye.

2) Microscopically

3) Cell suspension (tissue culture or Rose test) in cuvette.

a) Neural tumor - Ascites.

4) Need Transparent ganglion Thin

2) Methylene blue - Visible light

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Rate Transport vs Rate [illegible] discharge
Metabolism

1) Fluorescence
   1) Fluorochromasia
      1) Fluorescence
   2) No "
      1) Me Blue
      2) Naphthyl dec.
      3) Ed[illegible]
      4) Redox indicator
      5) pH "
      6) DPN ⇄ DPNH
         TPN

2) Assay
   1) Cell suspension in Cary. Rate Methylene blue. Lowage
      1) UV, visible
      2) Fluorescence
      3) Stim. neurons discharge suspension via.
         a) Norepinephrine
         b) Ac. Choline
      4) Inhibit neuron firing
         DEADA
      5) Electron grid
         Med →
         Med →
         grid for stim
         quartz plate
         Metal grid for stim

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Internal Control
Look for diff neural patterns (glow) when stim diff neurons.

Best 2) Microscope or eye. Approximation
1) Stimulating (recorded) single neuron or small gaulion.
1) Electrically
a) some neuron
b) other "

2) Microelectrodes or filter paper.
Noreph.
Ac. Chol.

2) Visible - UV, (Fluorescence)
a) Large neuron no microscope needed.

3) Polarization of fluorescence.
1) Acridines (tempt)
*2) Methylene blue.

4) May need to slow down nots.
Lower temp 3° 10° 15° 24° 37°

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5) a) Small vessel. Known amt. of Methylene blue or compd. transported + Inc. Remove samples at intervals. 5 sec & measure change on loss

10

15

30

60

a) Preliminary only.

b) Squid giant axon

1) Perfuse inside. Measure rate of amplitude, nonrelativistic $_{11}C^{14}$ compd etc.

6) 1) Use few ganglion.

* 2) Use Many ganglion or refusers.

a) Remove adjust.

b) Multiple filtration

Many adjust

1) Squid ganglion

2) Fix.

3) wash & Treat H^+ or alkali

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9/6/66

Idea: Rotation paper.

fluorescein discrete ~~diffuse~~ Eng. fluorescein + acetate

Outside ~~diffuse~~ inside inside cells

Nonfluorogenic. Fluorogenic

Study rate of exit from cell.

Use this technique for neural pathways

Study ~~cell~~ exit from cells or uptake dependent upon neuronal stimulation.

1) Need active transport (entry or exit) of fluorofor or precursor.

1) Active transport in. Little diffuse No exit pressure

2) Very rapidly converted (intracellularly in 6) to fluorescent material.

3) Transport proportional to rate of neuron firing.

4) Need natural neuronal monolayer to study.

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2) Pendulum compd!

3) Optical design to ~~[illegible]~~ slow down so can detect pulses (lead neuron firing) X.
High speed movie + computer.

For ext append

1) Flowcoffer assy.

1) Use many simple organisms good genetics & electrophysiology

1) Transparent nematode or isolated ganglion

2) Artemia?

3) Drosophila

4) Neurons in tissue culture

5) External (surface) neurons. best

1) Can be stimulated electrically or chem.

2) " " seen microscopically?

4) Myelinated neurons (Maybe have high conc. compd in myelin)?

7) Maybe use std neurophysiological prep. Inject fewer for (fluorescence) thing rate of exit from neuron as function of excitation.

* * * 8) Methylene blue etc. Rate of ~~[illegible]~~ [illegible]
Other redox dyes.

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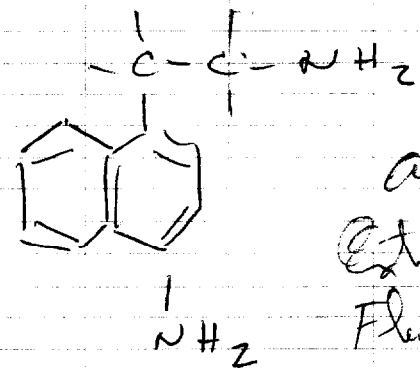
Methylene blue

a) Rate of uptake vs Rate neuron firing.

1) Right
2) Phase
3) Fluorometry
4) UV

b) Look up Ext. coefficient, Spectrum.



Aminonaphthylalanine

Ext 320

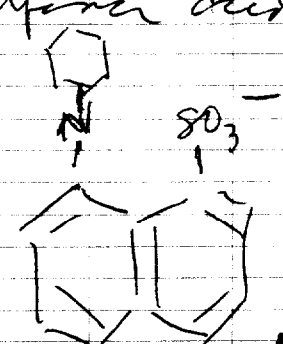
Flu 450

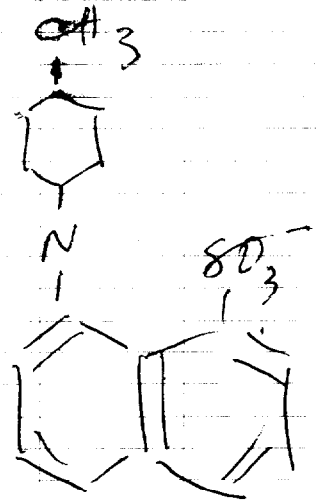
$< 10^{-4.17}$ measurable with O blank

~~Doesn't~~

Eosin - Protein

Sulfonic acid.





ANS

Doesn't flourish in H_2O

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20) Cell Glenner – Vital Stains Neurotisine
Fluorescent Other "

21) Assay –

1) Cell suspension, Rate of mytilus exit.

2) Mucosal ?

3) Tissue attached neurons.

X 4) Suspension mucosal organism
no good.

5)

Look up Fluorescent dyes
redox indicator

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$\frac{30}{120}$

$\begin{array}{r} 74 \\ \times\ 4 \\ \hline 296 \end{array}$ $\begin{array}{r} 74 \\ 3 \\ \hline 222 \end{array}$

Metal [illegible] blue

5500 – 7000 A
(6580)

6500 – 7000 [illegible]

Ref [illegible]

[illegible]

63, 69 (1941)

Molar ε_{5970} [illegible] $\times 10^{-7}$ M

= 5.76

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