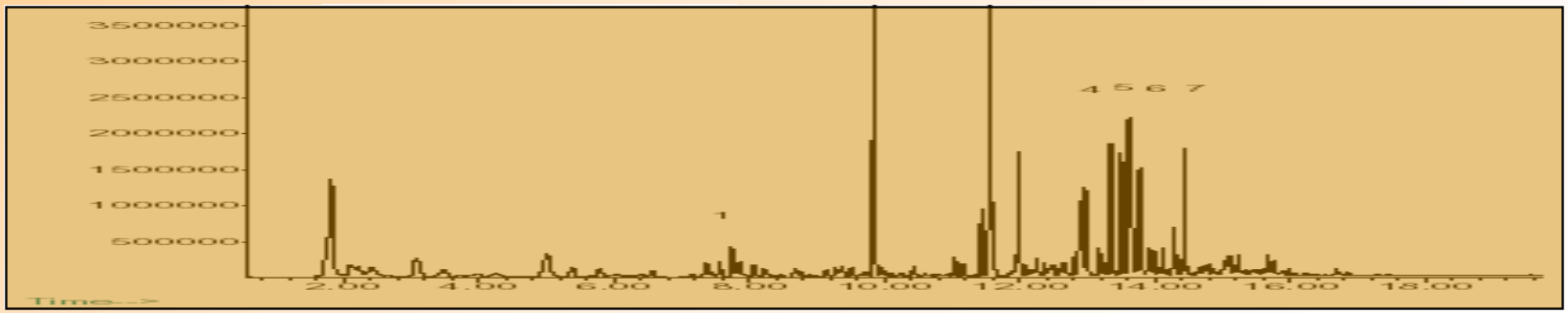
BIOMARKERS AND RESIDUES

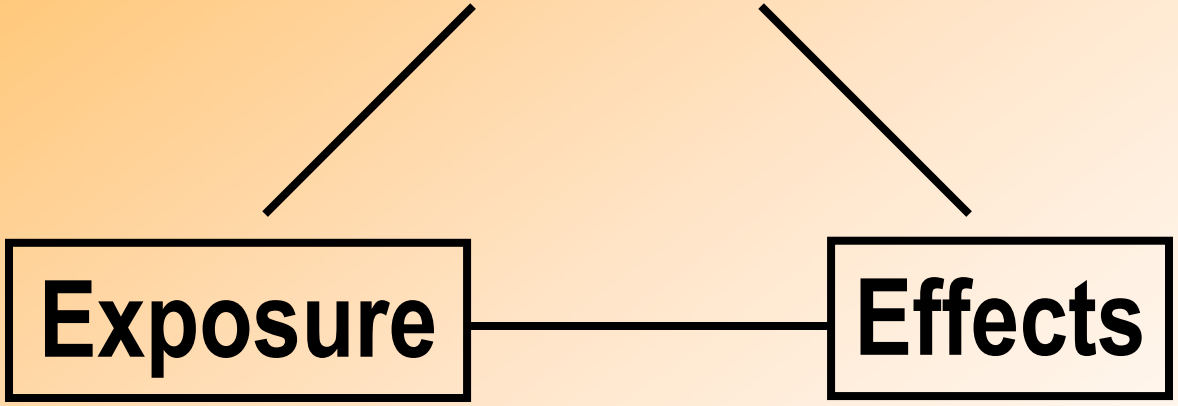


Chippendales®

presents

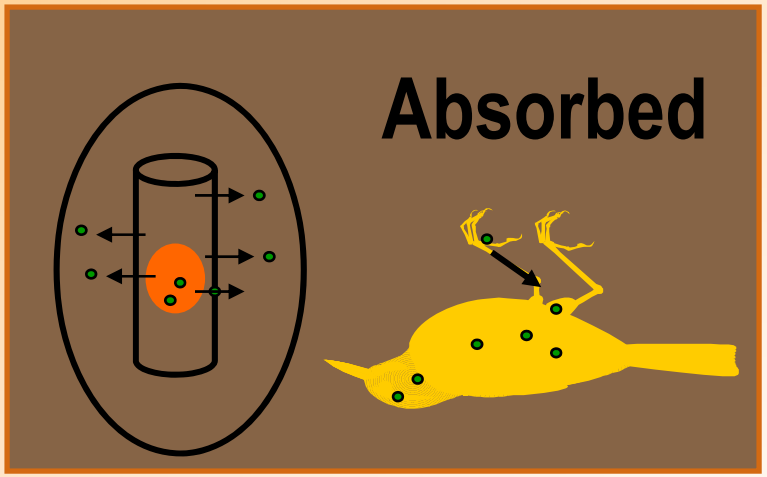
F e e l t h e h e a t

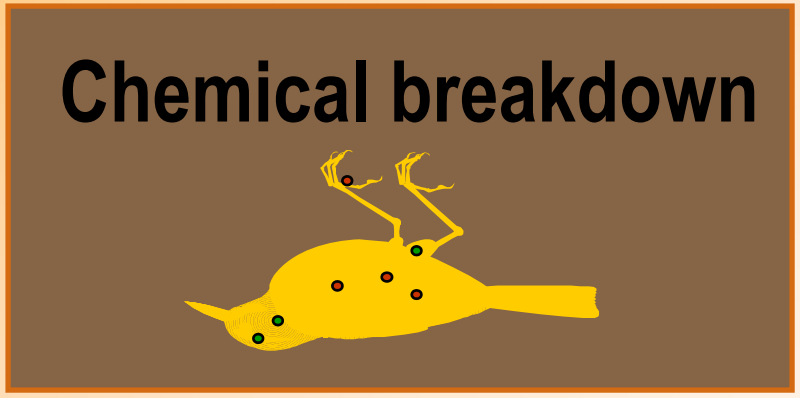
Biomarkers and Residues

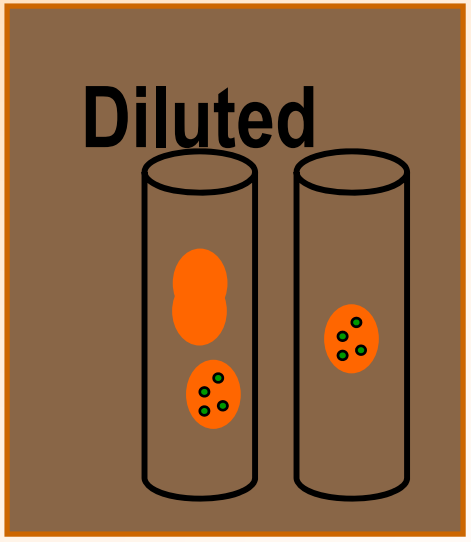


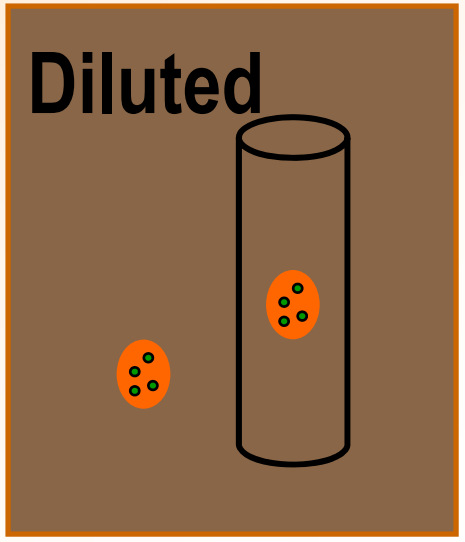
Residues

What you measure is what is left after being:

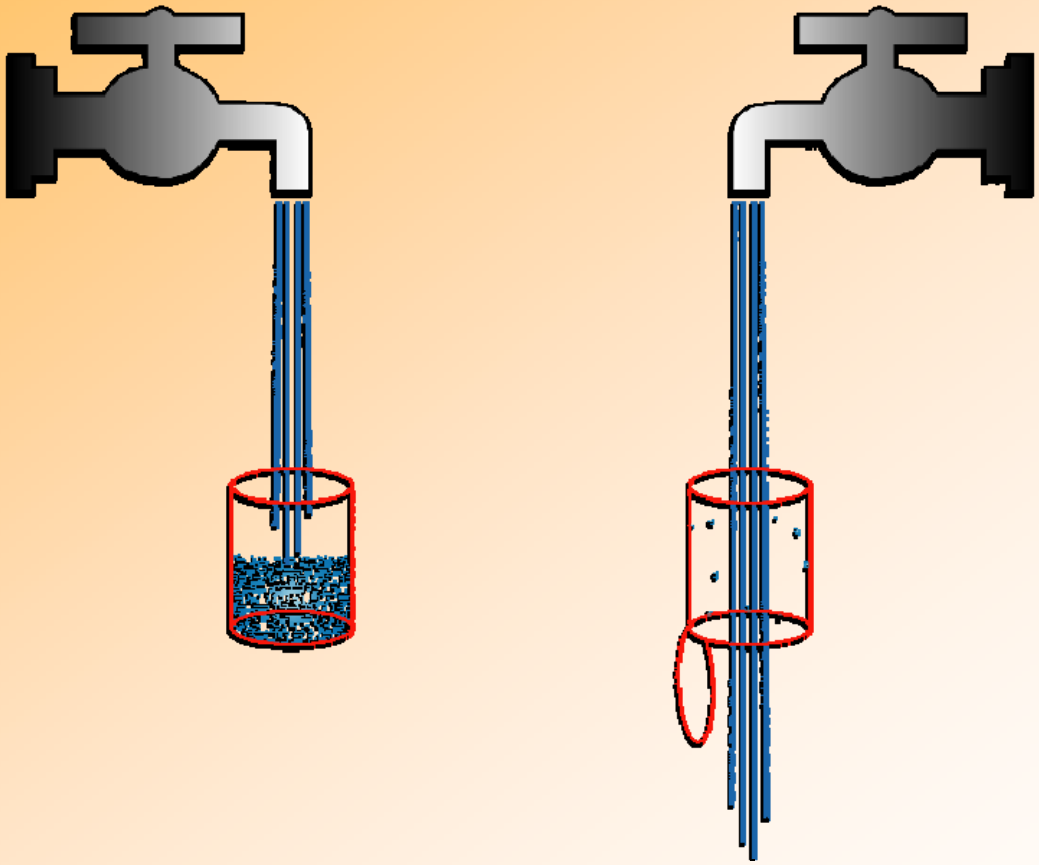








Two Residue Scenarios



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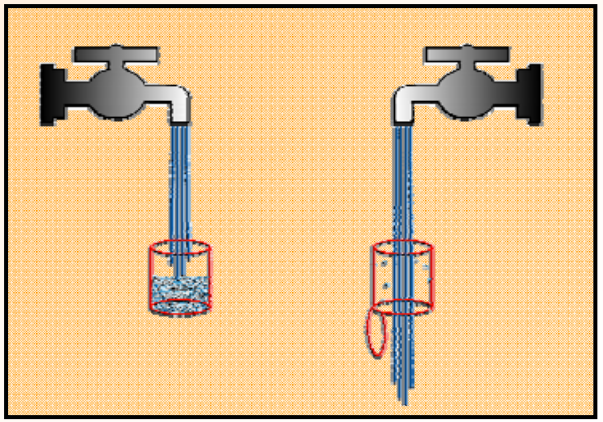
Residues

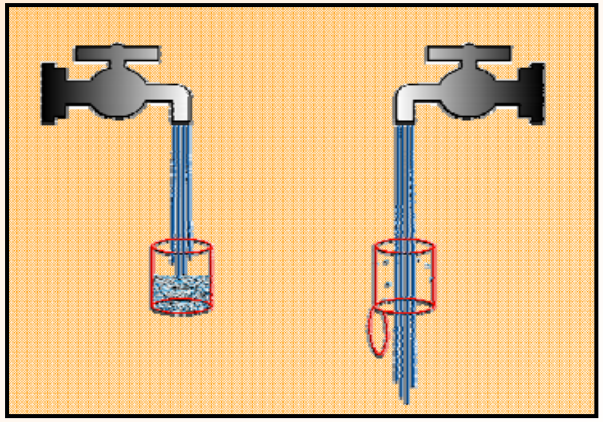
- **Do they relate to laboratory toxicity test values?**

- **Do residues measured in different matrices correlate with each other?**

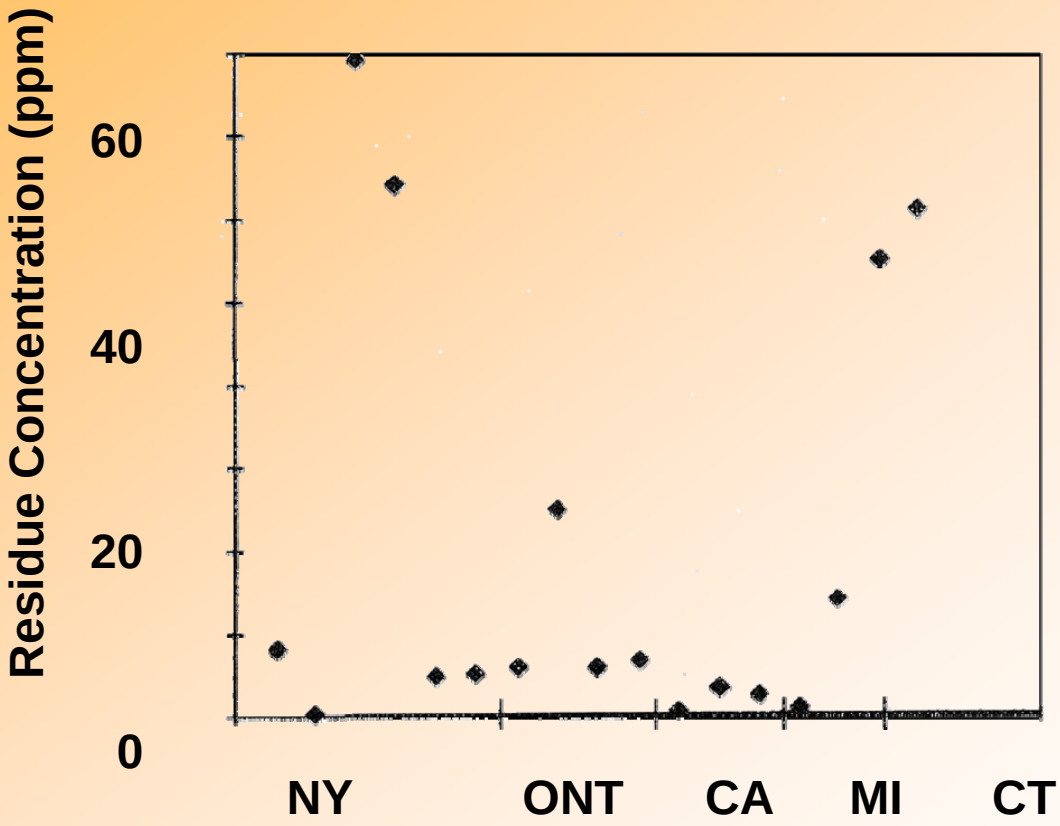


Residues

- **When are residue measurements useful?**



Diazinon residues in grass samples from upper gastrointestinal tracts of ducks or geese



Source: P. Mineau and K. R. Tucker, 2002. Improving detection of pesticide poisoning in birds. Journal of Wildlife Rehabilitation 25:4-13

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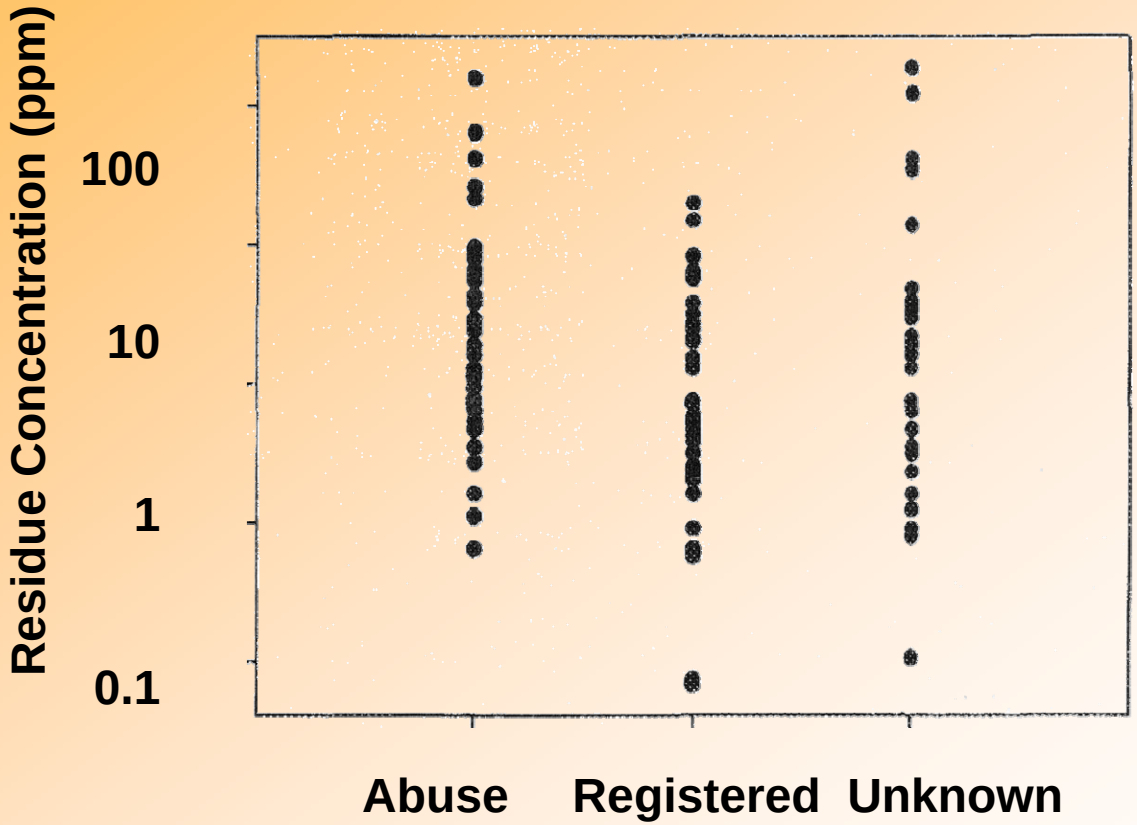
Carbofuran concentration from gastrointestinal tract of raptors



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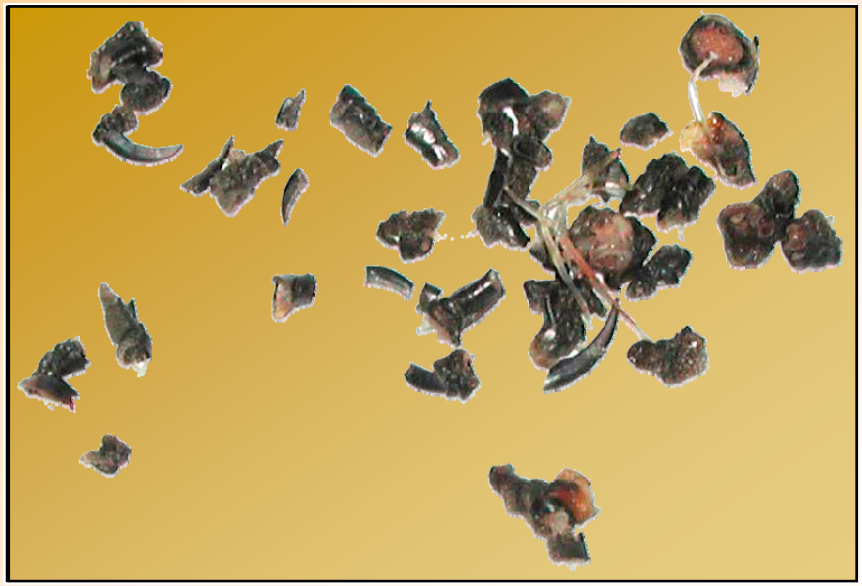

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D1

D2

D3

D4

D5

D6

Azinphos-methyl residues (ppm) from skin plus feathers of brown-headed cowbirds

Exposure time	ppm
0 hrs (control)	<0.4 (1)
36 hrs	11.3±1.4 (8) [6.1 – 18.0]
3 days	3.1±0.3 (9) [1.5 – 4.6]
7 days	2.6±0.4 (9) [0.9 – 4.2]

Detection limits for skin plus feathers = 0.4 ppm a.i.

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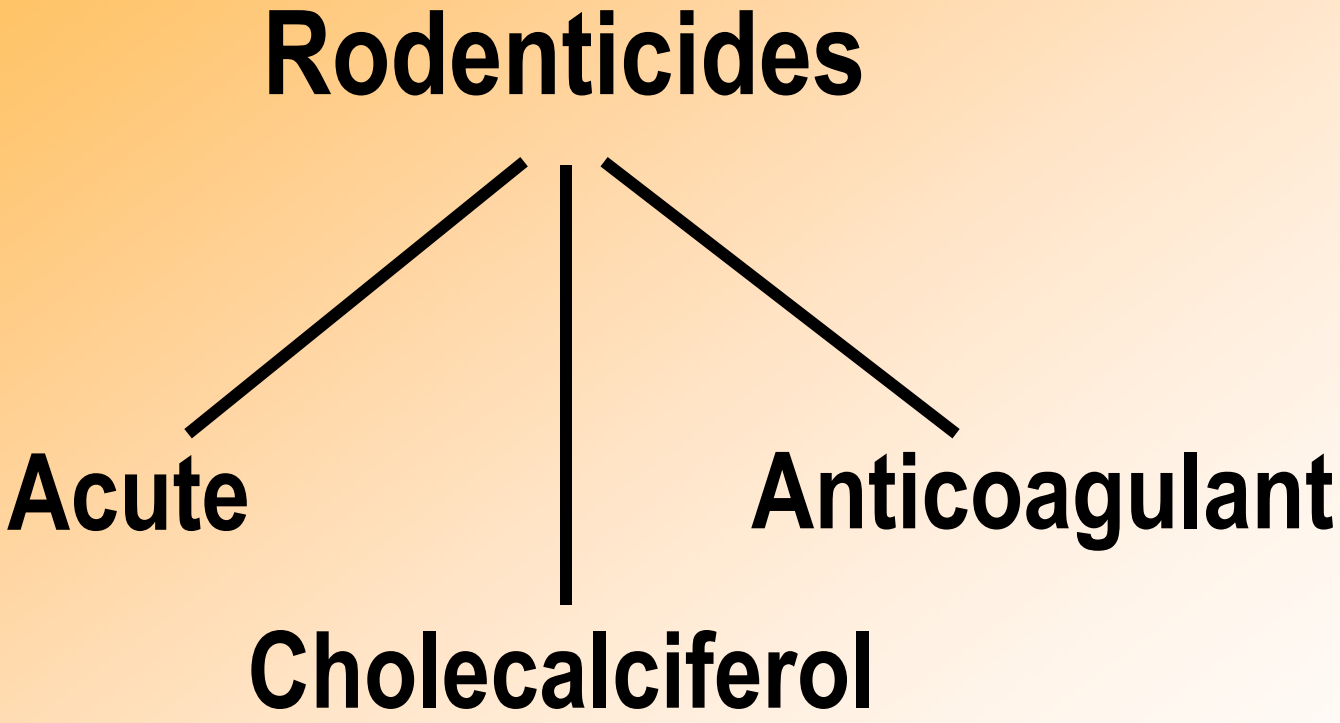

Rodenticides
Acute
Cholecalciferol
Anticoagulant

Anticoagulants

Coumarins

First generation

Second generation

Indandiones

First generation

Anticoagulants

First generation – multiple day feeding

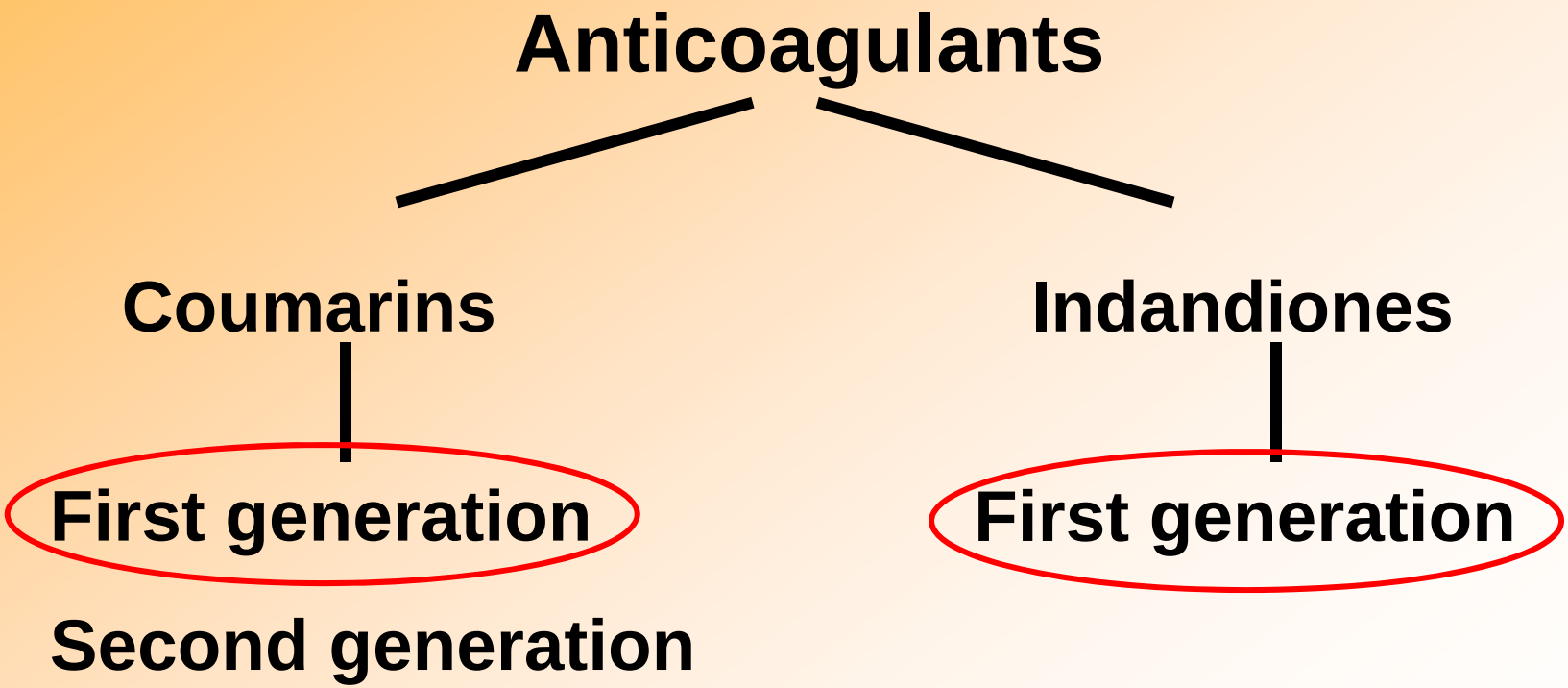
Second generation – single feeding toxicant

For both generations - mortality can occur up to 3 weeks after exposure.

Hematomas

New EPA Regulations for Rodenticides

- Expect increase usage of first generation anticoagulant rodenticides



Chronic vs. Acute Toxicity

Anticoagulant rodenticide	LD50- single dose (mg/kg)	LD50 total 5-day dose (mg/kg)
First Generation		
Chlorophacinone	20	0.95
Diphacinone	43	1.05
Warfarin	186	1.65
Second Generation		
Brodifacoum	0.2	
Bromadiolone	1.1	
Difenacoum	1.8	
Difethialone	0.6	
Flocoumafen	0.2	

Jackson, W.B., Ashton, A.D. 1992. A review of available anticoagulants and their use in the United States. Proc Vertebr Pest Conf 15:156-60.

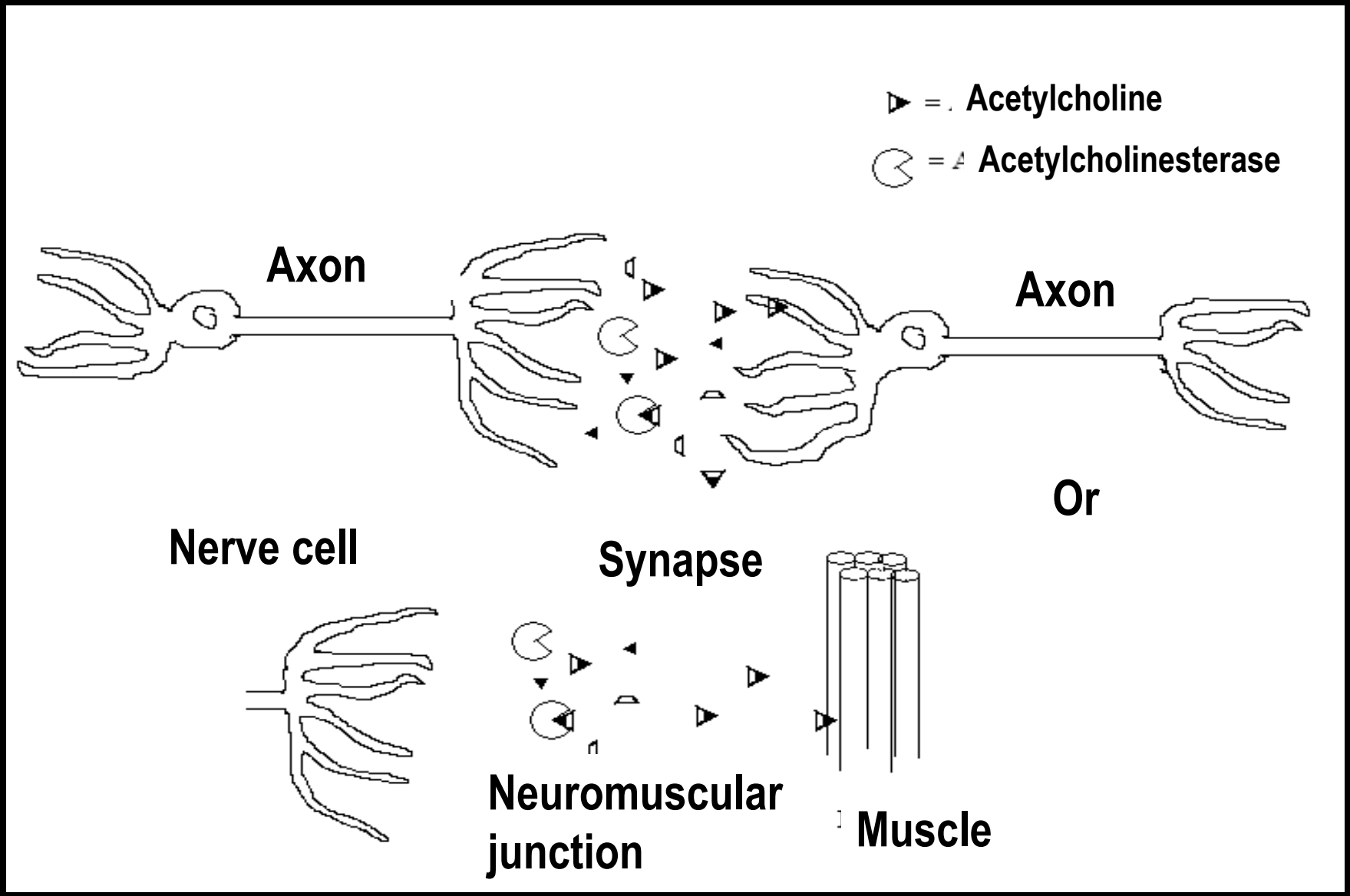
Insecticides

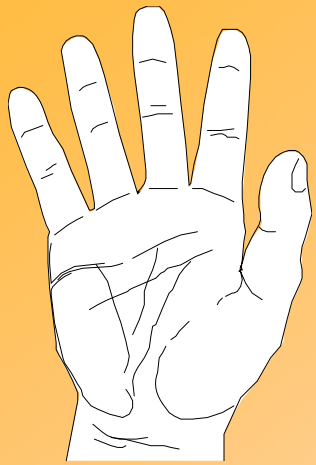
Modes of Action

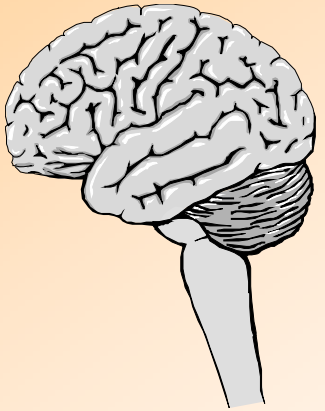
Affects the Nervous System

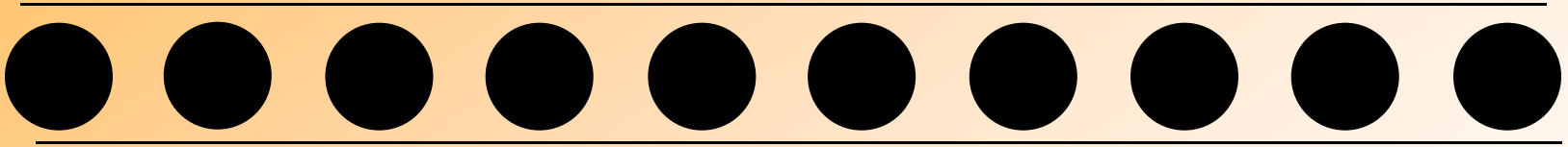
Affects Energy Production

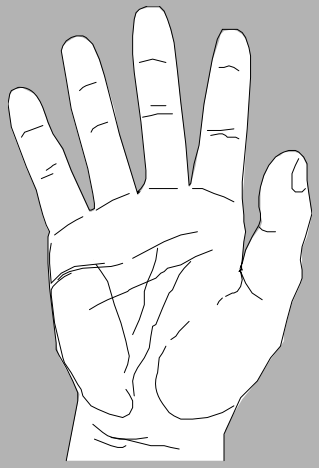
Overview of the nervous system

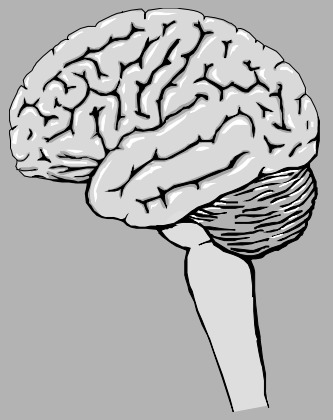


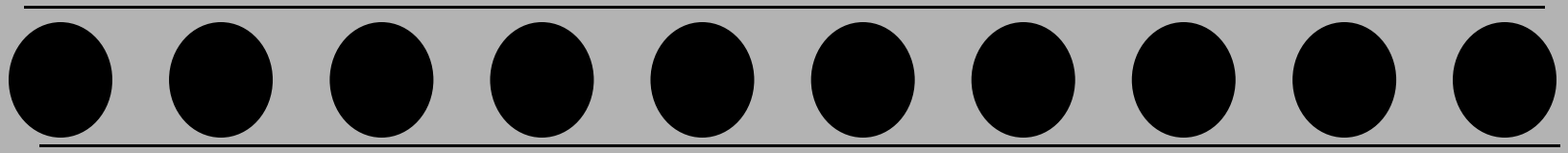


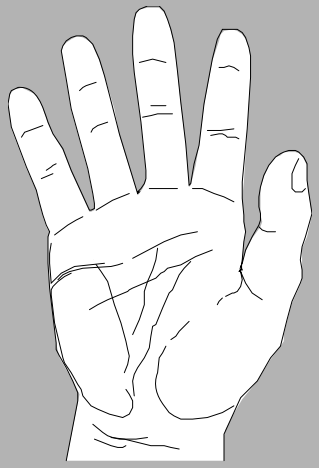


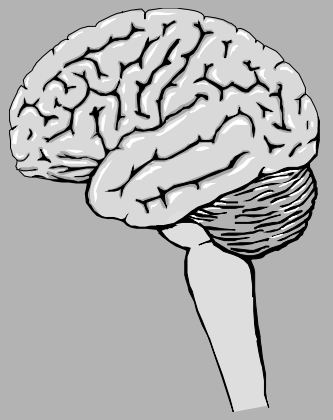


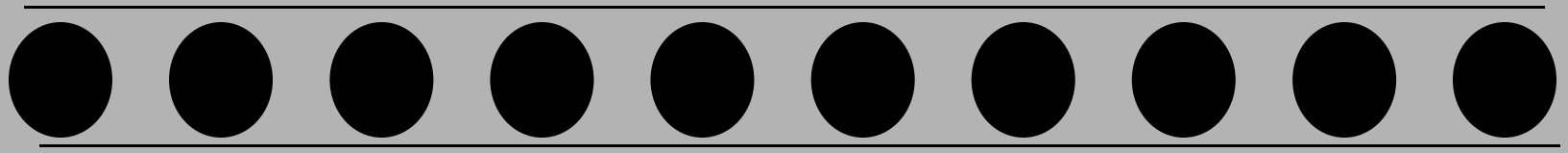


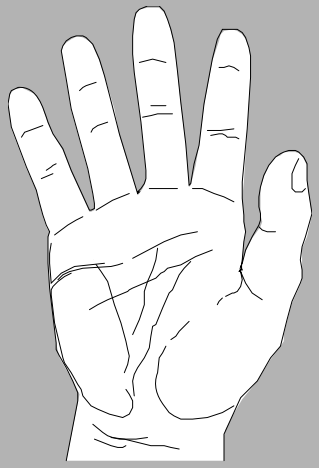


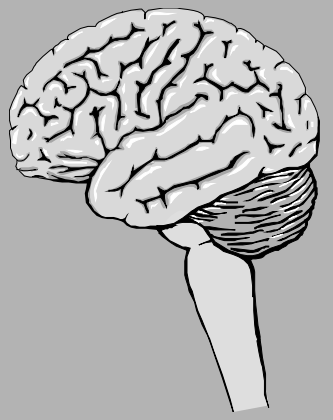


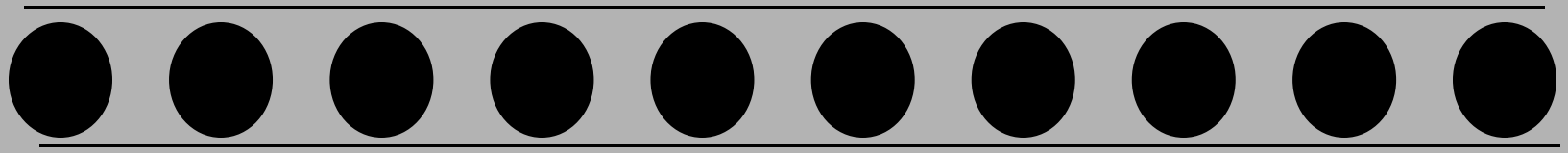


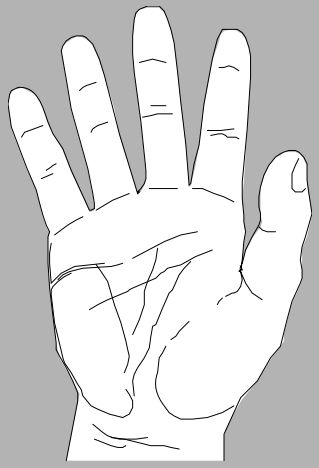


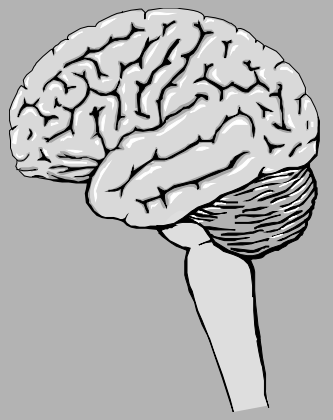


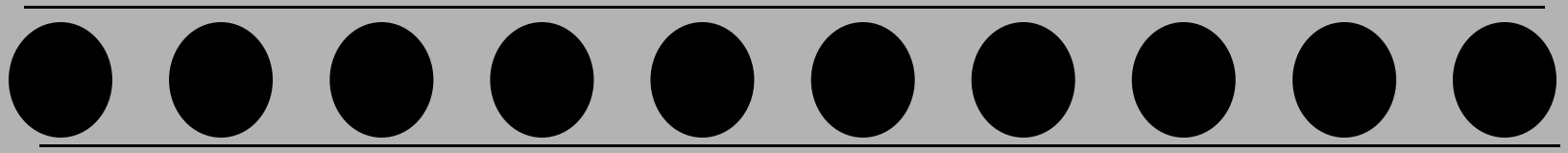


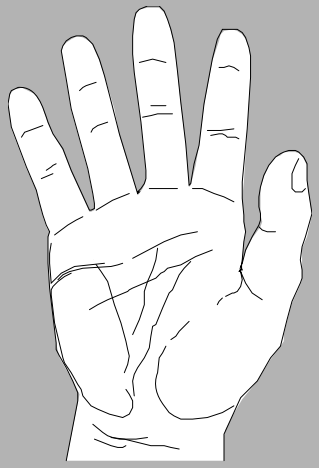


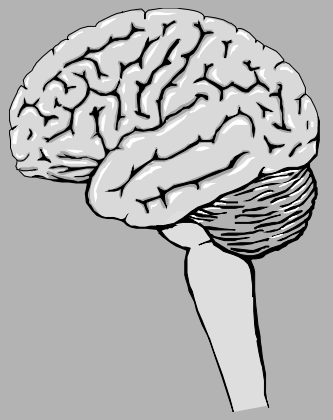


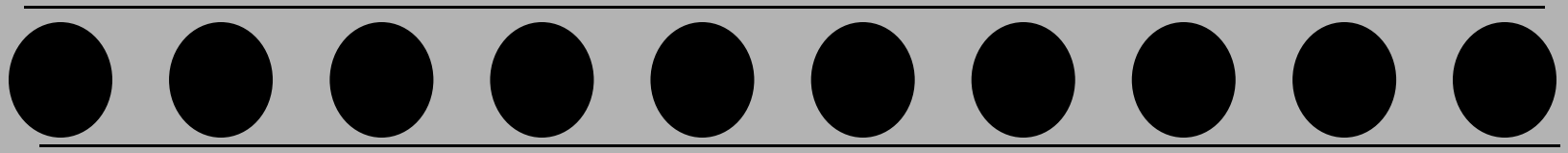


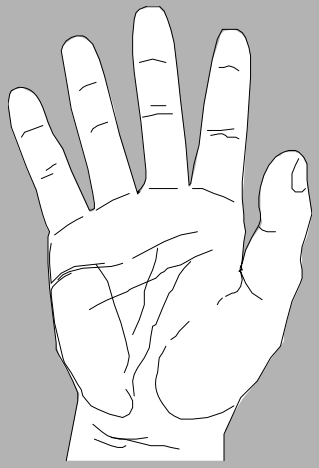


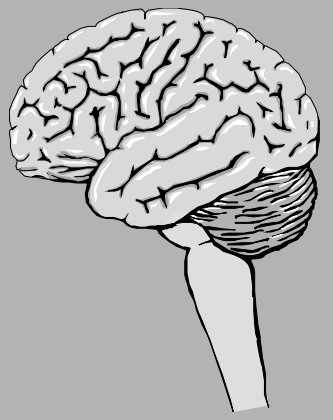


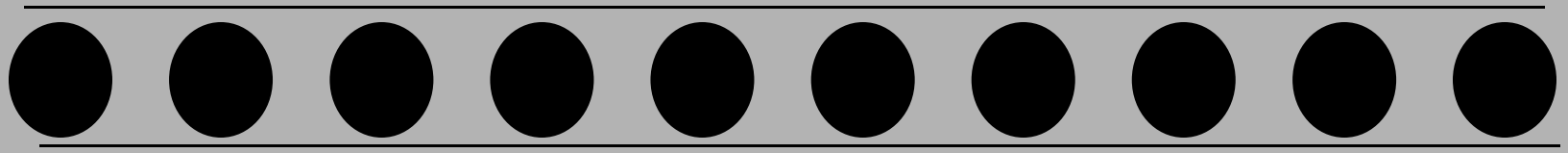


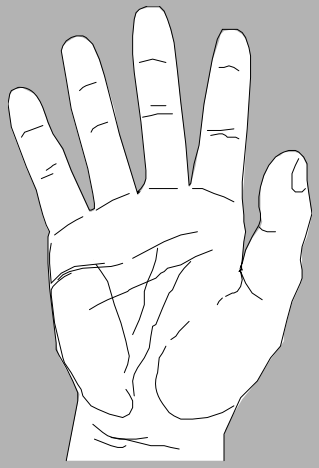


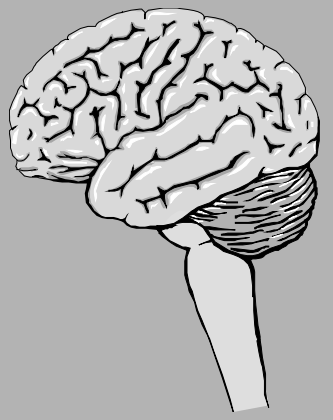


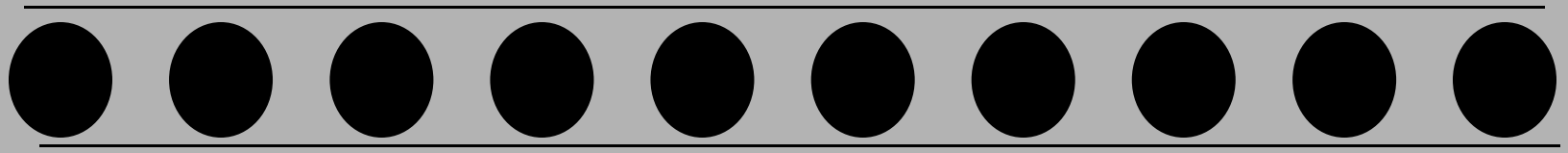


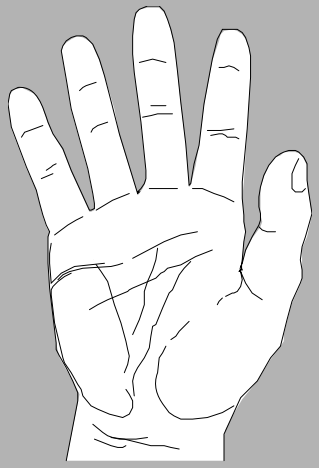


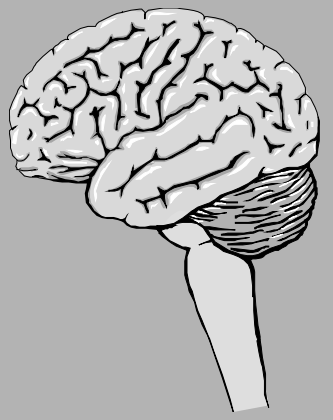


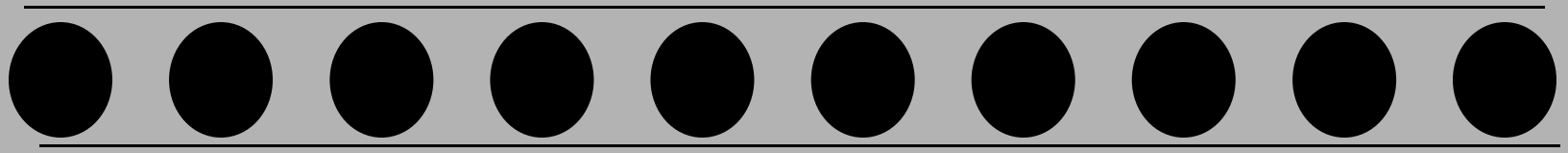


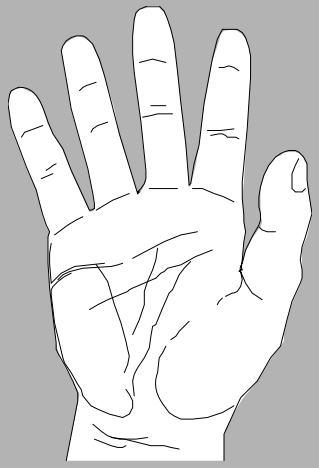


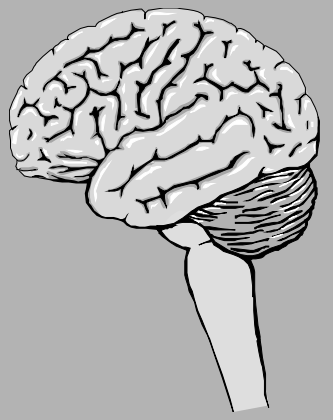


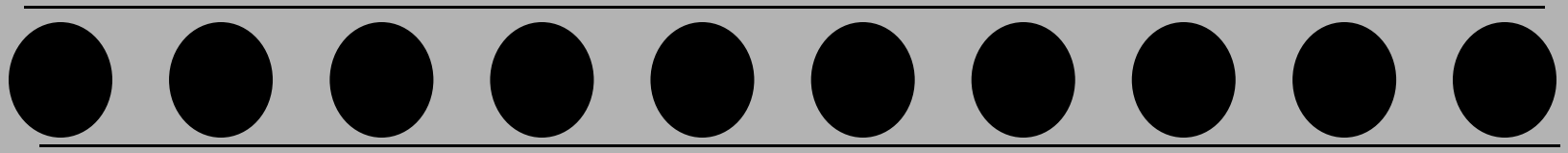


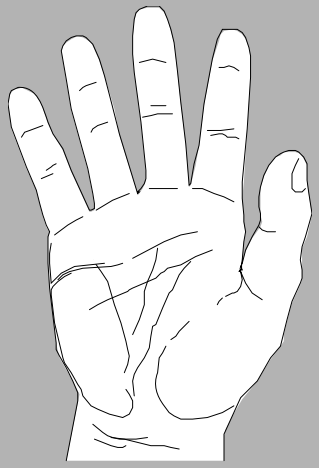


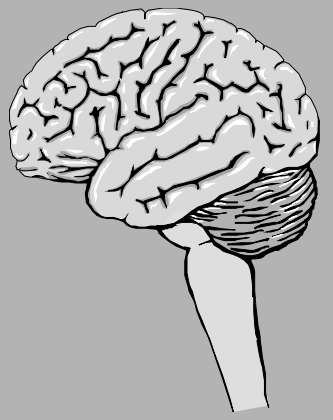


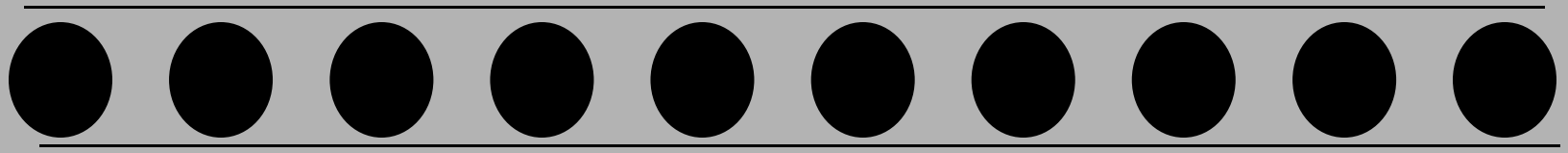


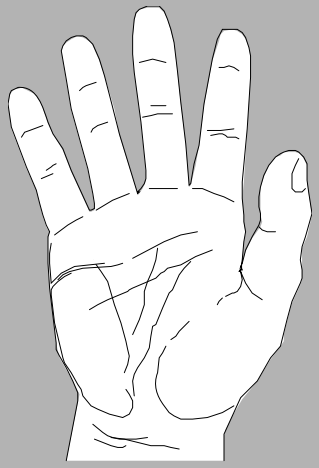


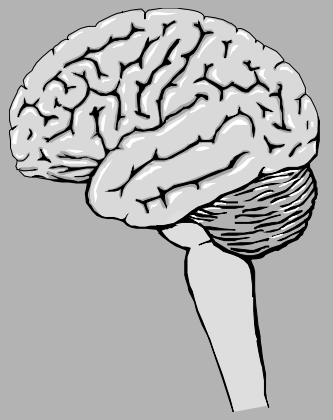


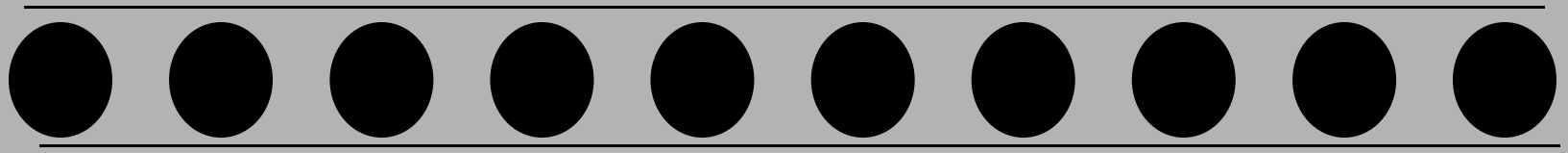


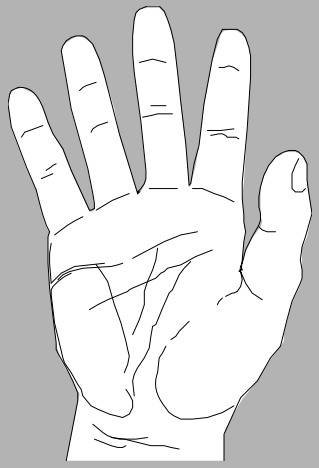


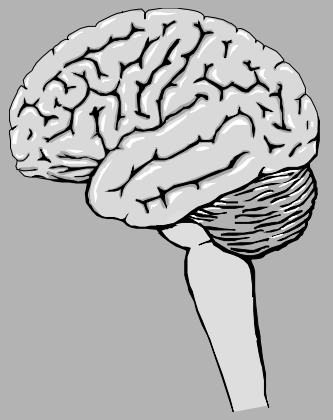


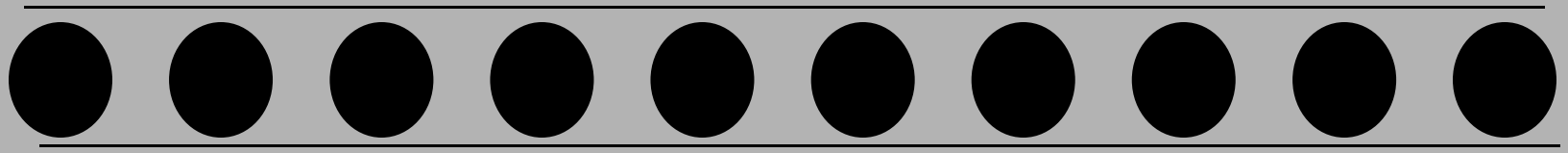


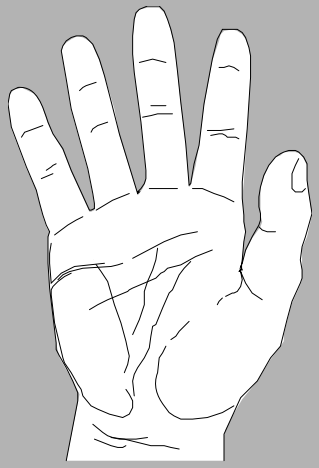


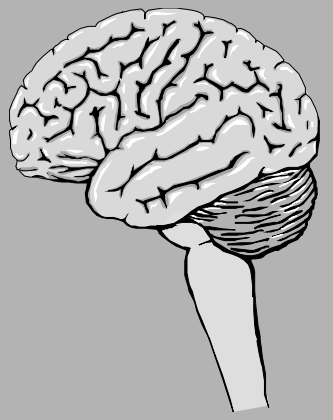


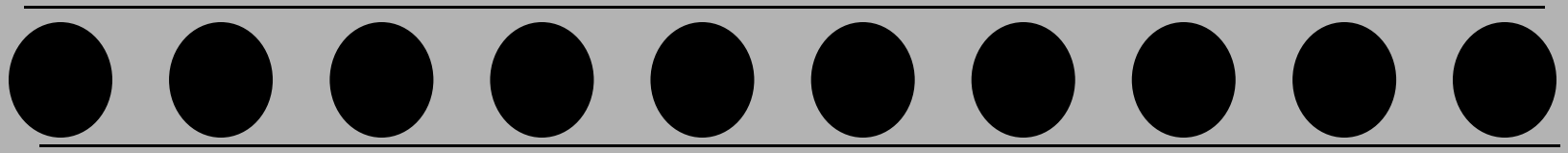


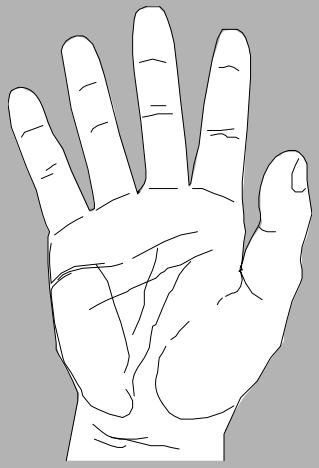


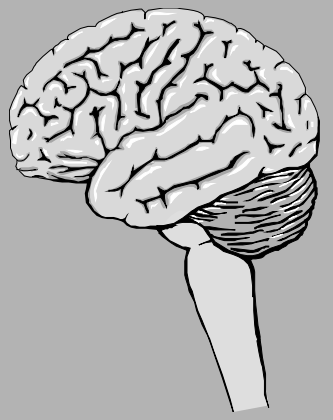


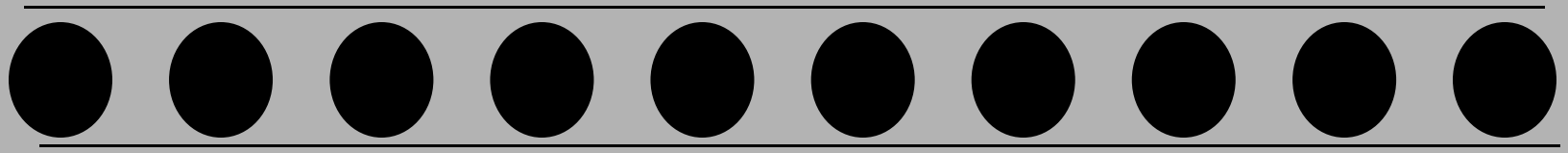


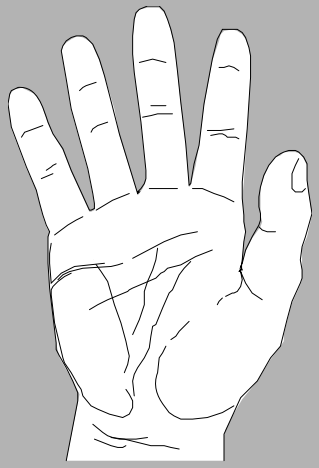


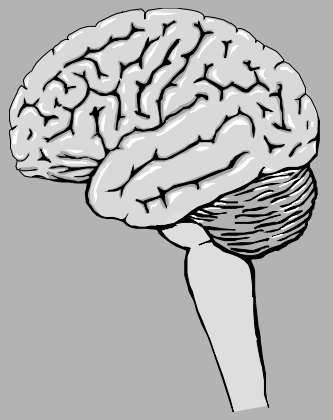


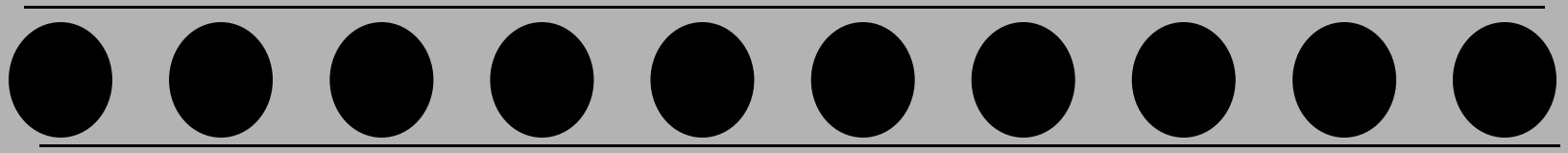


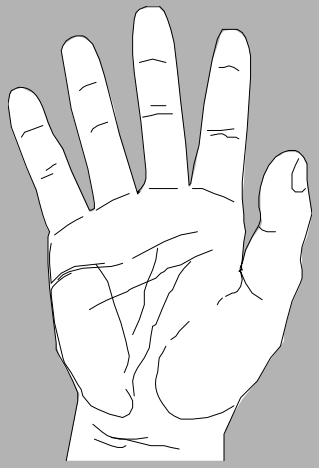


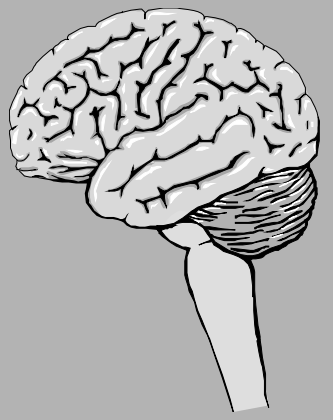


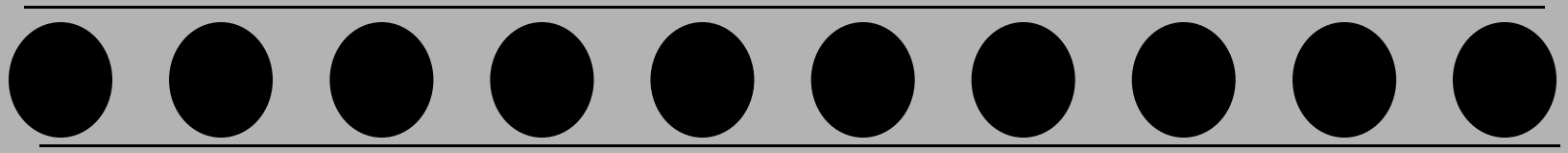


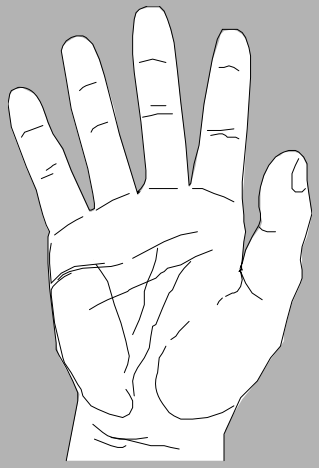


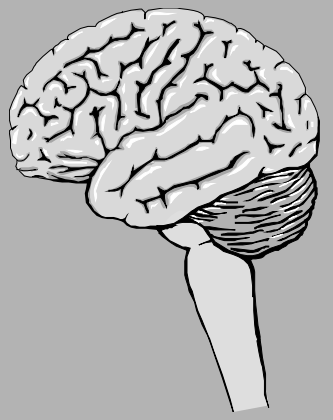


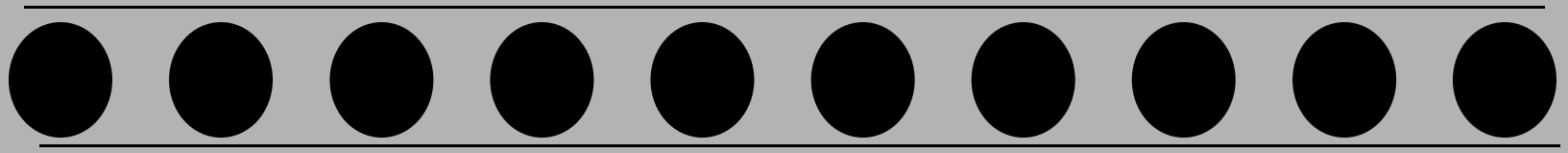


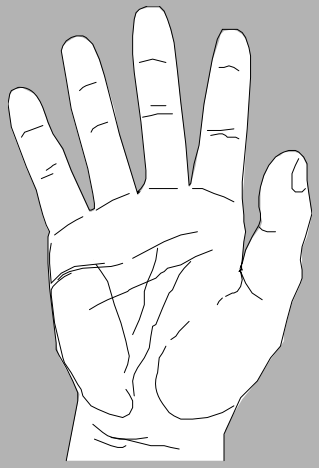


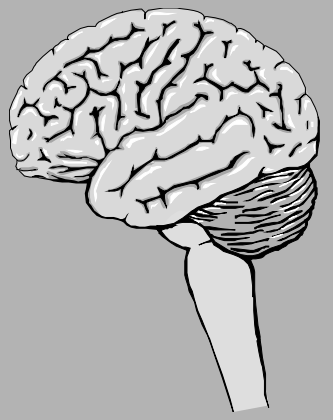


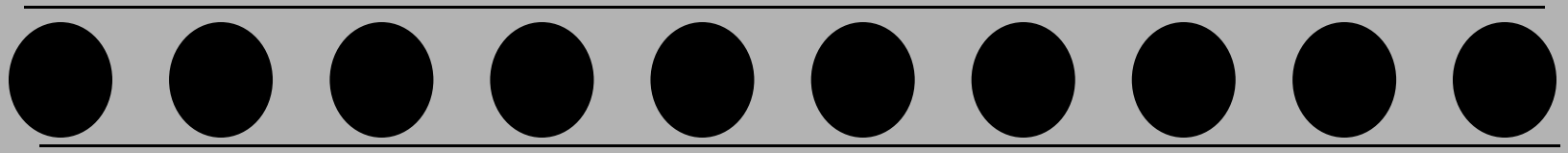


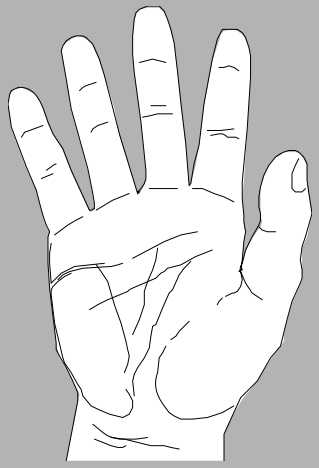


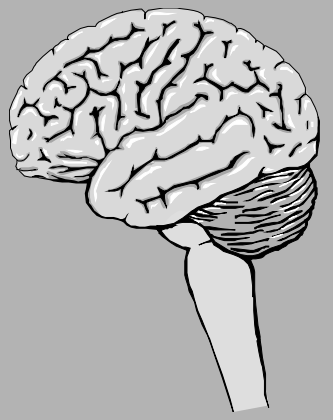


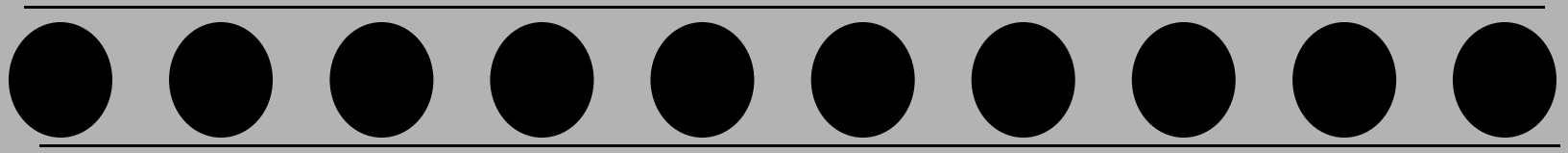


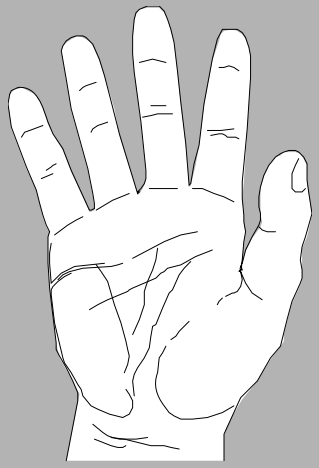


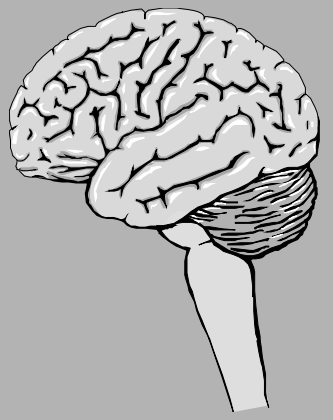


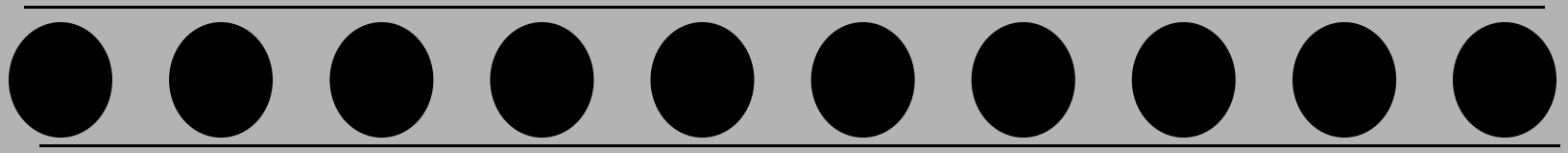


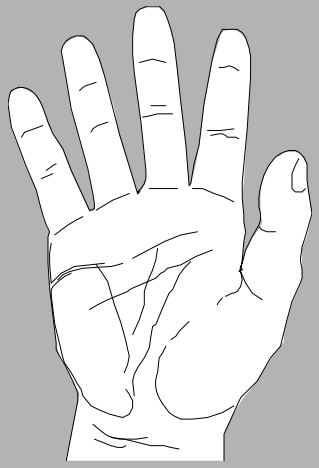


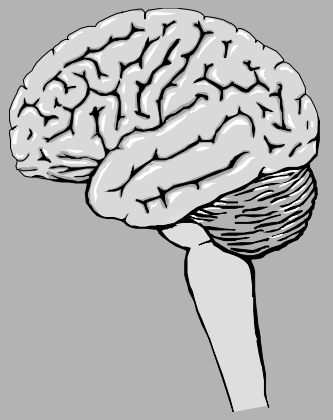


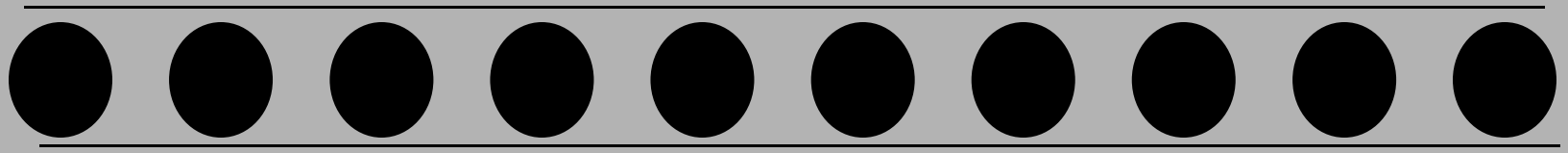


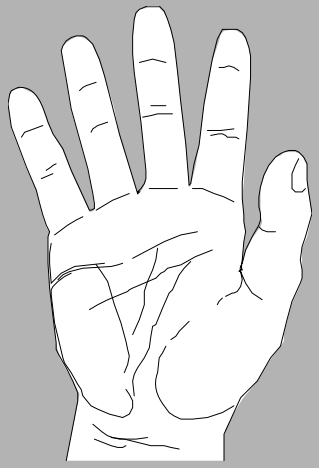


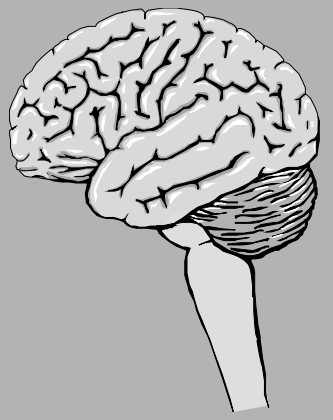


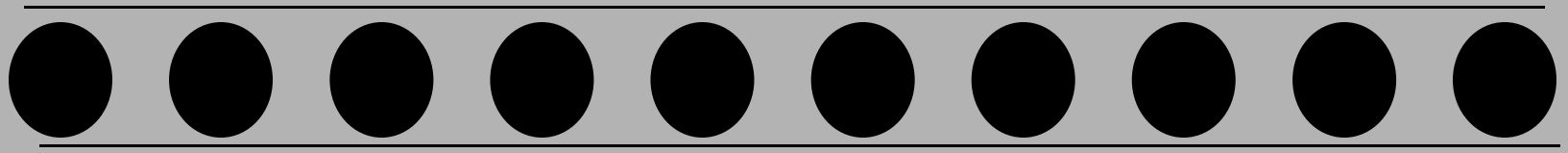


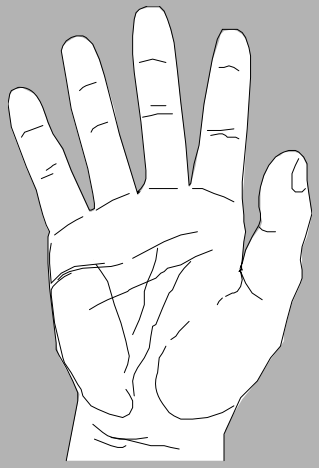


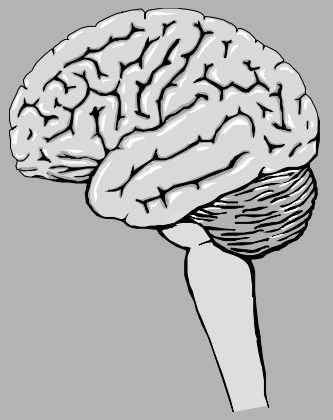


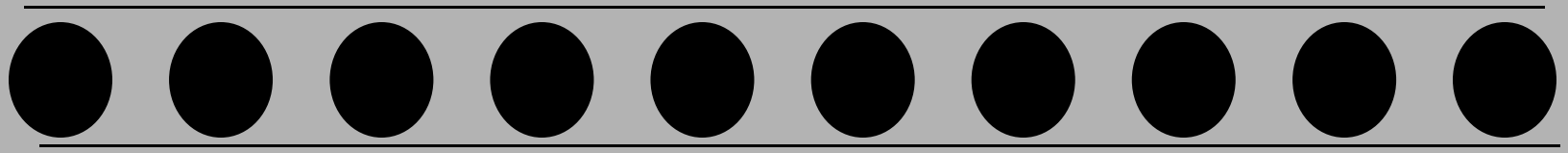


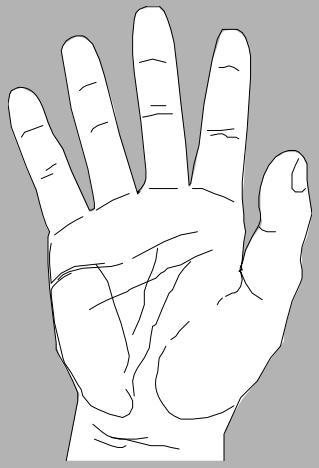


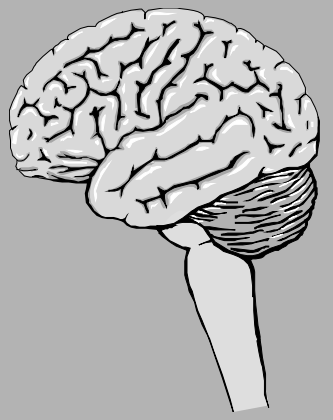


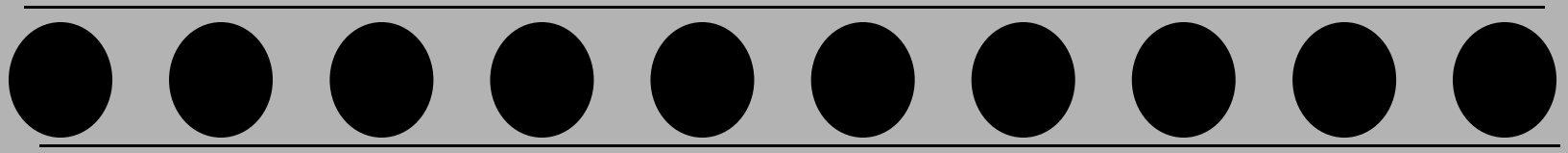


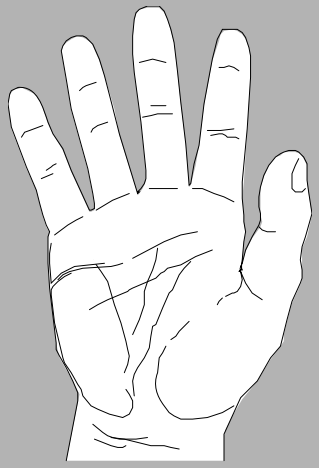


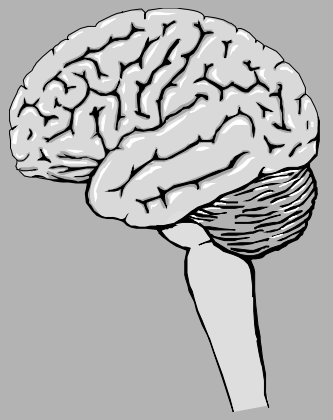


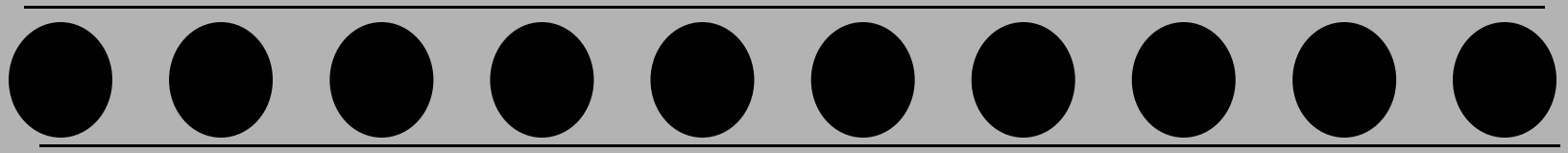


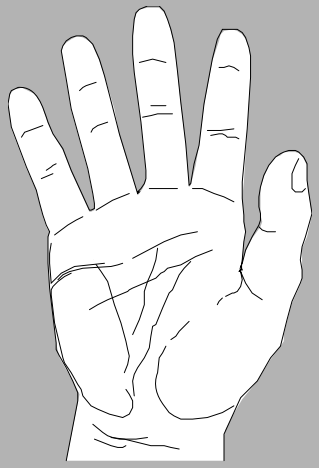


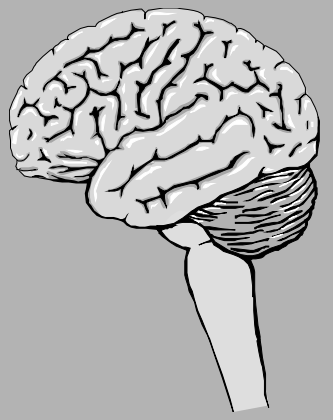


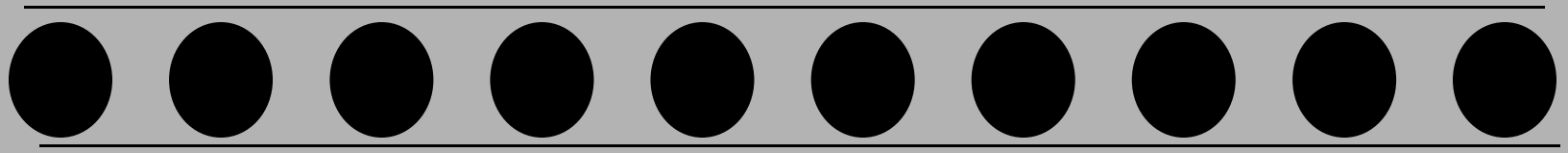


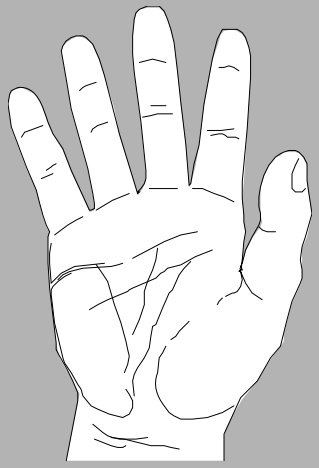


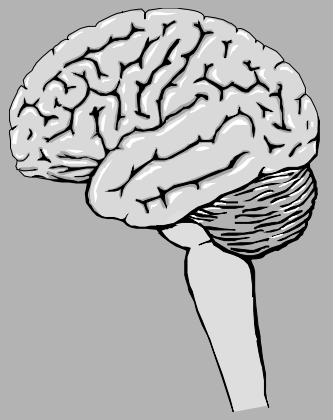


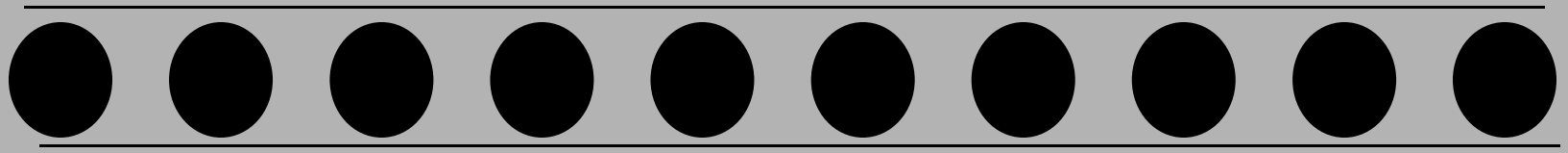


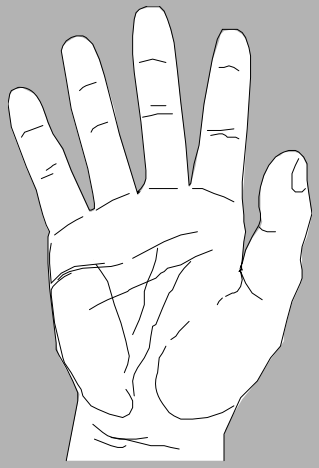


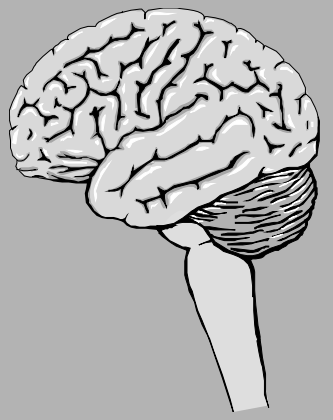


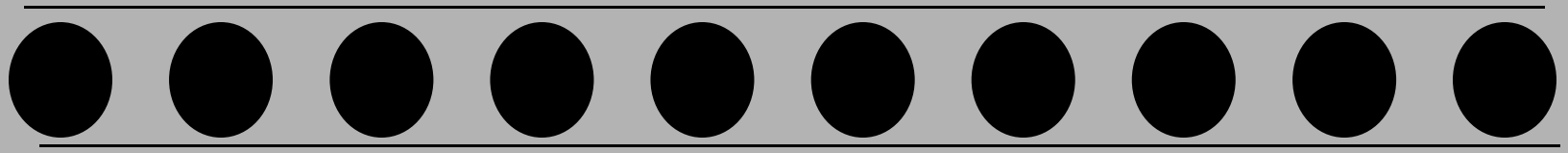


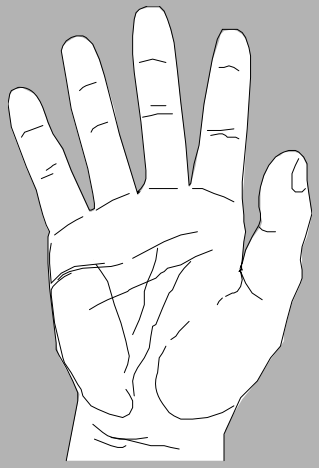


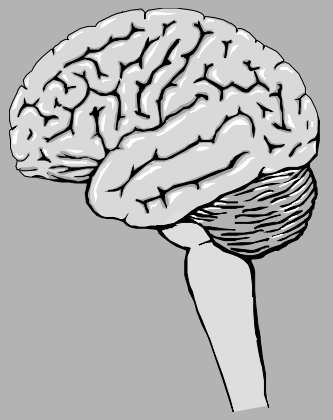


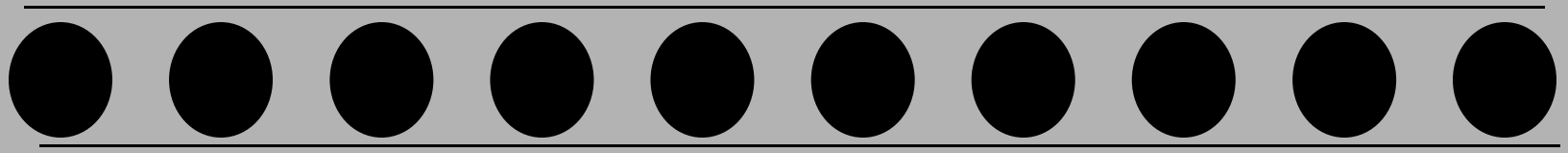


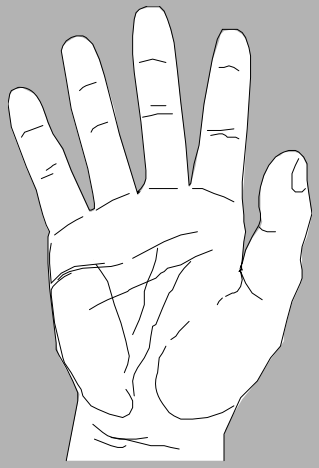


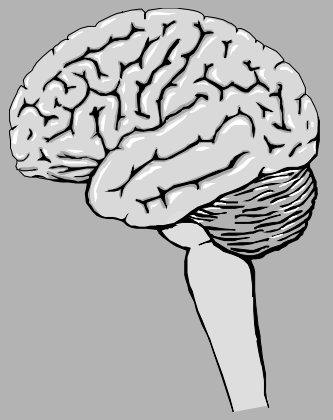


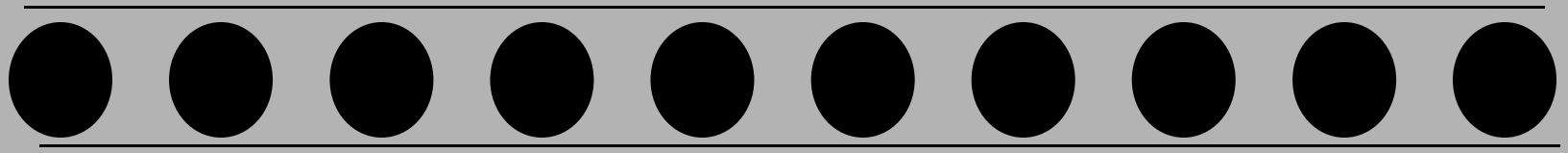


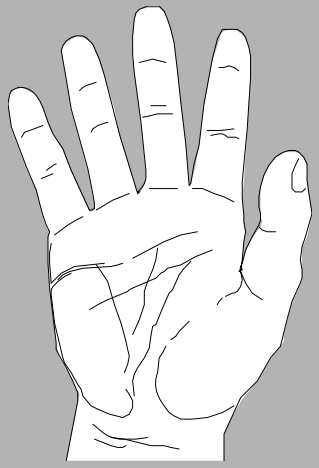


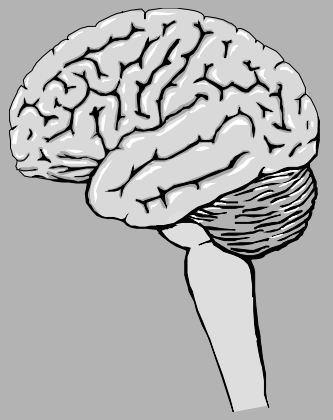


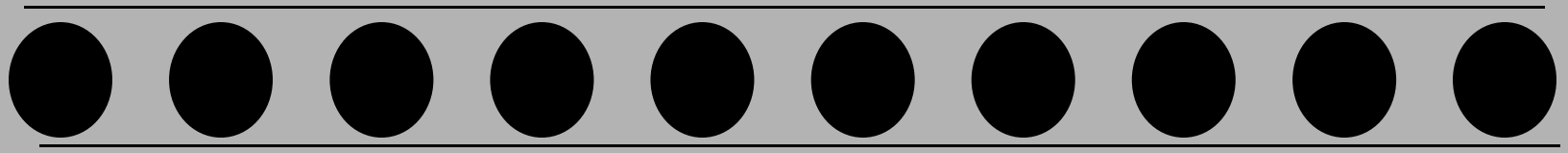


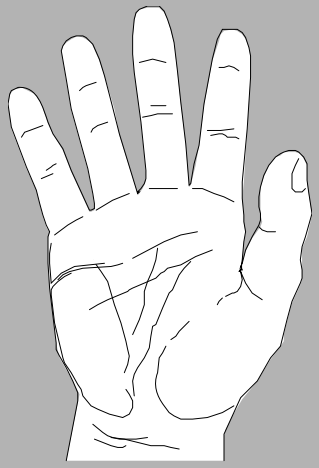


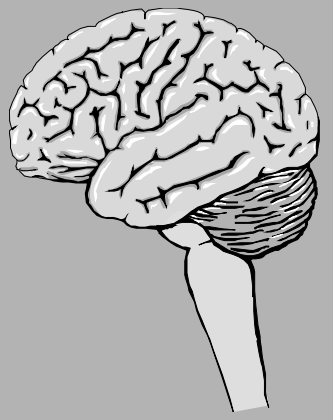


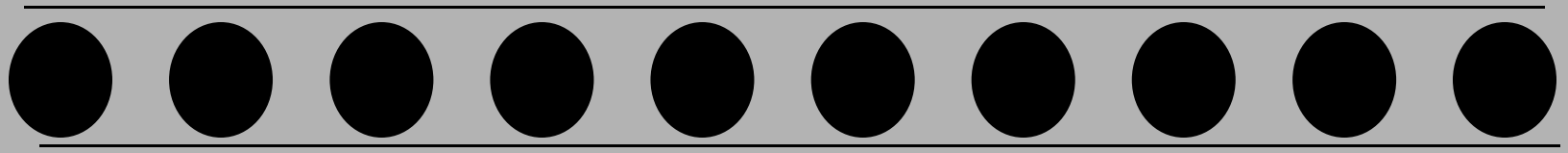




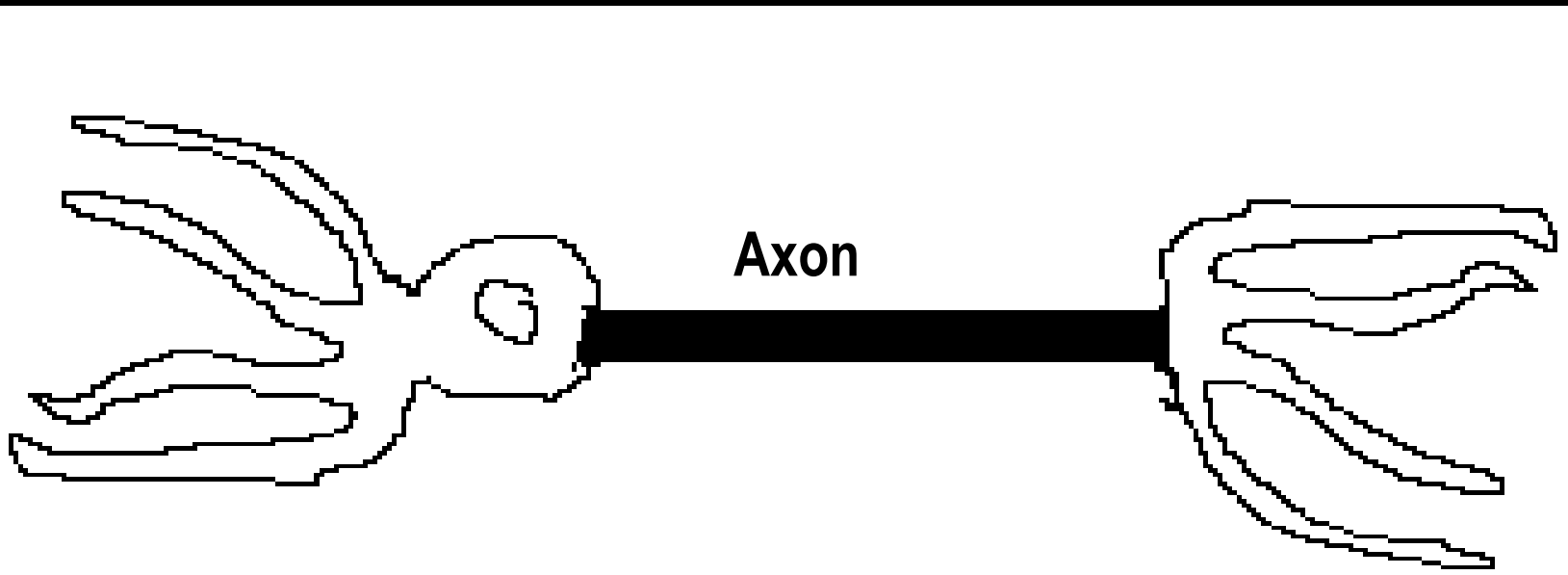






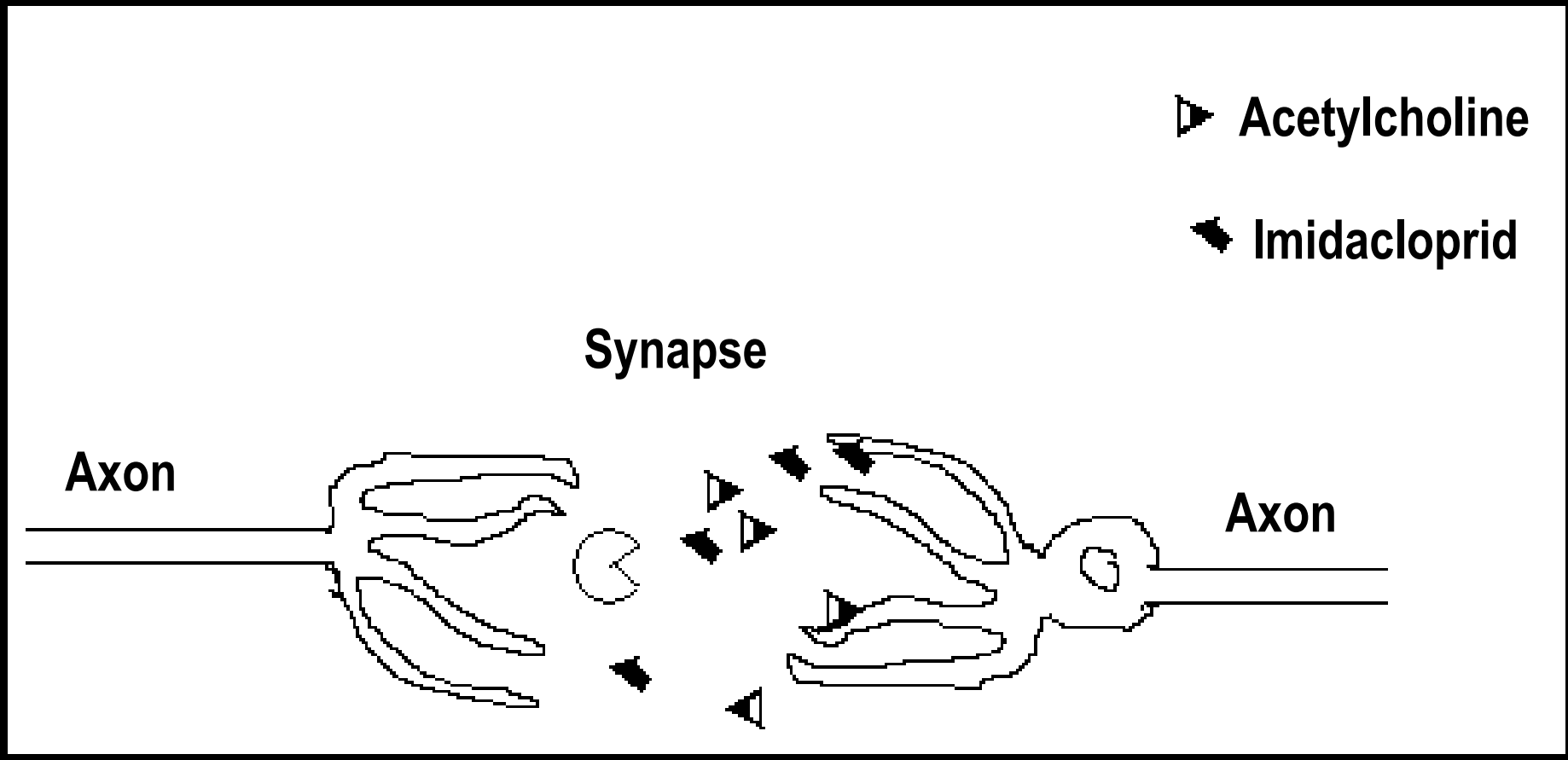


Organochlorine Insecticides

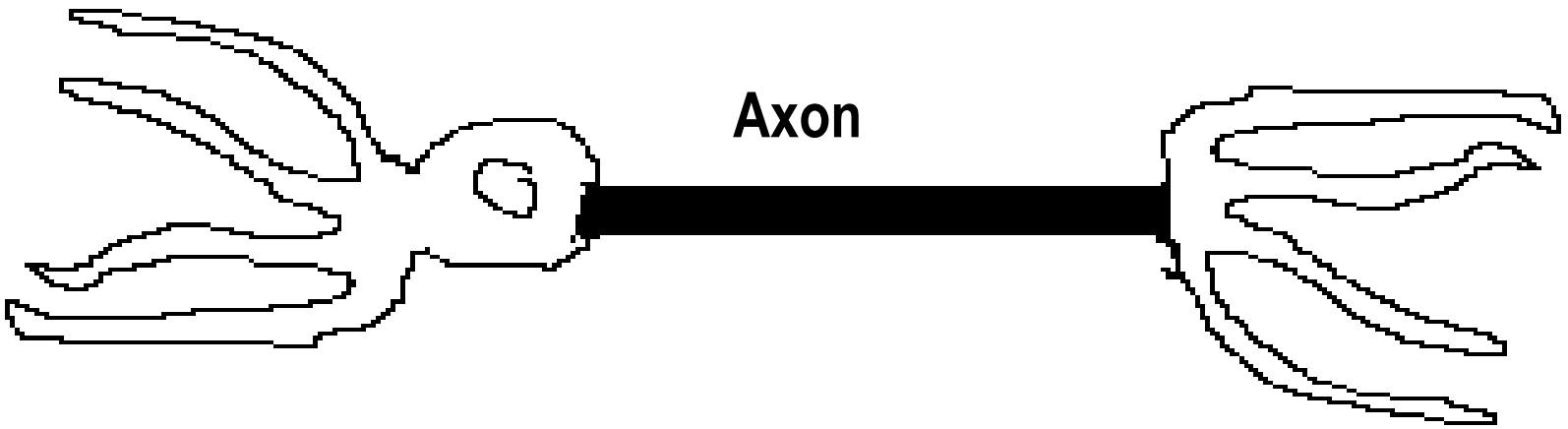


Nerve cell

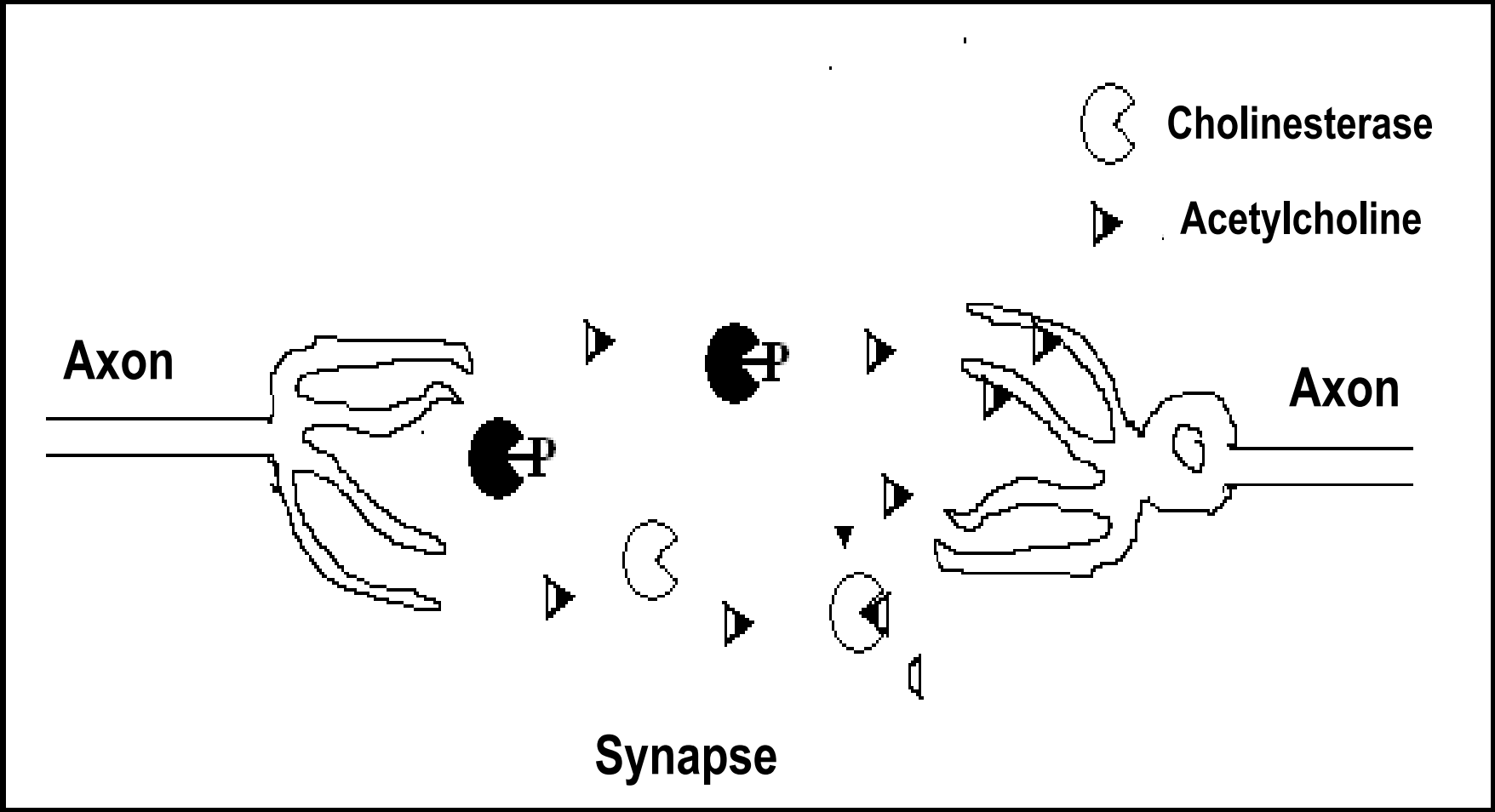
Nicotinoid Insecticides



Phenylpyrazole Insecticides



Organophosphorus and Carbamate Insecticides (Anticholinesterases)

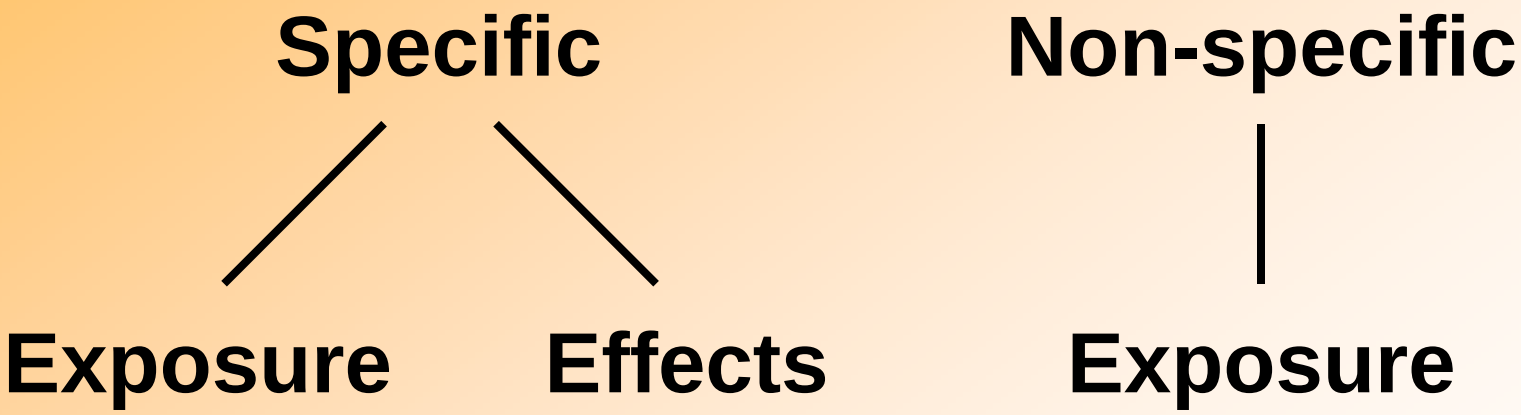


Direct vs. Latent Anticholinesterases

Direct inhibitors - some OPs and all CBs
Latent inhibitors - some Ops

Delayed Neurotoxicity

Biomarkers



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Species

Species	Mean Brain ChE Activity
American black duck	8
Least sandpiper	22
Common grackle	20
Barn swallow	12
Mourning dove	16

Units: µmoles of acetylthiocholine iodide hydrolyzed per min per g of tissue, wet weight at 25^0C.

Life History

Species	Young	Age (days)	ChE activity as % of adult
Mallard	Precocial	1	80
Starling	Altricial	4	25

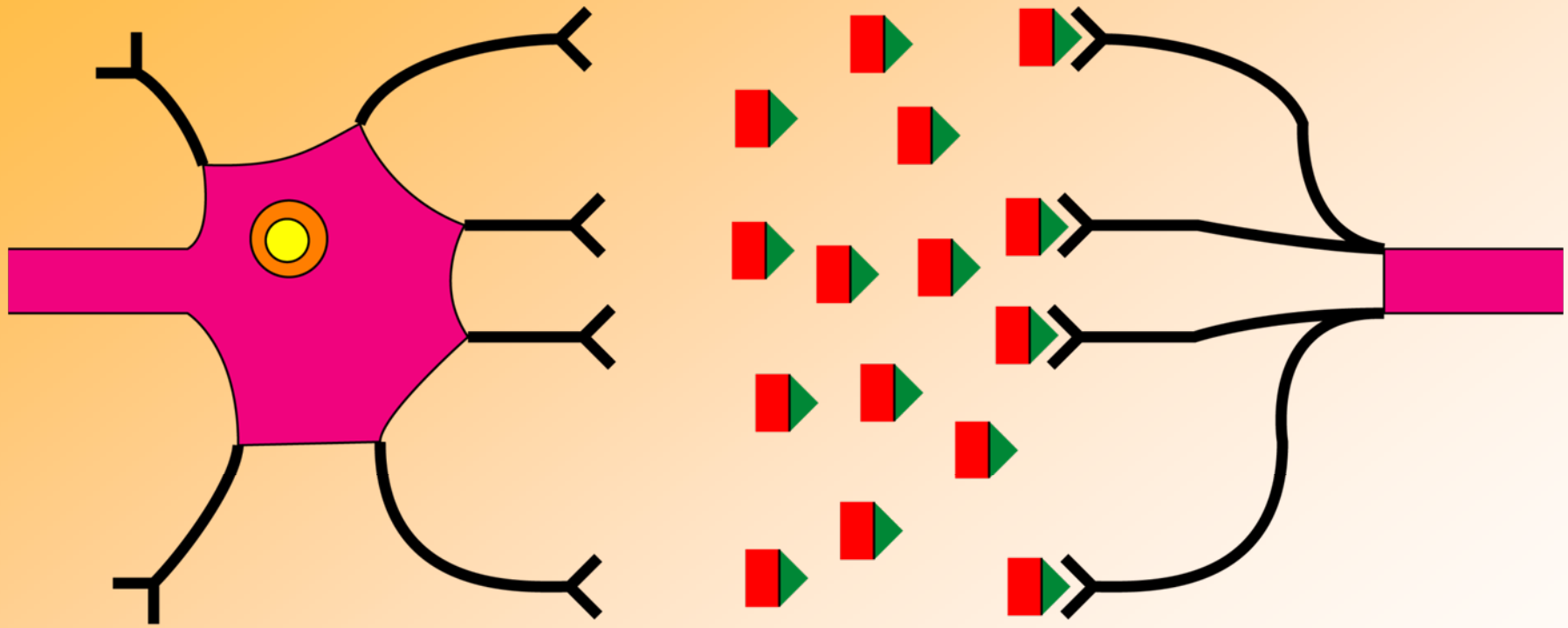
Units: µmoles of acetylthiocholine iodide hydrolyzed per min per g of tissue, wet weight at 25^0C.

Brain Regions

Brain Regions (white-throated sparrow)	Mean ChE activity
Basal ganglia	25
Hippocampus	15
Hypothalamus	14

Units: µmoles of acetylthiocholine iodide hydrolyzed per min per g of tissue, wet weight at 25^0C.

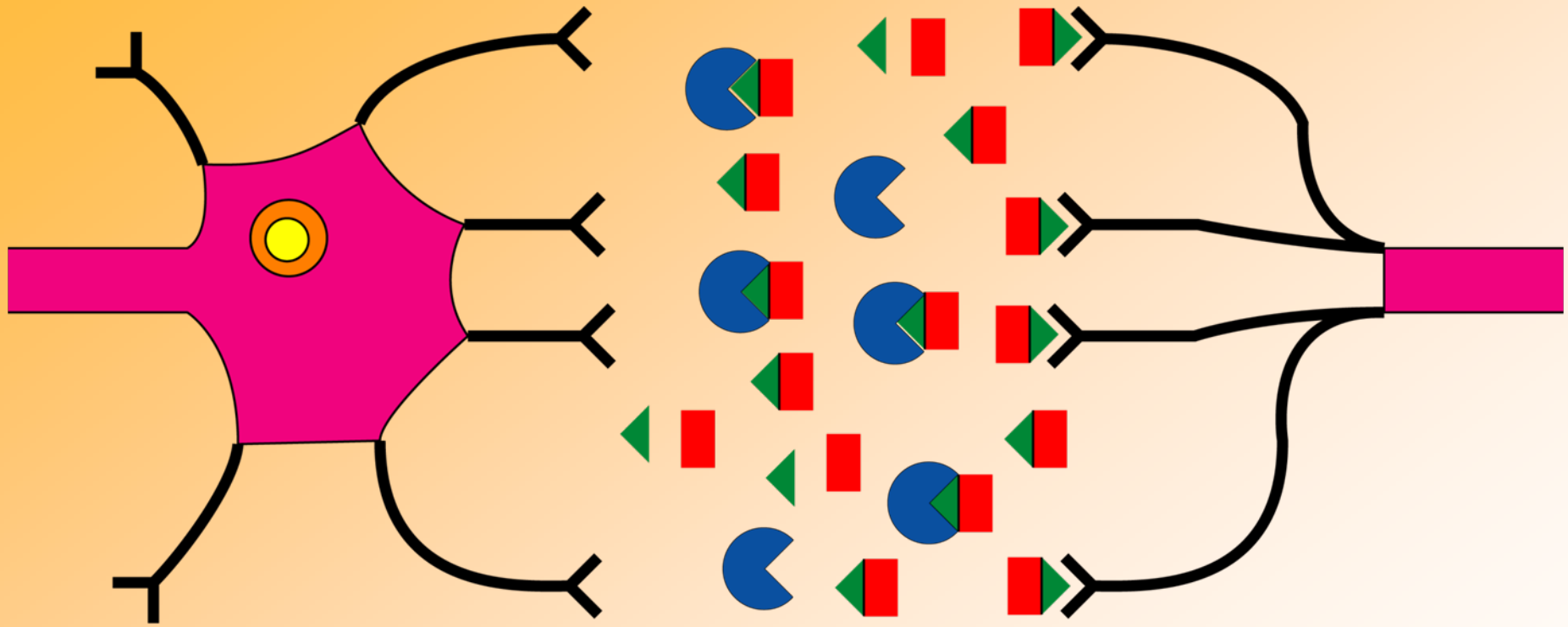
Normal neurotransmitter Activity



Neuron Synapse Neuron

= acetylcholine

Normal ChE Activity

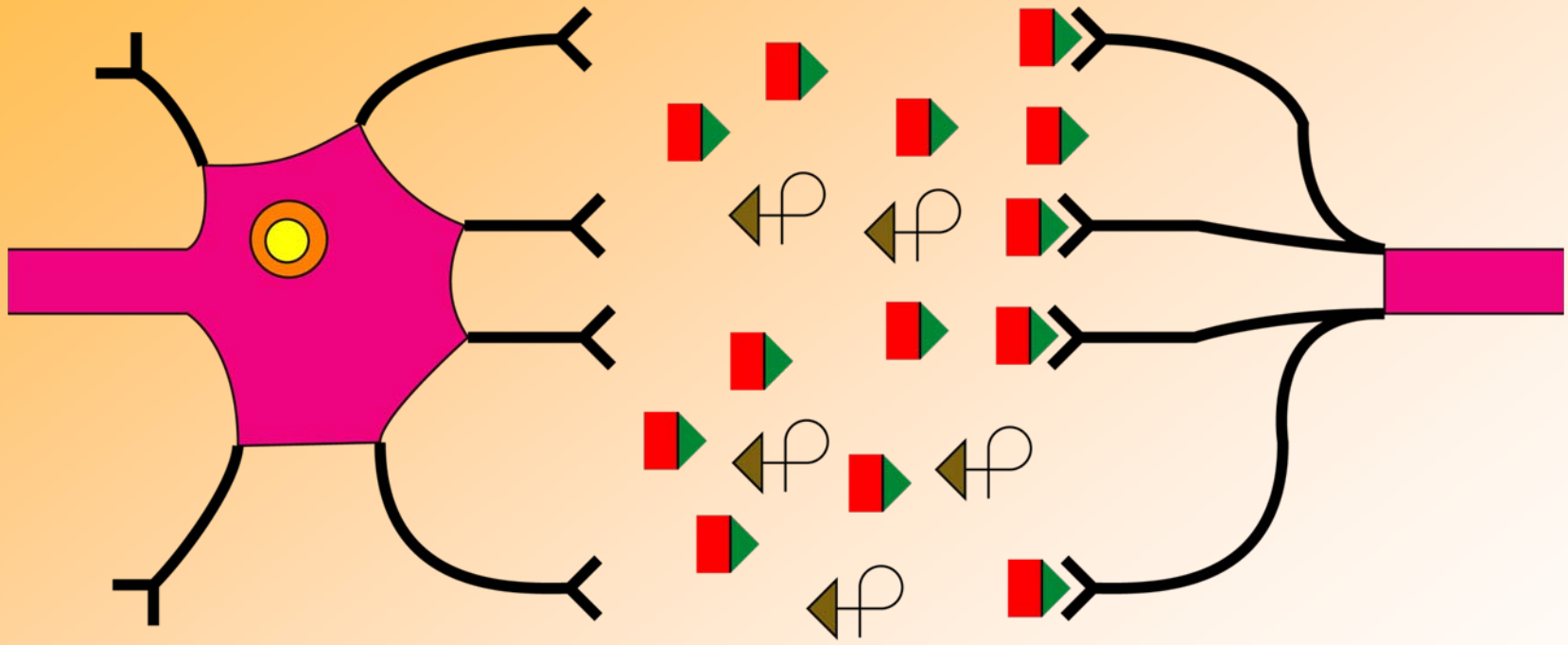


Neuron Synapse Neuron

= acetylcholinesterase

& = acetyl & choline

Pesticide Exposure

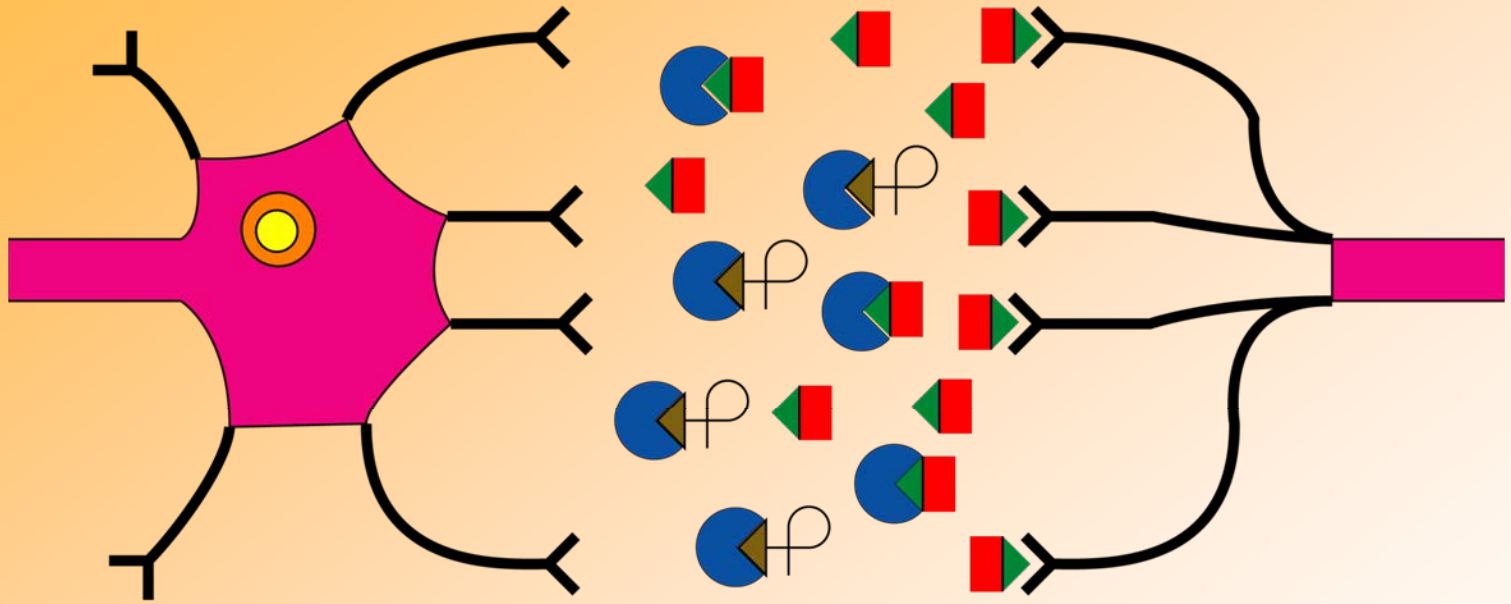


Neuron Synapse Neuron

= acetylcholine

= pesticide

ChE Inhibition



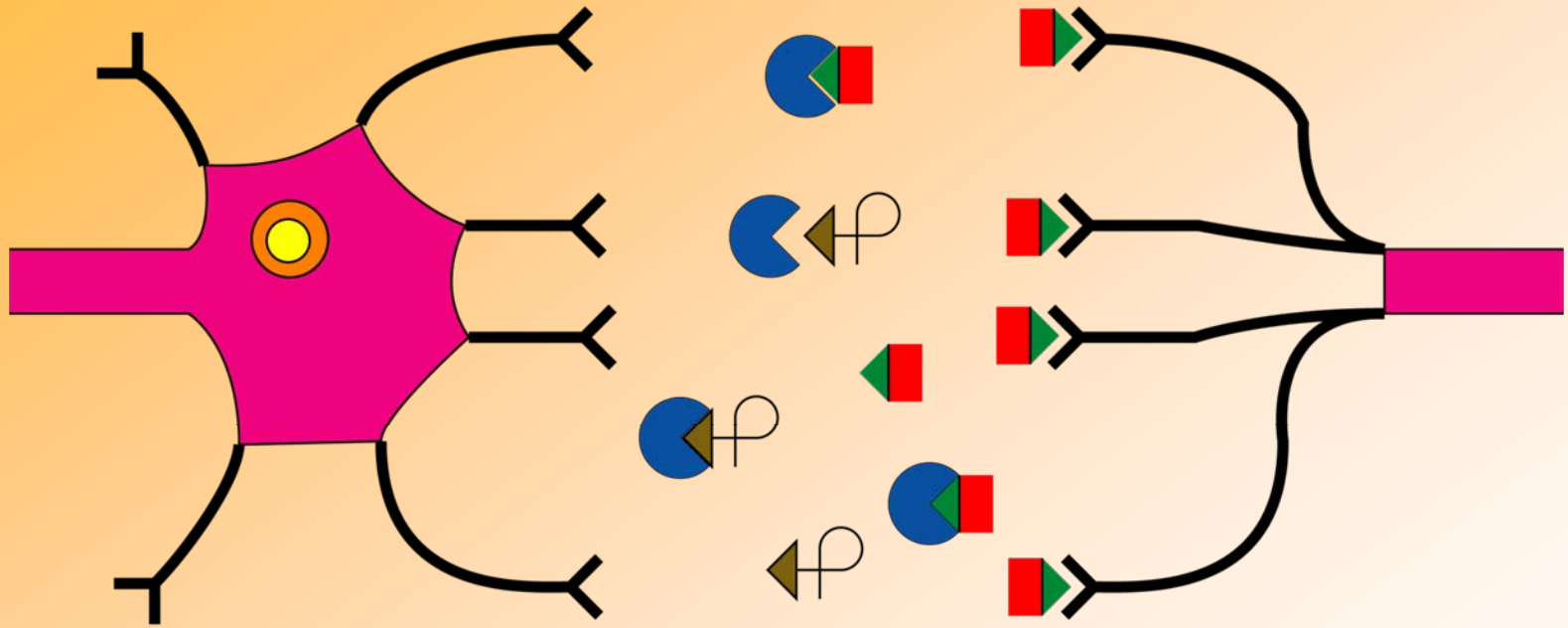
Neuron Synapse Neuron

= acetylcholinesterase

= acetylcholine

= pesticide

Recovery: Spontaneous Reactivation with CBs



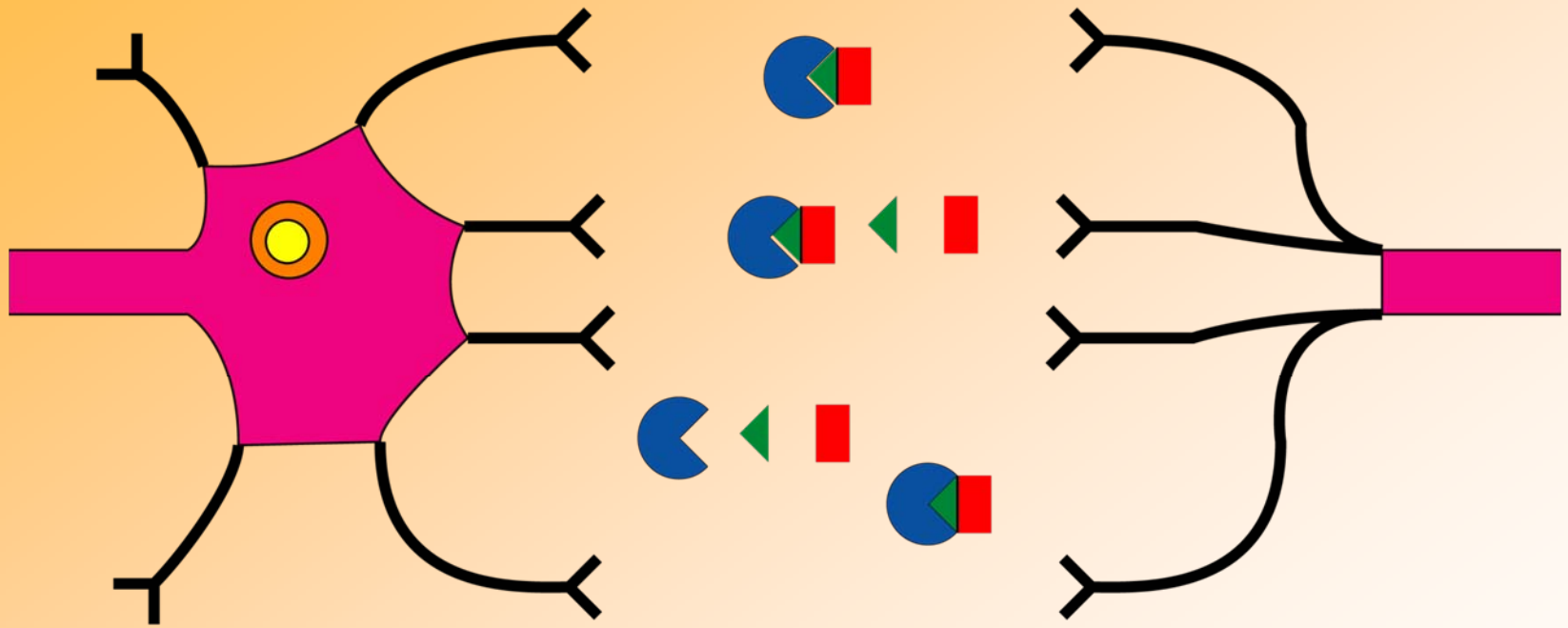
Neuron Synapse Neuron

= acetylcholinesterase

= acetylcholine

= pesticide

Recovery: New ChE synthesis with OPs



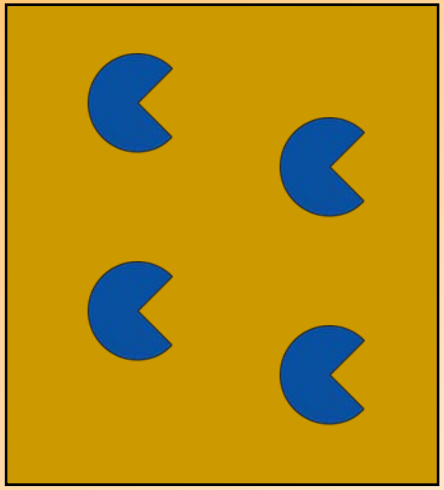
Neuron Synapse Neuron

= acetylcholinesterase

= acetylcholine

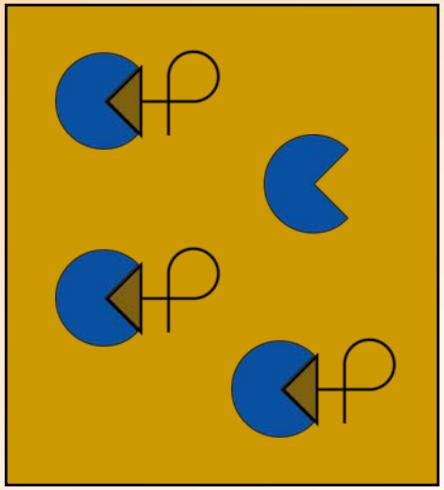
Cholinesterase Activity Measurement

Control



vs.

Exposed



25% activity of control

or

75% activity inhibited compared to control

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Traditional ChE Analysis

Advantage

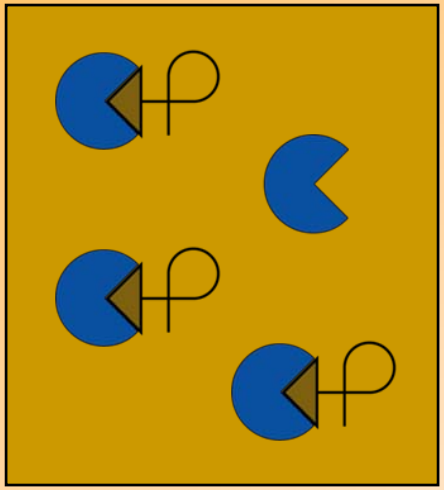
- Direct biomarker for OP and CB exposure and effects.

Limitations

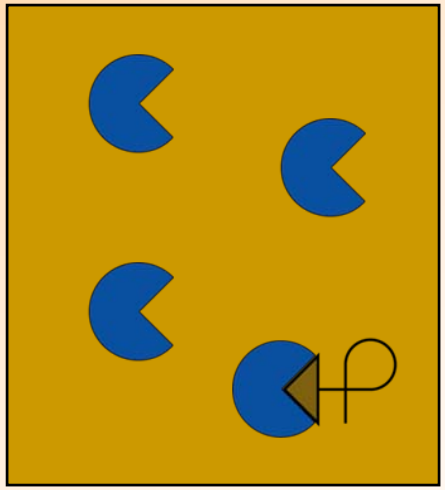
- Does not differentiate between OPs and CBs. Need residue analysis $$$$$.
- Only useful when appropriate control values are available.

Cholinesterase Reactivation

Exposed



Reactivated



66% reactivated

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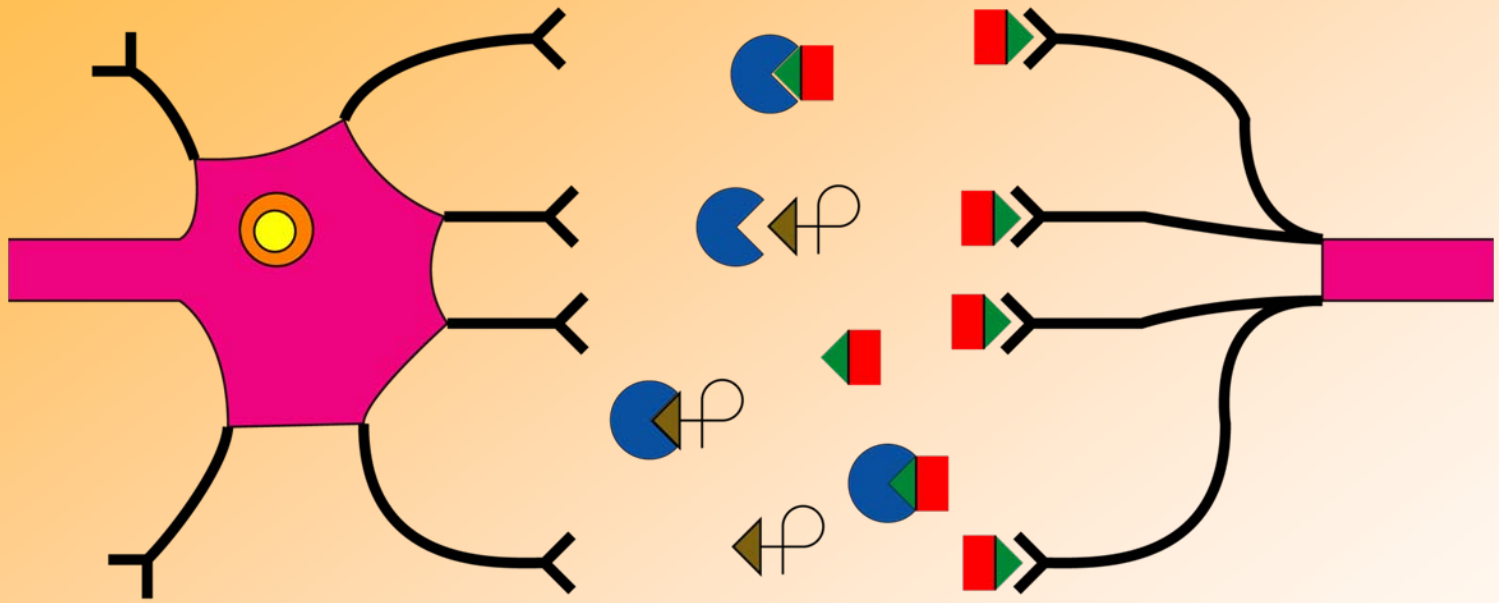
CB Reactivation

Incubate to get spontaneous reactivation



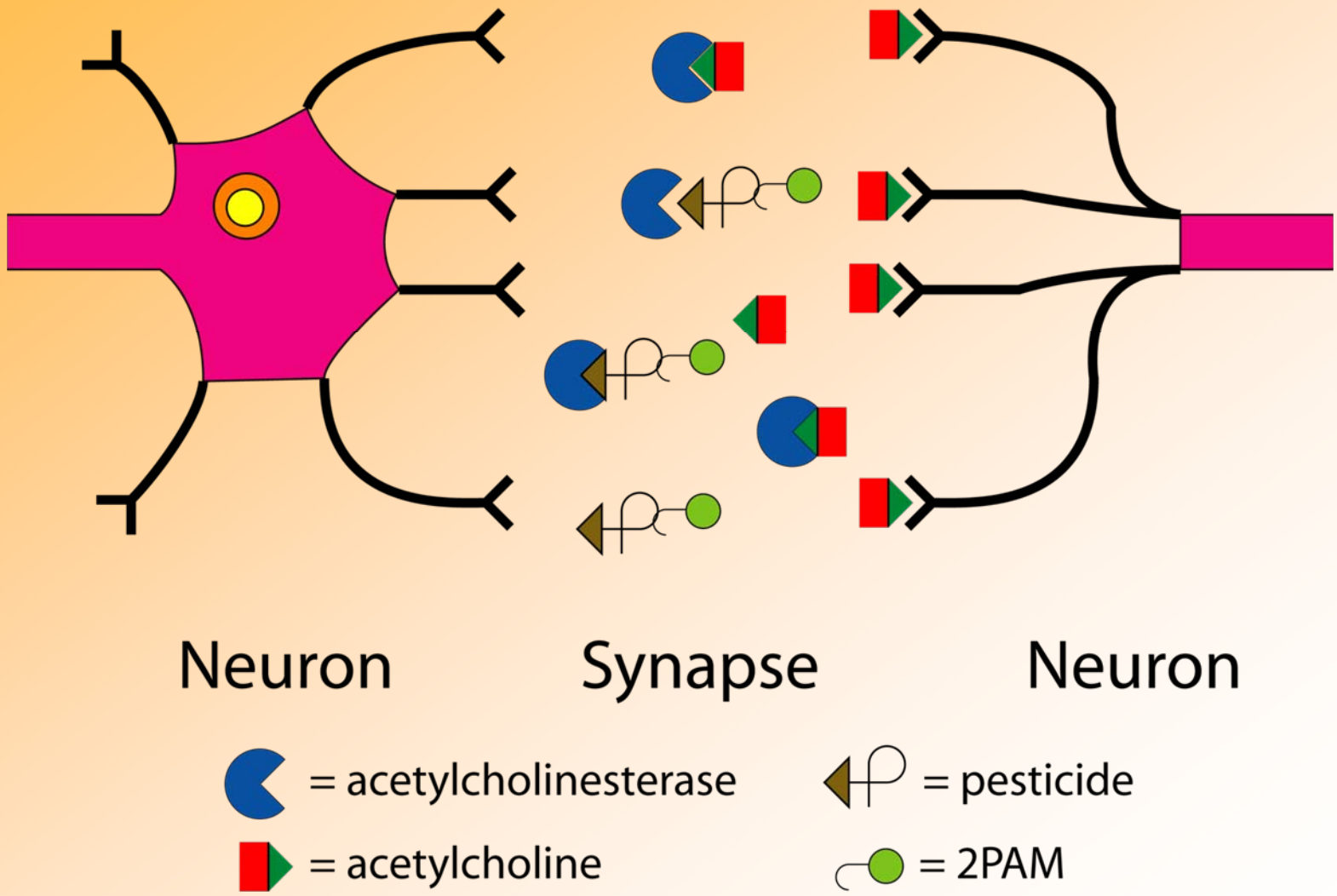
Neuron Synapse Neuron

= acetylcholinesterase

= acetylcholine

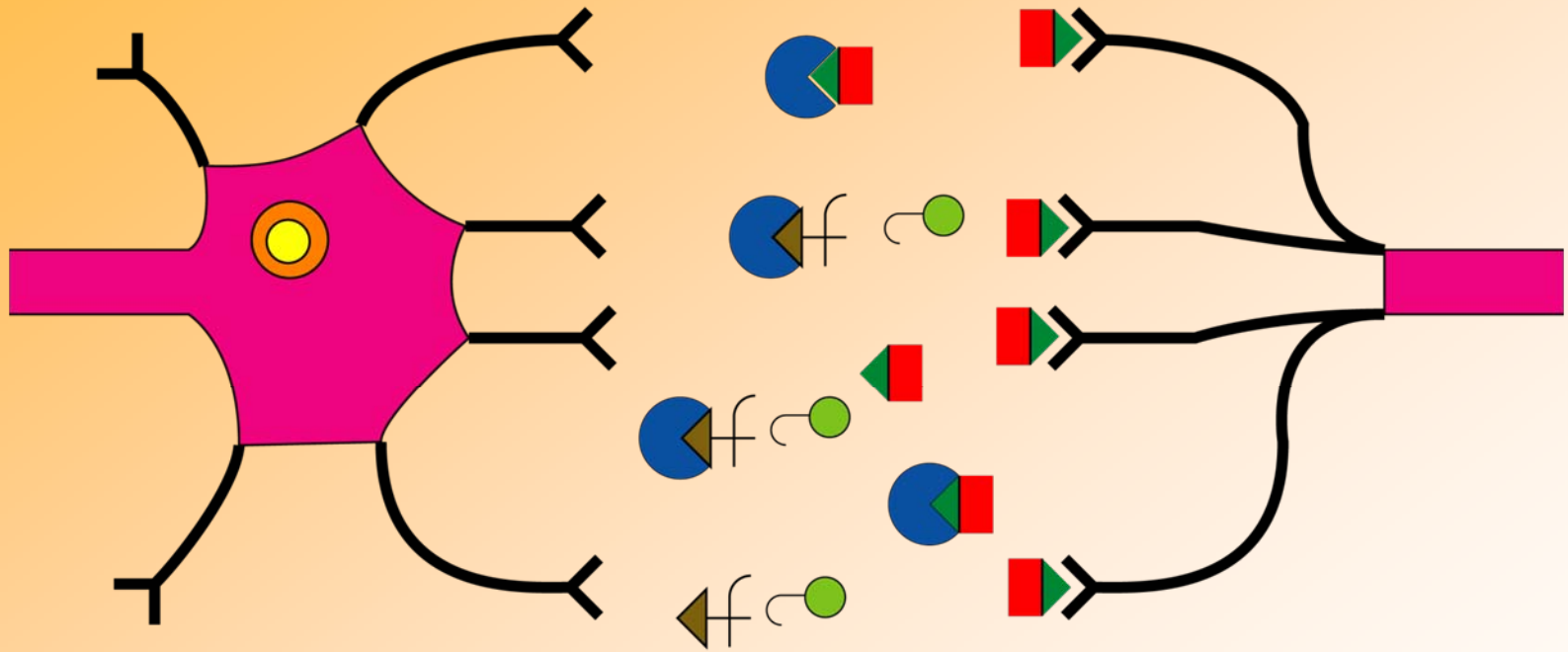
= pesticide

OP Reactivation: 2-PAM

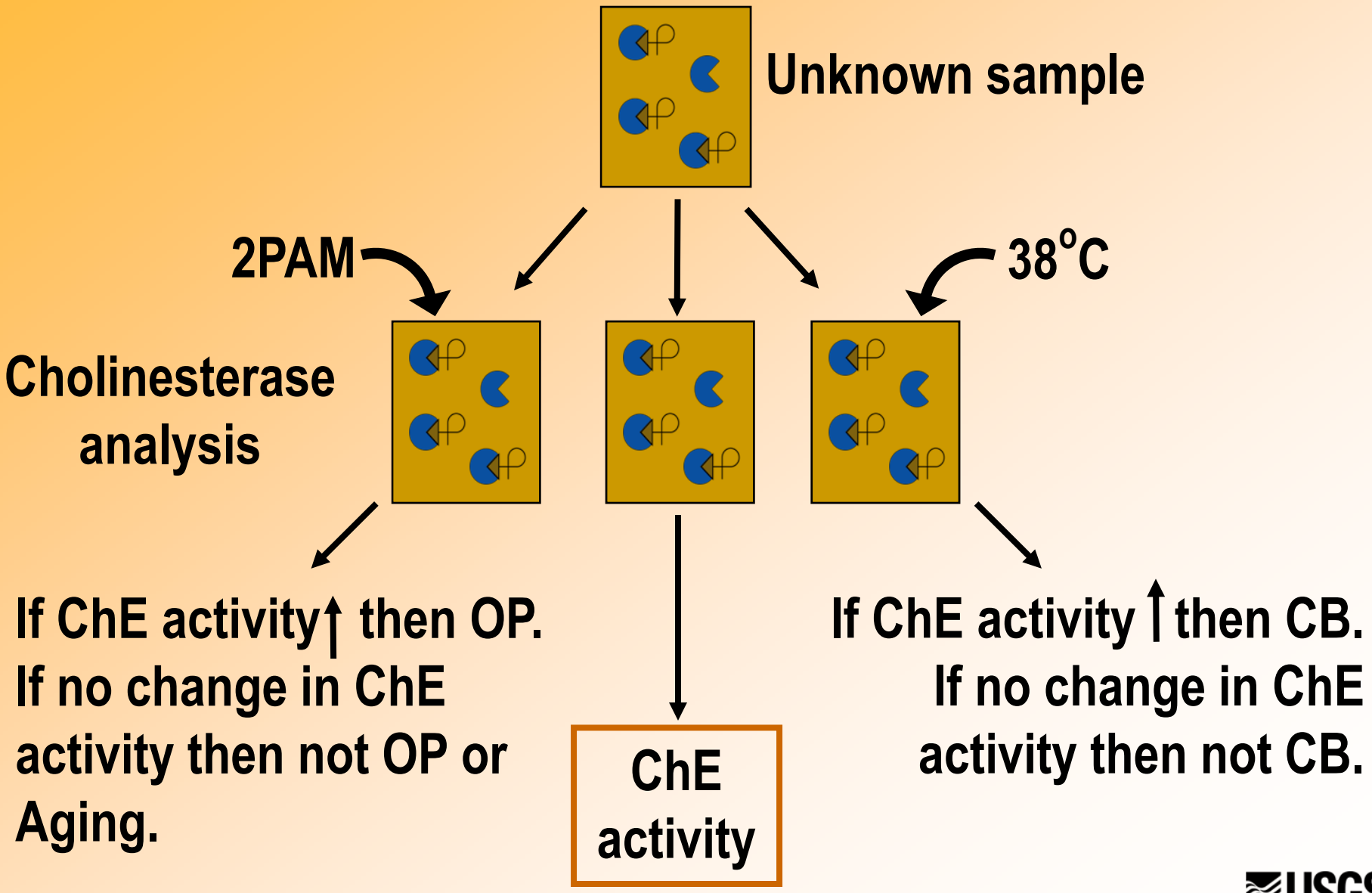


Aging of OPs

Dimethyl OPs ≤ 24 hrs
Ethyl-OPs within a few days



Cholinesterase Reactivation



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Cholinesterase Measurements

Plasma vs. Brain

Advantages and Disadvantages

Why is brain cholinesterase a specific biomarker?

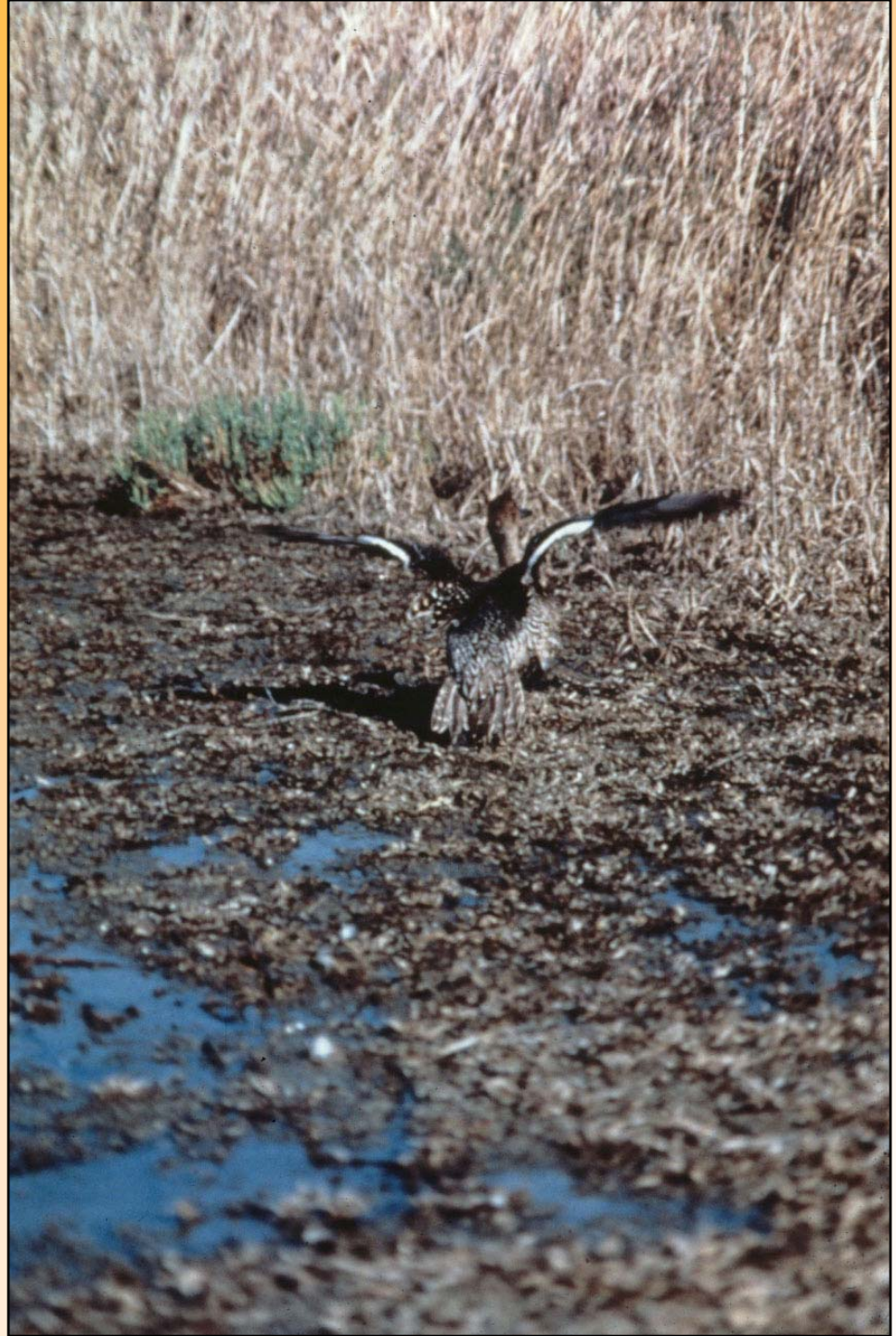


Photo by Tonie Rocke

Halogenated Pyrroles

Metabolic Pathway

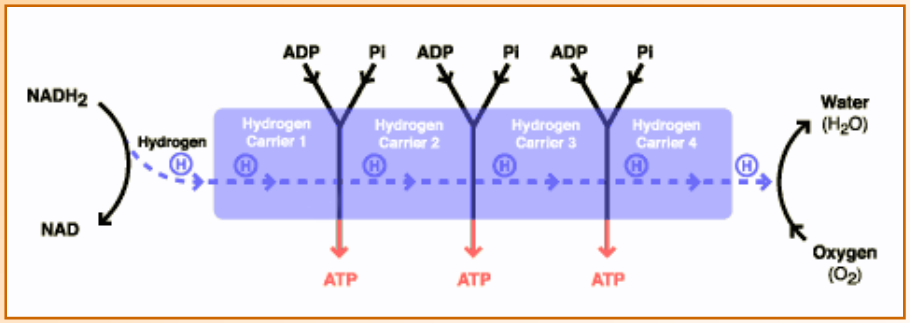
Glucose → Glycolysis

↓

Krebs Cycle

↓

Oxidative Phosphorylation



→ Energy

Implications

- Recovery of affected birds can improve the estimate of the magnitude of a mortality incident.
- The magnitude of a kill is a critical element of the prosecution.
- The magnitude of a kill can affect the sentencing guidelines and help understand the hazards from a pesticide use.

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