Announcements:

Last class!

Michael will hold 2 hours of OH tomorrow!

EC-Lab due at Final Exam.

Final EXAM – 11:30-2:30 Friday!!!

Please, porfavor --- complete your CAPE 😊

...ask questions ...discuss ...listen ...learn.
MOLECULAR MECHANISMS OF LEARNING AND MEMORY
Overview:

1. Neurobiology of memory
   Identifying where and how different types of information are stored

2. Hebb
   Memory results from synaptic modifications.

3. Study of simple invertebrates (Kandel)
   Molecular mechanisms lead to synaptic plasticity.

4. Electrical stimulation of brain
   Experimentally produce measurable synaptic alterations
Memory Acquisition – two stages

1. Acquisition of short term memory

2. Consolidation of long term memory

Memory formation
Short term memory is NOT working memory.

• Working memory requires rehearsal and has limited capacity.
• Working memory is vulnerable to distraction.
• Does not require lasting physical neural change.
• For example, trying to remember a phone number.
Short term memory survives distraction and has a large capacity.
Flow of Sensory Information into Long-Term Memory

- **Sensory experience**: Experiences are encoded by synaptic modifications.
- **Short-term memory**: Temporary synaptic changes are made permanent.
- **Long-term memory**: Only permanent modifications are retained.

Memory acquisition:
- Sensory experience to short-term memory

Memory consolidation:
- Short-term memory to long-term memory
Types of Memory

**declarative**
- Facts, events, places, faces
- Cerebral cortex

**procedural**
- Motor skills, habits
- Striatum/Basal ganglia
Responses to Faces in Inferotemporal Cortex

NOTE: IT neurons respond to complex shapes and images
IT responses to faces are selective
Featured Brain Tests

Fun website with memory & learning tasks
Do you never forget a face?
Are you constantly recognizing people from your past in unexpected places?
You might be a super-recognizer!

https://www.testmybrain.org/SupersRecruitment.html
Unique pattern or ratio of activity of neuronal activity

- Distributed memory
- No single neuron represents specific memory.

Advantage: Memories survive damage to individual neurons.

Graceful degradation of memories with gradual neuron loss

Physical change of memory → modification of synaptic weight

Experience dependent shift in neuronal selectivity
Model of Distributed Memory

No selectivity between responses to faces

Repeated exposure: changes in synaptic weights → familiar
Eric Kandel

- molecular mechanisms that lead to this synaptic plasticity

- What is physical basis of learning and memory
What Attracted Me to the Study of Learning and Memory in Apolpsis

by Eric Kandel

There was little to my early life that suggested the trajectory of scientific endeavors would eventually emerge from the antics of those tiny, beautiful creatures called Apolpsis, otherwise known as water snails. As a boy, I was more interested in reading Shakespeare and exploring the local flora and fauna of the marshes where I lived with my family. I was particularly drawn to the study of the natural world around me, and I spent countless hours observing the behavior of the creatures that lived there.

It was not until I was a student at Harvard University that my interest in Apolpsis began to take shape. I was fascinated by the way these small animals reacted to their environment and how their behavior changed over time. I began to experiment with different stimuli and discovered that Apolpsis had a remarkable ability to learn and remember. This discovery led me to pursue further research in the field of learning and memory.

I am a neuropsychologist and a neuroscientist, and my work has focused on understanding the neural mechanisms of learning and memory. In particular, I have been interested in how the brain forms memories and how these memories can be altered by environmental factors. My research has led to a better understanding of the neural basis of learning and memory, and has provided insights into the mechanisms of memory consolidation.

I have been fortunate to work with some of the most brilliant minds in the field of neuroscience, including Nobel laureates, and I am grateful for the opportunity to continue my work in this exciting field. I am committed to advancing our understanding of the brain and to applying this knowledge to improve the lives of people around the world.
memory:

Implicit

- Priming
- Procedural (skills and habits)
- Associative learning: classical and operant conditioning
- Nonassociative learning: habituation and sensitization

- Neocortex
- Striatum
- Amygdala
- Cerebellum
- Reflex pathways
Explicit

- Facts (semantic)
- Events (episodic)

Medial temporal lobe

Hippocampus

Medial temporal lobe
(Implicit) Procedural Learning – two categories
Learn a motor response in reaction to a sensory input.

**non associative**

Behavioral response over time to a single type of stimulus

Habituation & Sensitization

Repeated presentation of the same stimulus produces a progressively smaller response.

A strong stimulus results in an exaggerated response to all subsequent stimuli.
Most simple form of learning

Initial response to stimuli: very defensive -

Repeated exposure to stimuli: Response is muted - Eventually ignored.

Purpose: Animal needs to learn which stimuli to safely ignore

Eliminates inappropriate or exaggerated defense responses

Important for: Organizing perception
Sensitization – mirror image of habituation

After a noxious stimulus

the sensitized animal respond more strongly to all stimuli.

Purpose:
Instead of ignoring a stimulus – it is a form of learned fear. Survival.

It teaches the animal to attend and respond more vigorously to almost any stimulus

Konrad Lorenz: “An earthworm that has just avoided being eaten by a blackbird ... is indeed well advised to respond with a considerably lowered threshold to similar stimuli because it is almost certain that the bird will still be nearby for the next few seconds.”
Associative Learning—

**associative**

Form associations between events

Classical & Instrumental Conditioning

Learn that one **stimulus** (CS) **predicts** another (US) **stimulus**

Subject learns that a particular **behavior** is **associated** with a particular **consequence**

Classical Conditioning

Intrumental Conditioning

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*Savage Chickens* by Doug Savage
Aversive Classical Conditioning

A neutral stimulus should precede the aversive stimulus – that way the animal will come to predict it.

Pavlov: shock a dog’s paw. The shock caused the animal to raise and withdraw its leg – a fear response.

Pavlov found that after several trials in which he paired the shock with a bell – first sounding the bell then the shock – the dog would withdraw his paw whenever the bell sounded.

Classical conditioning an association is formed between a pair of stimuli that occur in rapid sequence.

Teaches the animal to associate an unpleasant stimulus with a stimulus that ordinarily elicits no response.
Synaptic strength is not fixed – it can be altered in different ways by different patterns of activity.

Habituation
Stimulus (tone or shock)
Response (degree of alertness)

Sensitization
Stimulus (S1-benign; S2 noxious)
Response (firing of cell)

Classical conditioning

Implicit learning
Human brain: 100 billion neurons
Aplysia brain: 20,000 neurons
The advantages of working with Invertebrate Models of Learning

- Small nervous systems
- Large neurons
- Identifiable neurons
- Identifiable circuits
- Simple genetics
The tale of the *Aplysia californica* and the Nobel prize!
Aplysia californica
Cooper, 1863

Order: ANASPIDEA
Family: Aplysiidae

DISTRIBUTION
Most of the Californian coast and parts of the Gulf of California.

PHOTO

A very large Sea Hare, reported by MacFarland, 1966 to reach 75 cm in length, but usually about half that. Has become very valuable laboratory animal for research into nervous systems and brain behaviour.

See the General Topics List for more information on Sea Hares.

Reference:
• Cooper, J.G. (1863). On new or rare mollusca inhabiting the coast of California - No. II. Proceedings of the Californian academy of Science 3(1): 56-60.

Authorship details

http://www.seaslugforum.net/aplycali.htm
Habituation Results from an activity-dependent presynaptic depression of synaptic transmission.
The gill-withdrawal reflex in Aplysia.

The mantle is held aside to show the gill in its normal position. The gill retracts when water is sprayed on the siphon.
Castellucci, Carew and Kandel (1978)
Repeated stimulation of the siphon skin leads to progressively less contraction of the gill-withdrawal muscles. 

One of the motor neurons that receives direct monosynaptic sensory input from the siphon is identified as L7 and this cell innervates the muscles that produce gill withdrawal.
Where is the physical change?

At the sensory nerve endings in the skin?
At the muscle, making it less responsive?
At the synapse between the sensory neuron and the motor neuron?
In this dorsal view of the abdominal ganglion, the six identified motor cells to the gill are brown and the seven sensory neurons are blue.

The sensory neuron carries the input from the siphon skin; the motor neuron makes direct connections onto the gill.
Habituation occurred at the synapse!

- Repeated electrical stimulation of a sensory neuron leads to a progressively smaller EPSP in the postsynaptic motor neuron.

There could be:
1. Less neurotransmitter release by the presynaptic axon or
2. Decreased post-synaptic responsiveness to the transmitter.

The sensitivity of the postsynaptic cell to neurotransmitter did not change.

After habituation, there are fewer quanta released per action potential!
HOMOSYNAPTIC DEPRESSION

Sensory neurons release glutamate \(\rightarrow\) generates fast EPSPs in interneurons and motor neurons

Motor cells discharge strongly causing a vigorous withdrawal of the gill.

If the stimulus is repeated...

EPSPs produced by sensory neurons in both interneurons and motor cells decrease...

From quantal analysis: it was determined that the amount of glutamate released from presynaptic terminals of sensory neurons decreased.

\(\rightarrow\) Fewer synaptic vesicles are released with each action potential

The sensitivity of the postsynaptic glutamate receptors did not change.
Habituation is non-associative learning.

- A diminished response to repeated stimuli.

- Importantly, the diminished response is not attributed to sensory adaptation nor to sensory or motor fatigue.
How much can the effectiveness of a synapse change?

How long can the change last?
Depression of synaptic potentials by long-term habituation

What is the basis for long-term habituation?

Castellucci, Carew and Kandel (1978)
Inactivation of synaptic connections by long-term habituation

Long-term habituation is caused by a decrease in the number of synaptic contacts between sensory and motor neurons.

The reduction in number of synapses persists and does not fully recover by 3 weeks.

Castellucci, Carew and Kandel (1978)
Sensitization involves presynaptic facilitation of synaptic transmission.
To cause sensitization of the gwr kandel applied a brief electrical shock to the head of Aplysia.

This resulted in exaggerated gill withdrawal response to stimulation of the siphon.

It was the modification of transmitter release in the sensory nerve terminal.

L29 synapses on the axon terminal of the sensory neuron.

L29 is activated by the head shock.
Sensitization in *aplysia*: a form of learned fear

In order for this type of sensitization to occur, an animal must *remember* a previous aversive stimulus, and they find that how long that memory lasts is a function of the number of repetitions of the stimulus.

In order for this type of sensitization to occur, an animal must remember a previous aversive stimulus, and they find that how long that memory lasts is a function of the number of repetitions of the stimulus.
Sensitization (like habituation) can be transient or long lasting.

A single tail shock will produce short-term sensitization → will last minutes.

DISHABITUATION: Sensitization will also overcome the effects of habituation and enhance the gill w/d effect.
How does sensitization and dishabituation enhance synaptic transmission?
It results from an enhancement in synaptic transmission in the same synapses that produced the synaptic habituation (depression).

How does sensitization and dishabituation enhance synaptic transmission?

Typically, modifiable synapses can be bidirectionally modifiable.
A Gill sensitization

Tactile stimulus

Sensitizing stimulus

Tail

Sensory neuron

Facilitating interneuron

Sensory neuron

Motor neuron

Gill

Siphon
Serotonergic interneuron
In my studies of *Aplysia* my work focused on the cellular substrates of the gill-withdrawal reflex that occurs when the siphon of the animal is touched (Figure A). This reflex undergoes sensitization (a simple form of learning) when a noxious stimulus is applied to the tail of the animal. I found that short-term memory results from a transient strengthening of preexisting synaptic connections, due to the modification of preexisting proteins, whereas long-term memory results from a persistent strengthening of synaptic connections brought about by alterations in gene expression, the synthesis of new proteins, and the growth of new synaptic connections. I discovered that the transient strengthening results in an increase
in the amount of transmitter released by the sensory neuron onto the motor neuron that controls the gill musculature. This increase is produced by activation by the tail stimulus of serotonergic modulatory neurons (Figure B part a). Serotonin increases the strength of the synapse between sensory and motor neurons by increasing the concentration of cAMP, an intracellular signaling molecule in sensory neurons that activates protein kinase A (PKA). When we similarly simply injected cAMP directly into the sensory neuron, it resulted in an increase in the release of the transmitter (glutamate) into
the synaptic cleft, thus temporarily strengthening the connec-
tion with the motor neuron (Figure B part b).

Beginning in 1980, the insights and methods of molecu-
lar biology enabled us to identify common mechanisms of
short-term memory in different animals and to explore how
short-term memory is converted to long-term memory. We
found that, following long-term sensitization, PKA moves into
the nucleus and activates gene expression, leading to the syn-
thesis of new proteins and a twofold increase in the number
of synaptic connections made by *Aplysia’s* sensory neurons
(Figure B part c). Moreover, the dendrites of the motor neurons,
which receive the signals from the sensory neurons, grow and
remodel to accommodate the additional sensory input.

Together, these early cellular studies of simple behaviors
provided direct evidence supporting Cajal’s suggestion that
synaptic connections between neurons are not immutable; they
can be modified in learning, and those anatomical modifications
are likely to subserve memory storage. In the gill-withdrawal re-
flex of *Aplysia*, changes in synaptic strength occur not only in
Recall:

**ionotropic**
- Fast and brief
- Mediate behaviors

**metabotropic**
- Start slow and last long
- Modulate behaviors
They change the balance of charge across the membrane quickly.
metabotropic

Start slow and last long

Modulate behaviors

→ They modify the strength of the synapse.

→ Their action can start local & spread wide.

→ Receptor → proteins → enzymes change genes etc.
G-protein coupled receptors

G-protein

Activate an effector which is typically an enzyme that produces a diffusible second messenger.
Serotonin is the culprit for presynaptic changes!
In its resting state, $G_s$, like all $G$ proteins, binds to a molecule of guanosine diphosphate (GDP).


$G_s \rightarrow \text{"stimulate cAMP synthesis"}$
Transmitter binding alters conformation of receptor, exposing binding site for $G_s$ protein.

The interaction of $G_s$ with a ligand-bound receptor promotes the exchange of the bound GDP for guanosine triphosphate (GTP), leading to a conformational change that activates the G-protein.

Diffusion in the bilayer leads to association of transmitter receptor complex with $G_s$ protein, thereby activating it for GTP-GDP exchange.

Displacement of GDP by GTP causes the α-subunit to dissociate from the $G_s$ complex

exposing a binding site for adenylyl cyclase on the α-subunit

The α-subunit binds to and activates the cyclase to produce many molecules of cAMP.

In its activated state Gs stimulates the integral membrane protein adenylyl cyclase to catalyze the conversion of adenosine triphosphate (ATP) to cAMP.
When associated with the cyclase, $G_{s\alpha}$ also acts as a GTPase hydrolyzing its bound GTP to GDP.

Hydrolysis of the GTP by the $\alpha$-subunit returns the subunit to its original conformation, causing it to dissociate from the cyclase (which becomes inactive) and re-associate with the $\beta\gamma$-complex.
The activation of the cyclase is repeated until the dissociation of transmitter returns the receptor to its original conformation.
Serotonin is the culprit for presynaptic changes!
The major target of cAMP in most cells is the cAMP-dependent protein kinase (also called protein kinase A or PKA).
Protein Kinase A
(cAMP-dependent protein kinase)

PKA is a heterotetrameric enzyme consisting of a dimer of two regulatory (R) subunits and two (c) catalytic subunits.

Kandel, et al (5th ed)
In the absence of cAMP, each R subunit binds to and inhibits the C subunits, leading to a conformational change that causes the R and C subunits to dissociate.

In the presence of cAMP, each R subunit binds two molecules of cAMP.

Kandel, et al (5th ed)
Dissociation frees the C subunits to transfer the gamma-phosphoryl group of ATP to the hydroxyl groups of specific serine and threonine residues in substrate proteins.
Facilitating interneuron

Siphon sensory terminal

5HT Receptor

$G_s$ protein

Adenyl Cyclase

cAMP dependent PKA

S-type $K^+$ channel (serotonin sensitive)

$Ca^{++}$ channel

Glutamate

Motor neuron
Decreased $g_K \rightarrow$ increased $g_{Ca^{++}} \rightarrow$ increased transmitter release

In the axon terminal, VGCC stay open as long as $V_m$ exceeds a threshold value.

A decrease in $K^+$ conductance causes the AP to be prolonged. The amount of time that VGCC is open increases the amount of transmitter release.
Classical conditioning of fear involves coordinated pre- & postsynaptic facilitation of synaptic transmission.
Classical Conditioning
— more complex learning form
— more than learning about the properties of one stimulus
— animal associates one type of stimulus with another.
Classical Conditioning
Results in:
1. greater &
2. longer-lasting
enhancement
Aphysia - GWR (gill w/d reflex)

Weak touch to siphon

CS

Gill W/D

UD

Strong tail shock
The setup:
CS gentle stimulation of the siphon
US tail shock
Pairing CS with US →
CR greater response to siphon stimulation.

• Pairing CS-US timing is critical!!!
timimg!!

classical cond.
To be *EFFECTIVE*,

the *conditioned stimulus* (siphon touch) must *precede and predict* the *unconditioned stimulus* (tail shock).
Activity-Dependent Facilitation

→ how does this work??
Weak touch to siphon CS

Gill W/D

UC

Strong tail shock

Touch siphon tail shock

1

2

Siphon touch tail shock

After an AP
AP triggers ↑↑Ca<sup>2+</sup>

Ca<sup>2+</sup> binds to calmodulin

adenyly cyclase

Sphynx touch tail Shock

after an AP
Ca^{2+} binds to calmodulin

AP triggers

Ca^{2+}

Adenylyl cyclase is primed for when 5HT is released.
What happens when 5HT is released?

Adenylyl cyclase is primed for when 5HT is released.

Adenylyl cyclase responds more vigorously.
What happens when 5HT is released?

- Adenylyl cyclase is primed for when 5HT is released
- Adenylyl cyclase responds more vigorously

⇒ More cAMP!

↑↑ Facilitation
Associative Learning in *Aplysia*

Gentle water jet to the siphon is the CS.

A shock to the tail is the US. The response measured is the withdrawal of the gill.

The US activates the same 5-HT cell (L29) that is active in sensitization.

Timing of the CS and US during three different types of training.
When Ca++ is elevated, adenylyl cyclase increases cAMP.

More cAMP → more PKA → phosphorylate K channels → decrease gK

Decreased gK → prolonged AP → increased Ca++ more transmitter released

Enhanced neurotransmitter release → increased postsynaptic response

The molecular basis for classical conditioning in *Aplysia*.

**US Alone** leads to sensitization via 5-HT

**Pairing CS-US** causes greater adenylyl cyclase response
What would happen if we reversed 1 & 2?
If tail shock is 1st then:

release

precedes

CaH influx
no potentiation!!

=> no classical conditioning.
Bottom line: adenyl cyclase is acting like a coincidence → detector *temporal order
Recall: classical conditioning is associative learning.

One learns about the predictive relationship between two stimuli.
Unpaired pathway:

Kandel, E et al (5th Ed) Principles of Neuroscience
Paired pathway:

Kandel, E et al (5th Ed) Principles of Neuroscience
Long Term changes in synaptic connections & synapses and NEW synapses!!
Short-term memory $\Rightarrow$ long-term memory consolidation is needed.
the consolidation is dependent on long-term 

\[ \uparrow \uparrow \text{ in cAMP} \]

(Repeated STT exposure)
cAMP leads to MAPK activation

mitogen-activated protein kinase (MAPK)
PKA → P CREB-1

Sensory neuron nucleus
CREB-1

binding

protein 1

element

response

CAMP
CREB-1 is a transcription factor
Regulation of Gene Expression by CREB

(a) CREB-2 binds to CRE, preventing transcription.

(b) CREB-1 binds to CRE, allowing transcription.

(c) Phosphorylated CREB-1 further enhances transcription.

Note: CRE is a recognition element.
The diagram illustrates the number of synaptic boutons per sensory neuron under different conditions: Control, Habituated, and Sensitized. The graph shows a significant increase in the number of synaptic boutons from Control to Habituated and further to Sensitized states. The images on the right provide a visual representation of these changes at a microscopic level, highlighting the differences in synaptic structure between the conditions.
Vertebrate Models of Learning

- Neural basis of memory learned from invertebrate studies:
  - Learning and memory can result from modifications of synaptic transmission
  - Synaptic modifications can be triggered by conversion of neural activity into intracellular second messengers
  - Memories can result from alterations in existing synaptic proteins
  - Cellular correlates of learning.
Declarative (Explicit) memory

- conscious recall of information about people, places, objects
2 structures in mammalian brains important for encoding & storing

hippocampus  Prefrontal ctx
working memory

long-term memory

declarative memory encoding
Explicit memory: different forms of LTP in the hippocampus
Recall long-term memories are dependent on long-lasting changes in synaptic strength.
Why the hippocampus?
Entorhinal cortex (EC):

Input from EC: **Perforant Pathway**

Multimodal sensory & spatial info

Output from CA1 back to EC!

(major output)
Perforant Pathways

1. Direct pathway -> CA1 (layer III EC)

2. Trisynaptic pathway (layer II EC)
LTP long-term potentiation

* all forms of LTP are induced by synaptic activity in the pathway that is being potentiated.

LTP is homosynaptic.
What is the role of NMDA receptor in the induction of LTP?

It depends which pathway!
Mossy fiber pathway LTP
Mossy fiber LTP – PKA mediated!

Blocking NMDA receptors had no effect on LTP

Large Ca++ influx into presynaptic terminal during tetanus

Ca++ $\rightarrow$ Ca/Calmodulin dependent adenylyl cyclase $\rightarrow$ increase cAMP

cAMP $\rightarrow$ activate PKA

PKA phosphorylate results in increased exocytosis $\rightarrow$ more xmttr released.

The diagram shows the pathway from Ca++ influx to increased exocytosis, mediated by PKA. It also notes that this is important for axocytosis.

Handwritten note: "Q$\exists$ reminds me of the gill withdrawal reflex in Aplysia"
Induction vs Expression

1. Induction of LTP
   - the biochemical reactions activated by tetanic stimulation
expression of LTP.

Long-term changes that take place at the synapse responsible for enhanced synaptic transmission.
Glutamate is released from Schaffer collateral terminals.

Both AMPA & NMDA receptors are activated.

Recall: NMDAR is voltage sensitive (Mg+ block) acts as a coincidence detector.
Normal synaptic transmission:
Induction of LTP:

1. NMDA open
2. Ca^2+ enter important for LTP.
Induction of LTP.

1. NMDA open
2. Ca++ enter

Important for LTP.

Downstream signaling pathways
Ca\textsuperscript{2+}/calmodulin-dependent protein kinase II (CaMKII), protein kinase C (PKC) & tyrosine kinases

→ downstream signaling pathways

→ induction of LTP

Induction open

Important for LTP?
induction of LTP:
Expression of LTP

Enhanced transmitter release

Glu

Mg²⁺

K⁺

Na⁺

K⁺

P

Insertion of new AMPA receptor
Properties of LTP in CA1

- Bliss and Lomo
  - High-frequency electrical stimulation (tetanus) of excitatory pathway produces LTP.

- Most excitatory and many inhibitory synapses support LTP.
- Schaffer collateral synapses
- Property of input specificity
- Spatial summation of EPSPs: cooperativity
  - LTP causes association of inputs.
How Ca$^{2+}$ Can Trigger Both LTP and LTD in the Hippocampus
Egg Carton Model of AMPA Receptor Trafficking at Synapse

(a) Initial steady state

(b) LTP

(c) New steady state

(d) LTD

(e) New steady state

= AMPA receptor lacking GluR1

= AMPA receptor containing GluR1

= Slot protein
Sleep consolidates your memories, give yourself the gift of sleep.
Your final exam -
Wednesday 8AM!
See you all then. ☺️.
Thank you!