MEDS 370 -- INTRODUCTORY NEUROSCIENCE

Introductory Class August 24, 2001 Dick Mains mains@uchc.edu x8894

MEDS 370 Syllabus

Course times: Fri 9 am, EG-052; Aug. 24, 2001 – Dec. 21, 2001

Course style: lectures; 2 short answer take-home exams in the middle & end of the course

SIGN IN – important !!! Neatly, especially e-mail !

Prerequisites: Working knowledge of cell biology and biochemistry; NO PRIOR TRAINING in neuroscience necessary. This course is designed for all Biomedical Science students and is encouraged for Neuroscience graduate students.

Faculty contacts: Les Bernstein (les@neuron.uchc.edu); Dick Mains (mains@uchc.edu); Doug Oliver (doliver@neuron.uchc.edu)

Organization of the course: in 3 blocks:

1] Action potential conduction and synaptic function
- how signals travel along axons; travel between neurons and targets
  - mix of electrophysiology, biochemistry, molecular biology
- Neurotransmitter synthesis, storage, secretion, action
  - what the signals do at end of axon; how signals got started

2] Development of the nervous system
- how it all gets wired up; how neurons achieve their mature identities

3] Systems neurobiology
- obvious from its title
- including intro to psychiatric and neurological diseases

Handout styles, lecture styles, taking notes: you will see that there are as many styles of lecture and handout as there are faculty in this course. Some handouts are in sentences, some in outline form. Faculty will use slides, overheads, powerpoint, the whiteboard. Often the most important things are in the handouts, *but taking notes is an **essential** skill to develop*, if you wish to get the most benefit from courses, from seminars and from meetings (e.g. Neuroscience and other departmental Research Seminars and Journal Clubs; Society for Neuroscience, Keystone Symposia, Gordon Conferences).

BUY ONE !!!

Textbooks are crucial: **at UCHC Med School Bookstore**

Recommended:

Kandel, Schwartz, Jessell 2000 Essentials of Neural Science & Behavior, 4th Ed., Appleton Lange

Also good:

Zigmond, Bloom, Landis, Roberts, Squire 1999 Fundamental Neuroscience, Academic Press

Purves et al 2001 Neuroscience, 2nd Ed, Sinauer

Matthews 1998 Neurobiology; molecules, cells, systems, Blackwell Science

Grading: Based on a written take-home (hence open book/notes) exams (**Given out Oct. 11, due TYPED Oct. 19; and given out Dec. 14, due Dec. 21)**

********** **Your effort matters, especially if you are having trouble**

Exams are simple straightforward written answers, usually a few paragraphs; we **will post** some similar exams **WITH ANSWERS** from last year's course and related courses at other institutions, accessible only from within the UCHC electronic network. Our goal is to teach you about neuroscience, not grade you, but unfortunately we do have to give grades. Our goal would be to give all A's.

Auditors
are welcome, in fact strongly encouraged. Especially postdocs, senior grad students, etc.

Lecture Outline:
Reading: Kandel 4th Ed. (2000) pages 5-86; 175-185; 253-277 (Ch. 1-4;10;14)
NUMBERS don't matter, the concepts DO matter

Why do we care so much about neurons? What makes neurobiology special?
- There are ~10,000 distinct types of neurons in the central nervous system [**CNS**]
  - and glial cells outnumber neurons about **10:1**
- The "average" neuron receives perhaps 10,000 synaptic inputs (range 0 - 1,000,000)
- >100 types of ion channels
- >100 different communication molecules (neurotransmitters)
- A major difference between humans and other animals is our nervous systems
- Hydra and other coelenterates have **Nerve Nets with all neurons ~ equal**
  - no hierarchy; all neurons are both sensory and motor/output
- **cephalization** = concentration of neurons at one end
  - goes with appearance of **interneurons** in flatworms
- Words to know for future: **Convergence ... Divergence**

What is **"special" about neurons**?
- action potentials? neurotransmitters? receptors? ion channels? polarity?
- **NONE** of these attributes is unique to neurons. Defining a neuron is not our goal.

Non-neuronal cells matter too
- targets of neurons (muscles, glands); glial cells; induction during development; effects on action potential (e.g. glial cells as K+ buffer); nutritive support

History of cellular/molecular neuroscience (**extremely** capsulated)

~1800	electrical basis of neuronal signaling recognized
~1900	cell theory of neurons proposed
1920s	neurotransmitters recognized as means of synaptic communication
1940s/50s	biophysics of electrical signaling initially understood
1950s	brain circuitry recognized; concept of topographical mapping
	EM revealed fine structure of axons, dendrites, synapses
	idea that synapse, not neuron, is fundamental unit of NS
1960s	initial understanding of synaptic integration, ± action potentials
1970s	second messengers initially identified

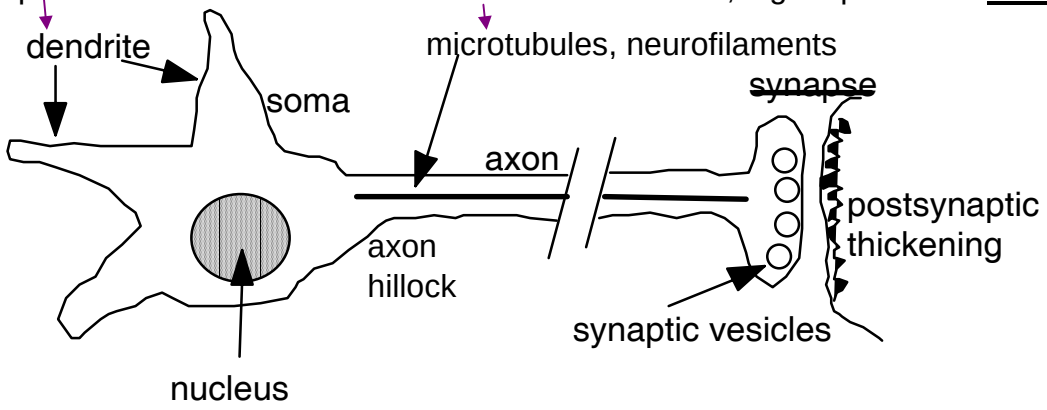
multiple signals; neuromodulators as well as neurotransmitters
beginning of cloning
1980s cloning of.....many things
1990s "Decade of the Brain"
2001 Now!

See how compressed this is into the present!!

Focus of this course is on **cells/molecules/development/systems/behavior/memory**, or simply ALL of neurobiology! There are later courses offered by this Program covering EACH these same topics in much greater depth.

The basic morphology of a neuron

spines
dendrite
soma
more than other cells; big emphasis on **transport**
microtubules, neurofilaments
synapse
axon
postsynaptic thickening
axon hillock
synaptic vesicles
nucleus



dendrites TAPER while axons are quite uniform in diameter away from branch points

Far prettier **diagrams** are found in the introductory chapters of the texts, notably Kandel. The best **electron micrographs** in introductory chapters are in Siegel and Purves. Really nice **camera lucida drawings** are in Zigmond.

More anatomically accurate drawings in the texts show the complexity of neuronal shapes in more detail. While invertebrate neurons are frequently unipolar, only a few autonomic neurons in vertebrates show this morphology, with *pseudo-unipolar* and many variations of *bipolar* being the more common. Dendritic patterns are widely varied among *multipolar cells*, with spinal motorneurons and sympathetic ganglion neurons having dendrites rather close to the cell body, while the dendrites of a retinal bipolar cell cluster a short distance from the cell body, and the input end of a dorsal root ganglion can be over 1 meter from the cell body. In the CNS, the dendritic arbors can be very elaborate (the usually cited champion is the Purkinje neuron in the cerebellum). In the brain and spinal cord, functionally distinct types of incoming signaling can arrive at different dendritic regions, as shown for the hippocampal pyramidal cell; another good example is the parallel fibers for CBLM Purkinje neurons, and climbing and mossy fibers for the same cells. Similar pyramidal cells are frequently the output neurons from many regions of the cerebral cortex.

Since neurons are often burdened with establishing and then maintaining dendrites and axons that may have 100 times the net volume of the cell body and 1000 times the surface area, neurons are understandably specialized for secretion. Massive amounts of protein

synthesis are devoted to the production of membrane proteins, synaptic vesicles, and cytoskeletal proteins.

Neurons first, now <u>the other 90% of the cells in the nervous system</u>:

Neurons are outnumbered ~10:1 in the nervous system by glial cells. Smaller nerve fibers can often be swept together and are thus ensheathed as a group by glial cells.

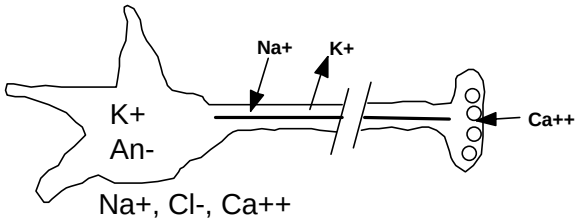
The different glial cells which form these different coverings for neurons are shown and diagrammed in the text. Oligodendrocytes are small glial cells found the central nervous system, where they can produce myelin in white matter regions or surround many axons. Schwann cells are larger glial cells found in the peripheral nervous system (**PNS**), which form myelin. Astrocytes are believed to have several roles in the CNS, including passing nutrients from blood capillaries to neuronal soma, inducing endothelial cells to form the blood-brain barrier, and buffering the potassium (K+) ions extruded from axons during nerve conduction.

@#* There is a whole lecture on glial cells in a few weeks in this course.

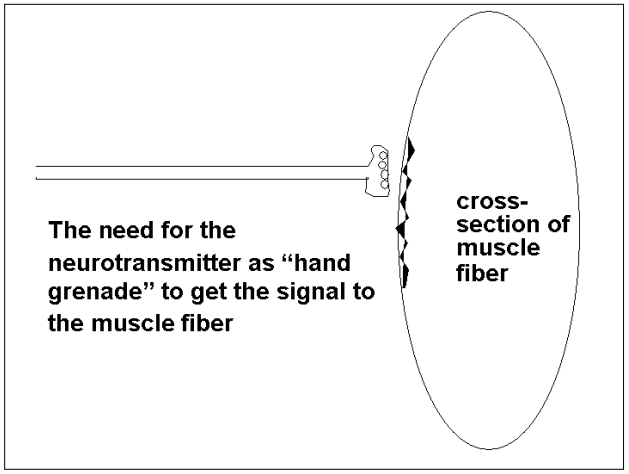
Synapses as electrical or chemical → vast majority in CNS, PNS are chemical

advantages of chemical synapses:

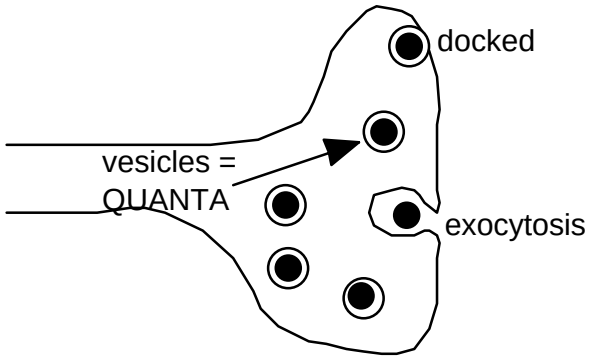
nearly as fast as electrical
amplification can be very big
blending (integration) possible
big variety of postsynaptic effects due to differences in receptors



Electrical pattern changes as action potential travels down the axon to the synapse:
Depolarize: [Ca^{++}] up: release <u>quanta</u> = vesicles = many NT molecules
So, why does the nervous system complicate the picture so much by using neurotransmitters anyway? Like trying to use poker to heat up a kettle; **use a grenade!**



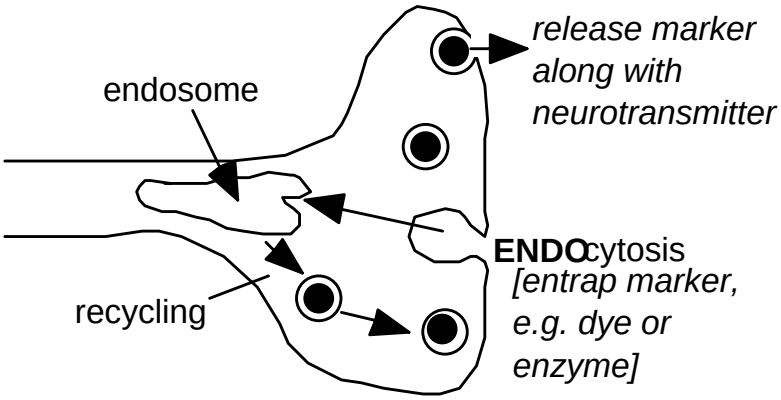
The big thing with **chemical neurotransmission** is to build in various **features**:
1. **<u>Safety</u>** of transmission, as in skeletal neuromuscular case **<u>10 - 25% NT is enough</u>**
2. **<u>Integration</u>** as in many CNS synapses, where the blending and summation are the most important.
3. **<u>Timing</u>** of immediate effects such as changes in ion fluxes **OR** multiple effects due to second messengers; **ALSO** longer term trophic effects on the targets



presynaptic terminal

Important terms:

Vesicles **EM, biochem**
Quanta **electrical**
Docking **EM, biochem'l**
Exocytosis **spill contents**
Endocytosis **cavicapture**
Recycling **morph, biochem**
Active Zone **morph, elect'l**
Releasable **functional**
Reserve **the rest**

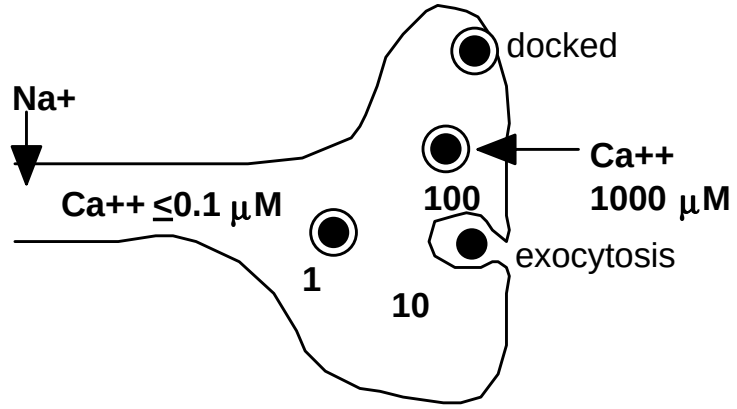


Calcium (Ca^{++}) crucial

Usually Ca^{++} comes **across plasma membrane** to start release, but Ca^{++} can also be liberated from **intracellular** pools. For example, the key calcium for long-term depression or long-term potentiation may come from intracellular stores, not transmembrane currents.

Exocytosis is followed by **endocytosis**, so that plasma membrane does not expand indefinitely, and to replenish vesicle membrane pool.
Endocytosis takes 1-10 sec, **may not involve clathrin**

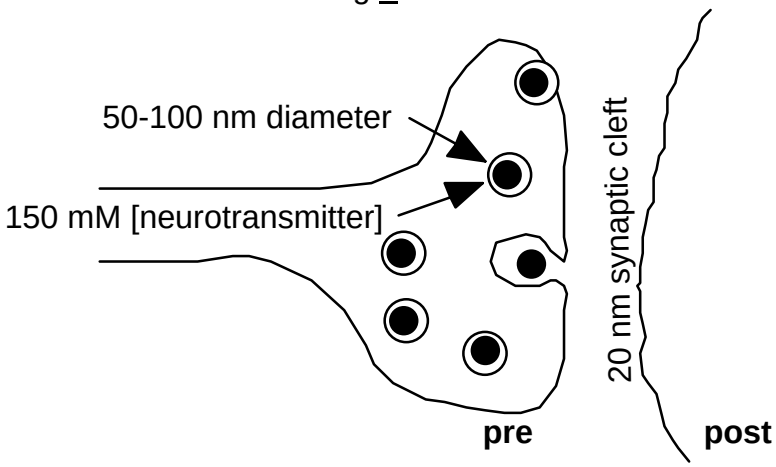
Ca^{++} entry can locally raise [Ca^{++}] 1000-fold, from <0.1 μM up to 100 μM or even higher, based on data from Ca^{++}-sensitive dyes. Release stimulated by Ca^{++} > 1 μM; **release=vesicle fusion lags Ca^{++} entry by only 60 μsec!** And current estimates put the distance from Ca^{++} entry to release site at 13-20 nm (well **under** 1 vesicle diameter).



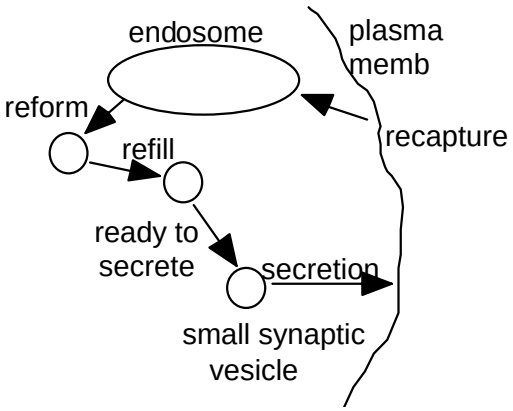
[Ca++] in presynaptic regions

Release shows cooperative dependence on [Ca^{++}] (roughly 4th power of [Ca^{++}]).
Estimates give 60 Ca^{++} channels open for 1 vesicle fused, and CNS synapses often operate at <1 quantum. **But**, once secreted, the concentration of neurotransmitter in the synaptic cleft can be 10's of mM!
note: **50-100 nm vesicle but 20 nm**

synaptic cleft! This gives a strong **hit-or-miss** character to CNS transmission, with the concentration of NT being ≥10 mM or zero!



So the picture is one of **constant recycling** of the components of the synaptic vesicle system. Let's make a **list** of some of the **key features and properties** of the components of the synaptic vesicle system, and then go over some of the evidence that the model is correct.



Vesicles Background

Average neuron has 10^6 to 10^7 vesicles
but any given terminal has only 50 vesicles

Vesicles in brain are 10 µM or 7% of CNS protein
PLUS peptide granules

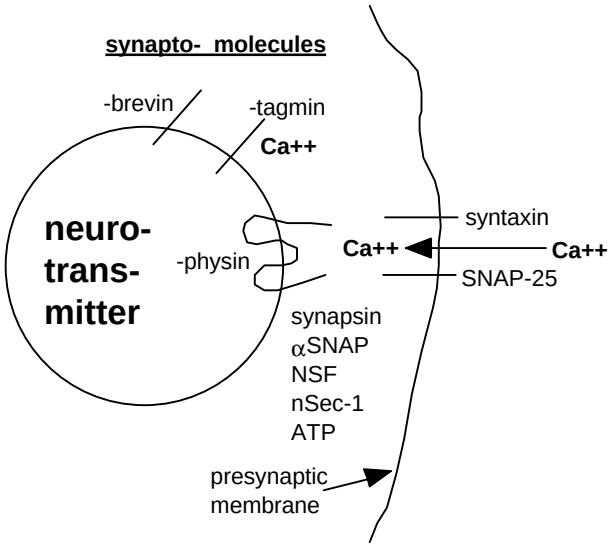
~10,000 lipid molecules/vesicle
lipid:protein = 3:1 by weight (higher than other membranes)

so only have about 3 x 10^6 Da of protein (3000 kDa) per vesicle
since proteins are either big (like channels) or multimers
then figure ≥ 200 kDa/function
---> **10-15 functions MAXIMUM** in vesicle membrane

that estimate fits with reality
≤20 different proteins
most are **families** of homologous proteins
protein families are quite distinct
very **high sequence conservation** among species
e.g. SV2 which is ~75% conserved elasmobranch fish->rat

IMPT concept; vesicles recycle ~100X; FAST perhaps 1 min
So vesicle lives only about 2 hours!!
that's still slow for a given synapse with 50 vesicles ==> release only 1 vesicle/sec !!

How do vesicles get ready to work?
concept of a **coiled spring or loaded gun**
spend time on how the spring gets coiled as well as release



"meet the proteins"
many are found in other cell types;
differences in AMOUNT
or in which member of a gene family expressed
******* SNAREs: t- and v- *******

other stuff in vesicles
ATP in CA and ACh vesicles;
AscA in CA vesicles
Often very high [divalent cation] of some type
Enzymes; routing questions – proteins had to be built in at start of vesicle's life

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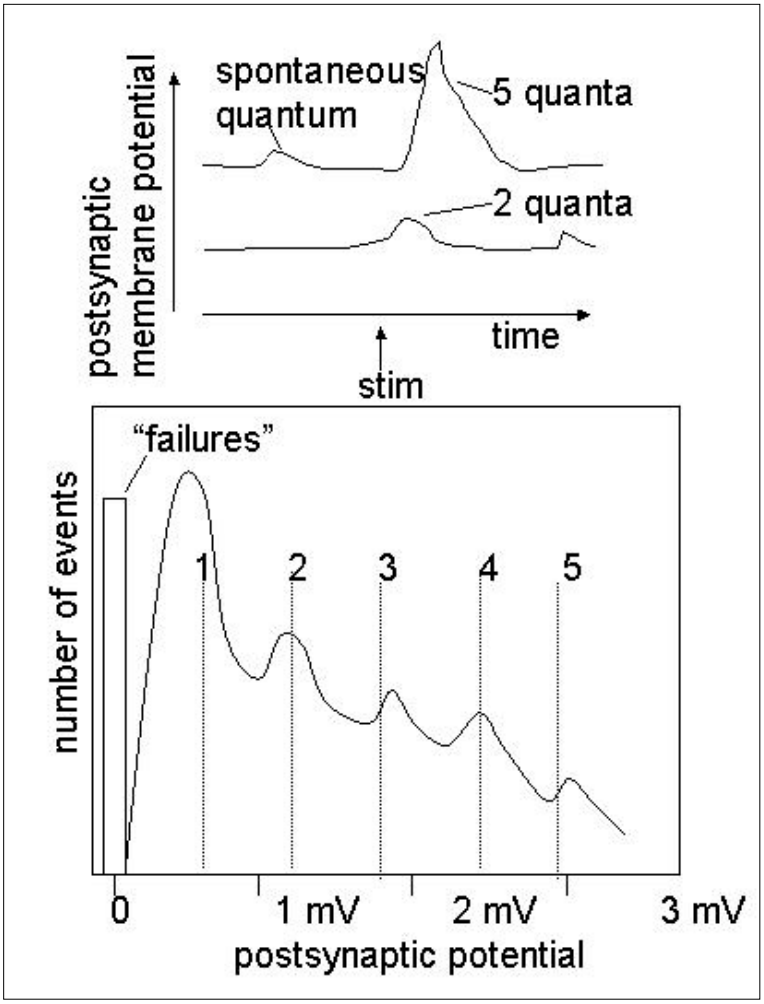
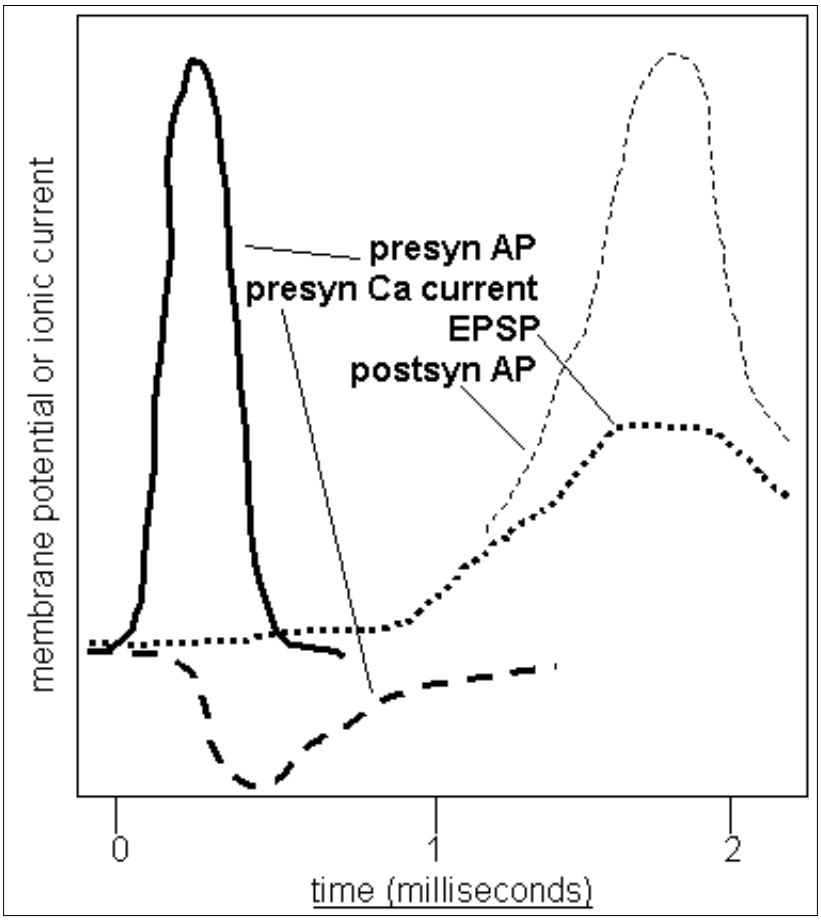
Now that we've visited neurotransmitter release from the **cell biological** point of view, let's go back and look at a few of the **electrophysiological approaches** to neurotransmitter release.

As modern cell biologists, we don't think of it as surprising that vesicle docking and fusion is "leaky" – that there is a slow basal rate of fusions. These "miniature potentials" are in fact the electrical equivalent of the vesicles seen in the EM and isolated biochemically. The electrophysiological term is "quanta". When a presynaptic action potential arrives, there is a coordinated release of many quanta, and when the numbers are not too huge, recordings over many stimuli give the results shown here:

The distribution is called "Poisson" and the usual explanation for this result is that the probability of release of any one quantum is very low (true) and that the quanta are released independently (only partially true, sometimes not true).

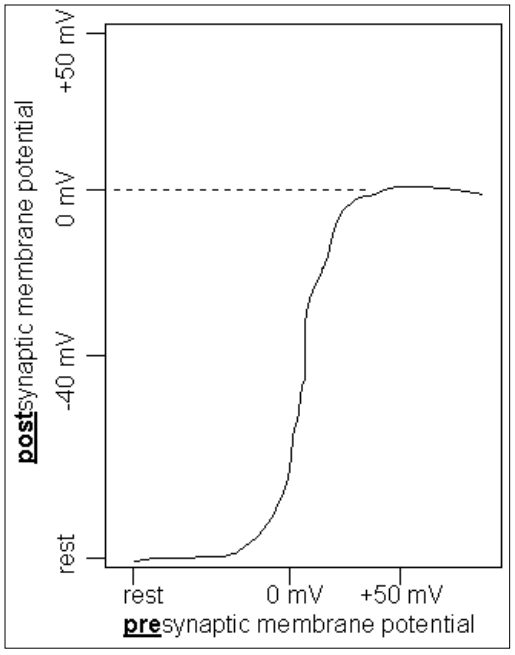


The timing of the arrival of the presynaptic action potential, the entry of calcium, and the release of neurotransmitter were all worked out by Katz and Miledi in a truly elegant series of experiments. The basic answer is shown here.



1. presynaptic action potential (entry of Na+) excites
2. calcium conductance in terminal, leading to
3. Ca++ entry (Ca++ current). This causes vesicle fusion for some of the docked vesicles, leading to
4. neurotransmitter release. Rapid diffusion across the synaptic cleft then allows the neurotransmitter to bind to the postsynaptic receptors and cause the opening [or closing] of ion channels. In the example diagramed, the postsynaptic effect is excitatory,
5. leading to an excitatory post-synaptic potential (EPSP)

The all-or-none (really all or little, remembering that the basal rate is not zero) release of neurotransmitter has another consequence. There is a threshold of presynaptic membrane potential that has to be reached before the calcium conductances are activated. So below that level of presynaptic depolarization, the postsynaptic view is that "nothing happened". Likewise, above a certain amount of neurotransmitter release, there can be no further postsynaptic response – the postsynaptic ion channels are passing enough current to reach the equilibrium potential for those ions, and more neurotransmitter will mean more postsynaptic conductance but no further postsynaptic membrane potential changes.



EXTRA STUFF NOT COVERED IN THE LECTURE

PLASMA MEMBRANE PUMPS and TRANSPORTERS

along axon Na/K and Na/Ca pumps; also Ca-ATPase
important? often told "take gradients as constant". Is that really OK?
yes, for a few AP

take 2 μ axon with 15 gNa / μ^2
pass 10,000 ions/AP = 1 msec x 10^7 ions/sec = 10^4 ions/AP
gives 0.5 mM rise in $[Na^+]_i$ per AP
OR for Node of Ranvier with 1500 gNa / μ^2, get $\Delta[Na^+]$ = 50 mM right under Node!!
so yes, treat as constant for a few AP
but **better get it pumped out !!!!!**

makes a point we will see over and over

pump Na^+ (plasma memb) or H^+ (vesicle), using ATP
and the rest of transport is usually downhill, riding the Na^+ or H^+ gradients

create the gradients using ATP --> PUMPS
Na/K ATPase
Vesicle H^+ pump

use the gradients to move things "uphill" across membranes
Membrane NT uptake and precursors to NT
vesicular transporters **all related to bacterial multidrug resistance transporter**
knockouts are usually fatal
Cannot increase filling vesicles with more cytoplasmic NT synthesis

BUT certainly can have release of a ½ - full vesicle

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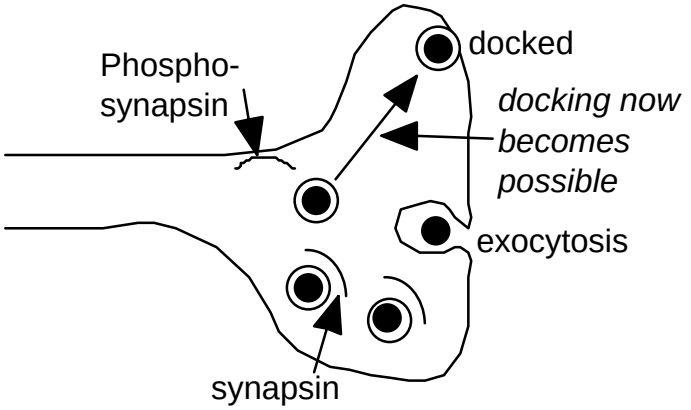
PROTEINS IN AND ON VESICLES, INVOLVED IN SECRETION
Example: Synapsin
only neurons, NOT endocrine cells; so far, only clear vesicles
52-74 kDa, 4 major isoforms, differ at COOH-terminals



major phosphoprotein in brain (0.4% of total)

binds to vesicles and binds vesicles to actin

idea of "local gel" in terminal/synapse

prevents secretion until release

release caused/allowed by phosphorylation of synapsin

Experiments: demonstrated with Mauthner cell and squid giant synapse

inject synapsin and block secretion
inject P'd synapsin and promote secretion

inject synapsin **antibody** and promote secretion
inject CaM kinase and promote secretion
isolate endogenous synapsin and show got P'd, using γ-[^{32}P]-ATP injection

So de-phospho-synapsin is a major <u>brake</u> on secretion!!

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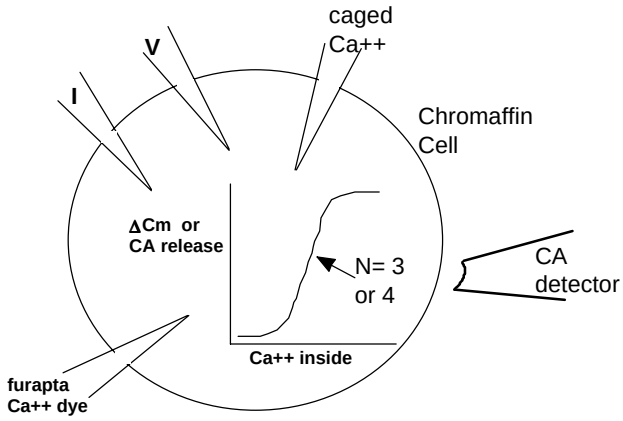
Membrane measurements during secretion: the adrenal chromaffin cell model

Isolated bovine chromaffin cells (synthesize catecholamines).
Record V_m, inject current, inject Ca^{++} -sensitive fluorescent dye, and inject caged Ca^{++} (dimethoxynitrophenamine)
Then flash with light
Ca^{++} released **inside**
Ca^{++} dye activated
C_m increases **memb**
CA = catecholamines released **outside**



Interesting questions remain about the time lags between increased $[Ca^{++}]_i$ and ΔC_m or CA secretion.

A **<u>rate-limiting step in secretion is dissolution</u>** of CA from vesicular matrix, NOT efflux from a small pore; may explain why ACh and Glu synapses are faster that CA synapses.