

Synaptic Transmission III: Transmitter Release

Dr. Eric Kandel

*(Reference Ch. 14 in PNS, 4<sup>th</sup> ed.)*

In this lecture, we consider:

- pre-synaptic factors in transmitter release;
- the properties of synaptic plasticity (which makes chemical synaptic transmission special)

**I. Transmitter Release from the Pre-synaptic Terminal**

**The Problem:** Action potential (AP) invades the pre-synaptic terminal → chemical substance is released. How does this occur?

What is it about the AP that is fundamental to transmitter release:

- Opening of Na<sup>+</sup> channels?
- Influx of Na<sup>+</sup> ions?
- Depolarization?
- Activation of K<sup>+</sup> channels?
- Efflux of K<sup>+</sup> ions?
- Repolarization?
- Some other unspecified step??

Along came Bernard Katz (from the Hodgkin & Huxley voltage-clamp experiments c.1950's)  
He took advantage of specific agents that were available at that time:

- Tetrodotoxin (TTX) *from salivary gland of Puffer fish* – blocks only voltage-dependent Na<sup>+</sup> channels; only works from the outside of the cell
- Tetraethyl ammonium (TEA) – blocks only voltage-dependent K<sup>+</sup> channels; only works from the inside of the cell

Using these agents, Katz & Miledi demonstrated fundamental principles about neural action:

1. Na<sup>+</sup> & K<sup>+</sup> channels are independent molecular entities (block one w/o affecting other)
2. The membrane is asymmetrical (TTX only worked on outside, TEA only inside)
3. Agents can be very specific, i.e. selectively blocking *one* type of ion channel (an idea useful in designing drugs that act on a specific target)

**The Experiment:** (Figure 14-1) Using the Squid Giant Synapse, they...

Passed current in the pre-synaptic neuron → producing a *pre-synaptic* AP → recorded a synaptic potential\*, which generated an AP in the *post-synaptic* cell

PART 1- Transmitter release depends on Na<sup>+</sup>???Block Na<sup>+</sup> channels

As TTX began to perfuse the synapse, the pre-synaptic AP gradually decreased and the (post)synaptic potential got smaller and smaller until it was eventually blocked (Fig14-1B)

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\*The synaptic potential is not an action potential; it is the change in membrane potential of a post-synaptic cell in response to a chemical signal (See Table 2-1, PNS) The synaptic potential of Squid Giant Synapse was so large that it always triggered an AP.

Artificial Stimulation = Pre-synaptic Depolarization = Transmitter Release

If you depolarize the pre-synaptic cell a small amount, no transmitter release. But if you depolarize past 40mV (threshold), you begin to increase transmitter release logarithmically. Ten-fold change in the amount of transmitter released for every 10mV of depolarization. (Fig 14-1C)

A depolarizing current was injected into the pre-synaptic cell in the presence of TTX.  
More current  $\rightarrow$   $\uparrow$  pre-synaptic depolarization  $\rightarrow$  AP  $\rightarrow$  synaptic potential (Fig 14-2A,B)

***A synaptic potential was still produced with passive depolarization. This clearly showed that the influx of Na<sup>+</sup> was not needed to release transmitter.***

PART 2 – If not Na<sup>+</sup>, then it must be K<sup>+</sup>, right???

TEA injected into pre-synaptic axon, then the cell is depolarized. This is what they observed:

- This passive depolarization is maintained since the K<sup>+</sup> channels are blocked – K<sup>+</sup> can't get out so there is no repolarizing force
- This maintained depolarization always produced a synaptic potential which now lasted longer because transmitter release was also maintained

***This meant that the efflux of K<sup>+</sup> was also not responsible for transmitter release.***

SO WHAT ELSE COULD BE RESPONSIBLE FOR TRANSMITTER RELEASE?

***CALCIUM!!!***

Ca<sup>2+</sup> is suspected because: Ca<sup>2+</sup> channels are dense in the pre-synaptic terminal of the squid axon; it carries a (+) charge; it is the messenger for the release of transmitter as well as other substances in other tissues of the body.

How does Ca<sup>2+</sup> do this? At what point during depolarization does Ca<sup>2+</sup> have to enter the pre-synaptic terminal?

**Experiment #2**

Katz used a neuromuscular junction (NMJ) blocked with TTX and removed Ca<sup>2+</sup> from media. Then he depolarized the neuron, variably applying Ca<sup>2+</sup> to pre-synaptic cell. Results:

1. Depolarize w/o Ca<sup>2+</sup> applied  $\rightarrow$  no transmitter release
2. Depol. just after Ca<sup>2+</sup> applied  $\rightarrow$  transmitter release (recorded in post-synaptic cell)
3. Ca<sup>2+</sup> applied just after depol.  $\rightarrow$  no transmitter release
4. Mg<sup>2+</sup> (which blocks Ca<sup>2+</sup> channels), then Ca<sup>2+</sup>, then depol.  $\rightarrow$  no transmitter release

Ca<sup>2+</sup> has to be present on the outside of the membrane just before the depolarization because depolarization allows Ca<sup>2+</sup> channels to open allowing the ions to rush in and mediate a 2<sup>nd</sup>-messenger role leading to transmitter release. Therefore...

**The Solution:** Pre-synaptic AP  $\rightarrow$  Ca<sup>2+</sup> channels open  $\rightarrow$  inward Ca<sup>2+</sup> current  $\rightarrow$  activates transmitter release  $\rightarrow$  (post)synaptic potential  $\rightarrow$  generates an AP in post-synaptic cell  
(This is true for both excitatory and inhibitory neurotransmitter release)

***The signal for transmitter release is the opening of Ca<sup>2+</sup> channels and the movement of Ca<sup>2+</sup> into the pre-synaptic terminal.***

This model was further proven by Rodolfo Llinás with voltage-clamp experiments using the squid in the presence of TTX. He showed that there was a graded release of transmitter.  
More  $\text{Ca}^{2+}$  current = more transmitter release = larger synaptic potential

So how does  $\text{Ca}^{2+}$  produce its action?

For the answer we look again to Katz and his neuromuscular junction...He observed:

- Spontaneous fluctuations in membrane potential - miniature synaptic potentials (msp's) [OR Miniature end-plate potentials (mepps)]
- If they blocked Ach receptors with Curare → no more msp's
- If they inhibit Ach-esterase → msp's prolonged

This suggested that these msp's may result from *the* unit of transmitter release, the *quantum* of chemical synaptic transmission. So he wanted to know...

How many molecules of Ach are in one "quantum"?

Apply a small amount of Ach → produce synaptic potential smaller than the msp

- Only 2 molecules of Ach are required to open one ligand-gated channel, producing a synaptic potential 1/1000 of the quantal synaptic potential previously observed (the msp)
- Katz estimated that 2,000-5,000 molecules of Ach would be involved in one quantal event and that synaptic potentials are made up of these quantal units
- There is a mechanism that releases these quanta spontaneously that doesn't require an AP

What role does  $\text{Ca}^{2+}$  play in this process?

The size of the spontaneous fluctuations were integral multiples of the quantal unit (Fig 14-6)

$\text{Ca}^{2+}$  doesn't act to increase the amount of Ach in a quantum, but synchronizes release by increasing the probability.

These quanta of neurotransmitter are packaged in synaptic vesicles in the pre-synaptic terminal

Quanta are released spontaneously at a very slow rate. The AP allows...

$\text{Ca}^{2+}$  influx → binding of vesicles to active zones → fusion of the vesicle membrane with the pre-synaptic membrane → exocytosis → release of vesicular contents

## II. The Molecular Details of Synaptic Vesicles

How are vesicles mobilized, restrained, targeted to the active zone, and exocytosed?  
(Figs 14-13 – 14-15)

Mobilization molecules:

Synapsin – binds to the cytoskeleton and the vesicle, keeping the vesicles away from active zone  
 $\text{Ca}^{2+}$  activates  $\text{Ca}^{2+}$ -dependent kinase → phosphorylation of synapsin → frees vesicles from cytoskeleton

Trafficking:

Rab3 – a GTP-binding protein that binds to vesicles and guides them to the active zones

Docking & Fusion:

SNARES – v-SNARES (on vesicular membrane): synaptobrevin (a.k.a. VAMP); t-SNARES (on cell membrane): syntaxin and SNAP-25

Binding of these SNAREs takes place in the presence of  $Ca^{2+}$  → fusion of membranes → exocytosis

NSF & SNAP then attach to the v-SNARE/t-SNARE complex, unwinding the SNAREs and allowing the vesicles to be recycled

**III. Chemical Synaptic Transmission is Modifiable by Experience (PLASTICITY)**

Two general processes: Intrinsic and Extrinsic

**A. Intrinsic signals:**

## 1. Changes in membrane potential

There is a small leak of  $Ca^{2+}$  in the pre-synaptic terminal of many neurons

- can be turned off if you hyperpolarize the cell
- can be enhanced if you depolarize the cell

These changes affect the amount of transmitter released (Fig 14-16)

- the hyperpolarized cell would produce a smaller synaptic potential
- the depolarized cell would produce a synaptic potential large enough to generate an AP (post-synaptic)

***Membrane potential controls  $Ca^{2+}$  channels and therefore  $Ca^{2+}$  influx and transmitter release***

## 2. Changes in activity in the pre-synaptic neuron (Fig 14-17)

- If you speed up the rate of firing of the AP (creating a tetanic stimulation) → ↑ in  $Ca^{2+}$  influx
- This cannot be readily handled by the buffering capability of the pre-synaptic terminal, so the mitochondria and ER take up this excess  $Ca^{2+}$
- If you give the cell a high enough stimulus, the mitochondria become saturated with  $Ca^{2+}$  and begin to release some back into the cytoplasm when the tetanus is over. There will be residual  $Ca^{2+}$  in the pre-synaptic terminal.
- This residual  $Ca^{2+}$  will add to the  $Ca^{2+}$  entering the cell. Subsequent APs produced at the regular rate will yield enhanced post-synaptic potentials (POST-TETANIC POTENTIATION!)

***This is the simplest case of memory storage!*** The neuron remembers that a train of APs has been generated, then it plays out this memory for a period of time.

**B. Extrinsic Signals**

In addition to ion channels, the pre-synaptic terminals have receptors for transmitters from other sources and, in some cases, auto-receptors for their own transmitter (creating a feedback loop)

## 1. Pre-synaptic modulation of transmitter release (Fig 14-18)

- a. Inhibition – an inhibitory neuron acts on the pre-synaptic terminal → ↓ in  $Ca^{2+}$  → ↓ transmitter release

- b. Facilitation – a modulatory transmitter acts on the pre-synaptic terminal → turns off  $K^+$  channels → prolongation of AP → prolonged  $Ca^{2+}$  current → ↑ transmitter release

Why do we need pre-synaptic modulation if we already have post-synaptic excitation/inhibition?

Inhibitory input on a *post*-synaptic cell will affect all excitatory input that comes in.

However, with *pre*-synaptic inhibition (or facilitation), you can change the input of one cell without affecting the input of others.

A post-synaptic input will affect the whole neuron, but the pre-synaptic input will selectively shut off one set of terminals and the other set of terminals will not be affected