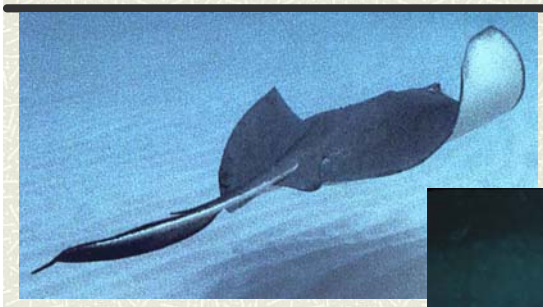
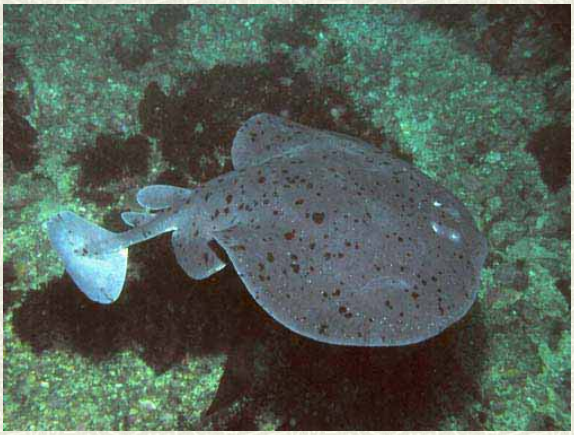
ZOO 332H1S - Lecture 5
(AJE 2003)



FAST SYNAPSES

Torpedo californica

Torpedo Ray (*Torpedo californica*)



Torpedo Ray - Torpedo rays (*Torpedo californica*) are identifiable by their flat grey bodies and black spots. Interestingly, these animals catch their prey by stunning them with a jolt of electricity! (photo: Daniel Gotshall)

Today

- Electrical synapses - fastest
- Chemical synapses secrete neurotransmitters that modulate post-synaptic ion channels
- Ion channels are related molecularly, but come in many flavours
- Post-synaptic response depends on nature of ion-channel, not transmitter
- Contribution of synapse in determining post-synaptic response depends on position

Electrical and chemical synaptic transmission

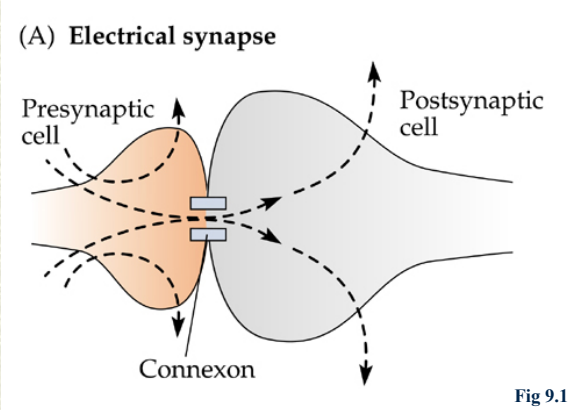


Fig 9.1

cont…Electrical and chemical synaptic transmission

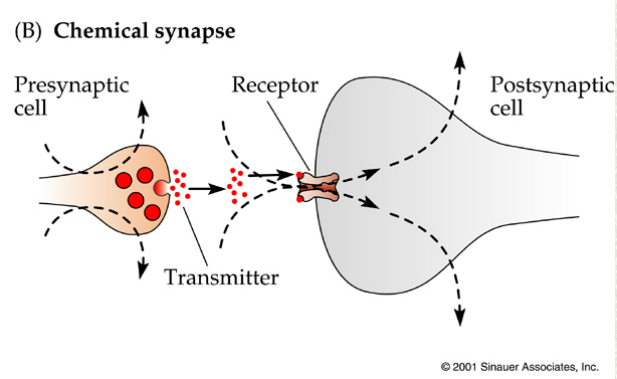
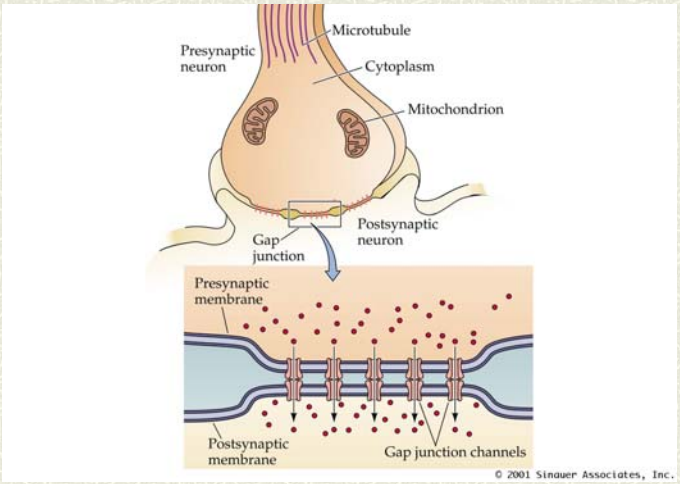


Fig 9.1

cont…Electrical and chemical synapses

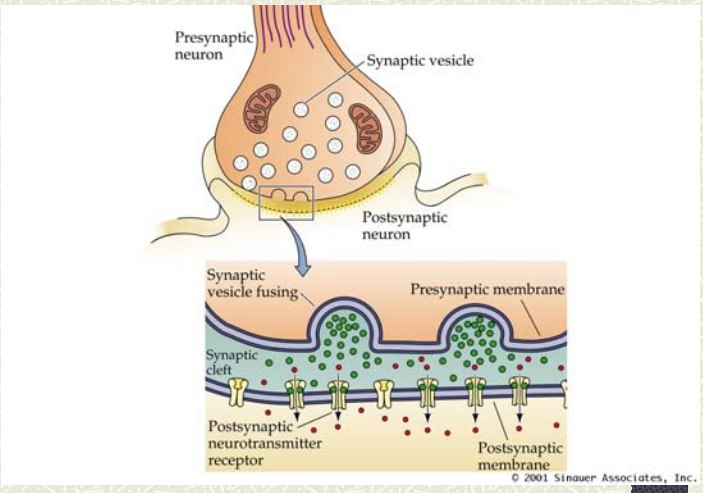
Electrical



Purves *et al.* (2001) - Fig.5-1

cont….Electrical and chemical synapses

Chemical



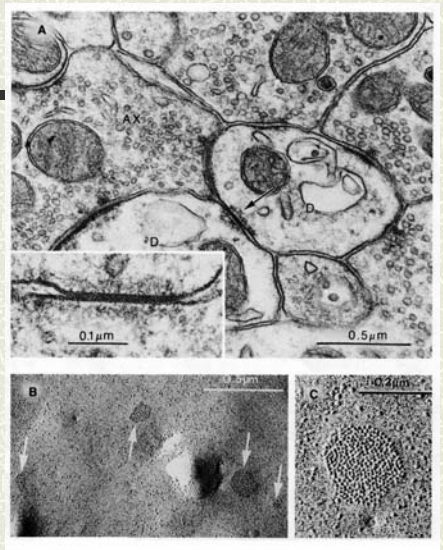
Purves *et al*. (2001) - Fig.5-1

EM of electrical (and chemical) synapses

A - two dendrites in inferior olivary nucleus of the cat

B - freeze-fracture through the presynaptic membrane nerve terminal in ciliary ganglion of a chicken

C - high mag of B (cluster of closely packed particles about 9nm in diameter)

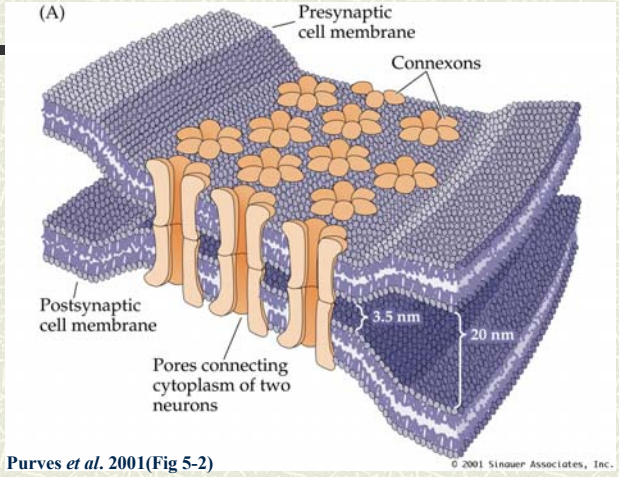


Kuffler, Nicholls, & Martin (1984)

Electrical synapses

Connexons -

- hexameric structure of six conne**xin** proteins (on each side, pre- and post-synaptic)
- Also known as "gap junctions"
- rapid transmission (<0.1ms)



Purves *et al.* 2001(Fig 5-2)

- 1st in crayfish; mammalian example: hormone secreting neurons in hypothalamus (synchronization of secretion into circulation)

cont...Electrical synapses

Modulation:

- low pH
- intracellular calcium (Ca^{2+})
- voltage
- 2nd messengers

Pore Size:

- about 1.5nm diameter when open (3.5nm between pre- and post-synaptic cells) – Lucifer Yellow

Connexin proteins in the heart

(don't try to read this here - it will be a handout)

RESEARCH NEWS

DEVELOPMENTAL BIOLOGY

Connexin Knockout Provides a Link to Heart Defects

If anything proves the adage "You don't know what you've got 'til it's gone," it's the knockout mouse. Such mice, created by using gene-engineering techniques to inactivate, or "knock out," specific genes, have become a boom industry for researchers who want to pinpoint the role of genes whose functions in the animal are unknown. The most recent case in point: A Canadian-US research team has produced the first knockout of a gene encoding a connexin, a member of a family of proteins that form the cell communication links known as "gap junctions" (see p. 1331).

The result turned out to be both more—and less—than the researchers expected. On the positive side, it may provide a new model for understanding congenital heart defects, such as pulmonary stenosis. Every year in the United States about 32,000 children are born with heart defects, and this is one of the most common. On the other hand, researchers were surprised to find that knocking out this particular connexin, known as connexin43 (Cx43), had less catastrophic effects on fetal mice than they expected—which may lead researchers to re-evaluate some of their ideas about the role played by connexins in early embryonic development.

The fact that the disruption was fairly mild surprised even the Canadian team itself, which was led by developmental biologists Janet Rossant of Mount Sinai Hospital in Toronto and Gerald Fishler of the University of Western Ontario in London. In fact says Rossant, the team chose to knock out the Cx43 gene because that protein is expressed in mouse embryos with as few as eight cells, and researchers assumed a protein expressed so early in the embryo's history must be crucial for normal development.

"We wanted to knock out the main gap junction in these animals to study signaling in early embryos," Rossant explains. Gap junctions, water-filled channels that connect the cytoplasmic compartments of two coupled cells, are thought to be important conduits for the transmission of many kinds of cell-to-cell signals, including those that tell cells what developmental paths to follow.

But the embryos lacking the Cx43 gene didn't die immediately, as the researchers thought they might. "Since this protein is expressed so early in development, one would have thought a priori that [it] would be necessary for survival. But they found out that the organisms did not only survive, but made it to birth," says Norton Gilula of the Scripps Research Institute in La Jolla, California, whose group, along with Kidder's, showed that the Cx43 gene is expressed in early embryos.

The knockouts were not normal, however. Shortly after birth, the animals died with labored breathing and blue coloration, suggesting they were unable to get oxygen from their lungs into the bloodstream.

Bridging the gap. Connexin43 (bright stain) can be seen in this sagittal mouse embryo.

Autopsy of the affected mouse pups showed why. The right ventricle, the chamber of the heart that pumps blood through the pulmonary artery to the lungs, was abnormal. The region where the pulmonary artery exits the heart was filled with overgrown tissue that obstructed blood flow, preventing blood from reaching the lungs. Such a defect "would pose no problem," Rossant says, since both because in utero the animals could get oxygen from the mother's placenta, but afterward it would be lethal.

The heart defects shown by the knockout mice resemble congenital pulmonary stenosis in humans, a condition in which anatomical blockage in the heart's right ventricle prevents normal blood flow to the lungs. Further evidence that Cx43 plays a role in heart development comes from Scott Britz Cunningham, William Fletcher, and their colleagues at the Jerry L. Pettis Memorial Veterans Administration Medical Center in Loma Linda, California, who have recently linked mutations in the Cx43 gene to another congenital condition, called visceroatrial heterotaxia. Although this condition causes a spectrum of abnormalities, some very mild, it can also cause serious, even lethal, heart defects, such as the reduction of the normal four chambers to two.

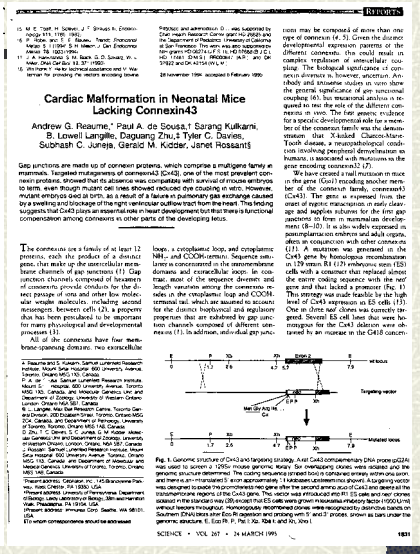
What does this overall picture—mice that survive to term, with a full set of organs, including a beating heart, cells to contract in it, both to a severe heart problem—tell researchers about embryonic development? One thing is that these studies are not the first to find that a connexin defect can cause a relatively specific developmental abnormality, rather than being fatal for the early embryo. About 15 months ago, two teams, one led by Jo Ann Bargiello of the University of Pennsylvania School of Medicine in Philadelphia and the other by N. Farwerden of the University of Aberdeen Medical School in the United Kingdom, found that one form of a hereditary condition called Charcot-Marie-Tooth (CMTX) disease has been linked to mutations in the gene for another connexin, connexin32 (*Science*, 24 December 1993, p. 2059). In this case, the defect appears to be limited to the cells that form the linings around many peripheral nerves, which degenerate in late childhood.

Exactly why the effects of the connexin knockouts and mutations are limited remains unclear. That is particularly puzzling, says Dan Goodenough of Harvard Medical School, whose lab cloned the Cx43 gene in 1985, because several groups, including his own and that of Gilula, have found that in normal animals, Cx43 is expressed throughout development in many types of tissue. Cx32 is also expressed in several kinds of tissue. Paul de Sousa, another member of the Canadian team, notes, however, that since gap junctions are made up of two connexins, and one or another of them might have been able to substitute for Cx43 in some tissue types, although apparently not in the right ventricle of the heart.

The next step, Rossant suggests, is to examine other connexins to see if their expression goes up during development to compensate for the missing Cx43. And the connexin studies won't be limited just to mice. Gap junction researchers will want to check for missing connexins in patients with congenital heart abnormalities to confirm that they have a role in these conditions as well as in CMTX. Meanwhile, at least two additional knockout knockouts are on the way, as they were announced at a meeting held in Île des Embiez, France, in early March. Researchers expect that these will further clarify connexin activities. "The knockout animal is a very important experimental animal, because it gives you the ability to study each connexin to see what the cells [lacking it] can't do," Goodenough says. Indeed, these new tools are sure to win a warm acceptance—especially if the tantalizing links to human disease hold up.

—Amy Stone

Amy Stone is a free-lance writer based in Atlanta.



REPORTS

Cardiac Malformation in Neonatal Mice Lacking Connexin43

Andrew G. Reaume,* Paul A. de Sousa,† Sarang Kulkarni, B. Lowell Langille, Daguang Zhu,‡ Tyler C. Davies, Subhash C. Juneja, Gerald M. Kidder, Janet Rossant§

Gap junctions are made up of connexin proteins, which comprise a multigene family in mammals. Targeted mutagenesis of connexin43 (Cx43), one of the most prevalent connexin proteins, showed that its absence was compatible with survival of mouse embryos to term, even though mutant cell lines showed reduced dye coupling in vitro. However, mutant embryos died at birth, as a result of a failure in pulmonary gas exchange caused by a swelling and blockage of the right ventricular outflow tract from the heart. This finding suggests that Cx43 plays an essential role in heart development but that there is functional compensation among connexins in other parts of the developing fetus.

The connexins are a family of at least 12 proteins, each the product of a distinct gene, that make up the intercellular membrane channels of gap junctions (1). Gap junction channels, composed of hexamers of connexins, provide conduits for the direct passage of ions and other low molecular weight molecules, including second messengers, between cells (2), a property that has been postulated to be important for many physiological and developmental processes (3).

All of the connexins have four membrane-spanning domains, two extracellular loops, a cytoplasmic loop, and cytoplasmic NH_2- and COOH-termini. Sequence similarity is concentrated in the membrane-bome domains and extracellular loops. In contrast, most of the sequence diversity and length variation among the connexins resides in the cytoplasmic loop and COOH-terminal tail, which are assumed to account for the discrete biophysical and regulatory properties that are exhibited by gap junction channels composed of different connexins (1). In addition, individual gap junctions may be composed of more than one type of connexin (4, 5). Given the distinct developmental expression patterns of the different connexins, this could result in complex regulation of intercellular coupling. The biological significance of connexin diversity is, however, uncertain. Antibody and knockout studies in vitro show the general significance of gap junctional coupling (6), but mutational analysis is required to test the role of the different connexins in vivo. The first genetic evidence for a specific developmental role for a member of the connexin family was the demonstration that X-linked Charcot-Marie-Tooth disease, a neuropathological condition involving peripheral demyelination in humans, is associated with mutations in the gene encoding connexin32 (7).

We have created a null mutation in mice in the gene (Gja1) encoding another member of the connexin family, connexin43 (Cx43). The gene is expressed from the onset of zygotic transcription in early cleavage and supplies subunits for the first gap junctions to form in mammalian development (8–10). It is also widely expressed in postimplantation embryos and adult organs, often in conjunction with other connexins (11). A mutation was generated in the Cx43 gene by homologous recombination in 129 strain R1 (12) embryonic stem (ES) cells with a construct that replaced almost the entire coding sequence with the *neo* gene and that lacked a promoter (Fig. 1). This strategy was made feasible by the high level of Cx43 expression in ES cells (13). One in three *neo*^{r} clones was correctly targeted. Several ES cell lines that were homozygous for the Cx43 deletion were obtained by an increase in the G418 concen-

Fig. 1. Genomic structure of Cx43 and targeting strategy. A rat Cx43 complementary DNA probe (pCZA) was used to screen a 129/Sv mouse genomic library. Six overlapping clones were isolated and the genomic structure determined. The coding sequence (shaded box) is contained entirely within one exon and there is an untranslated 5′ exon approximately 1.4 kilobases upstream (open box). A targeting vector was designed to delete the proximal-coding sequence after the second amino acid of Cx43 and downstream sequences, including the 3′ untranslated region. Homologous recombination in ES cells and transmission of the targeted allele (58) ensured that ES cells carrying a single mutated allele (1/200) and without foreign integrated, homologous recombined clones were recognized by Southern blot analysis (DNA) digested with Eco RI (probe A) and Hind III (probe B). Shown as bars under the genomic structure: E, Eco RI; P, Pst I; Xb, Xba I; and Xh, Xho I.

Crayfish Giant Axon (GA) to abdominal motor axon

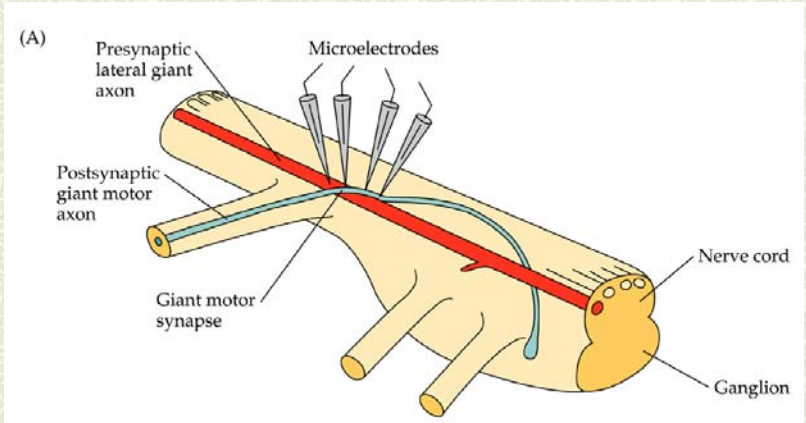
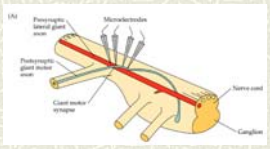


Fig 9.2

cont...Crayfish GA to abdominal motor axon



- the preparation
- intracellular recording presynaptically (GA) and postsynaptically (motor giant)
- rectification (unusual in electrical synapse)

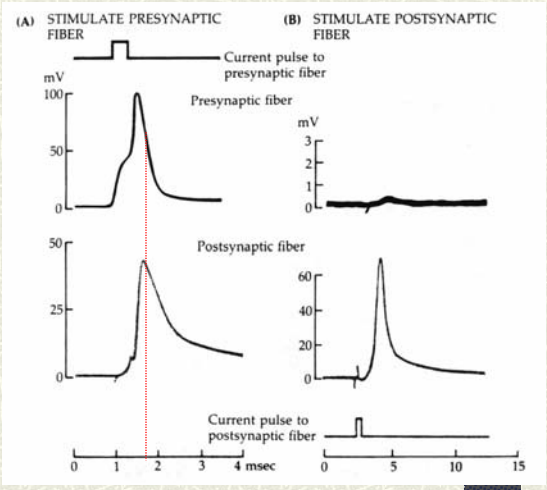
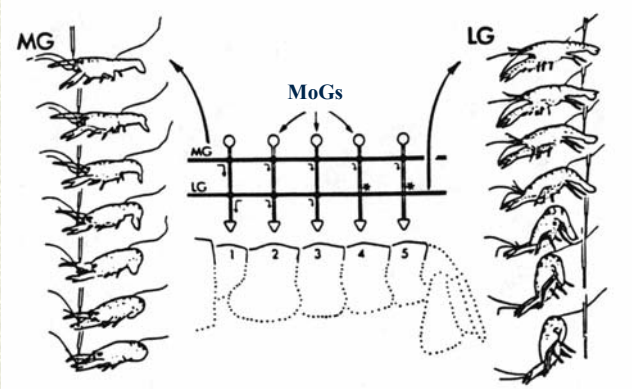


Fig 9.2

Electrical synapses - crayfish giant axon (GA) to abdominal motor axon (MoG)

- the animal
- the giant axons
- excitation
- the behavioural response



From: Krasne & Wine (1984) in Neural Mechanisms of Startle Behavior, RC Eaton (Ed.)

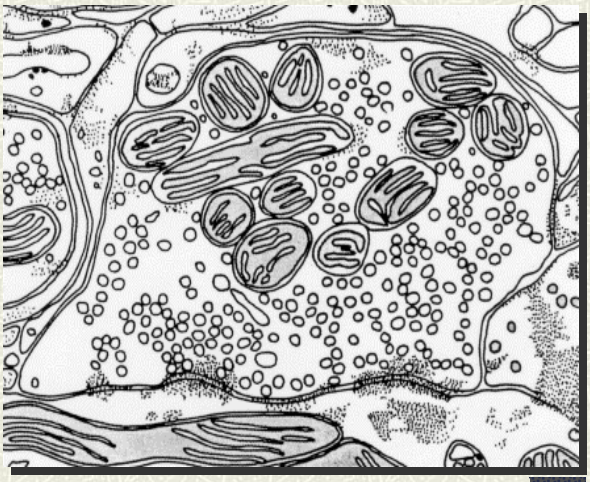
Figure legend from reference for previous slide

Figure 1. Forms of giant-mediated tailflips. When the MGs fire, all segments flex and the abdomen curls and propels the animal backward. When LGs fire, caudal segments remain straight and cause the thrust to be directed mainly down, thus pitching the animal forward. Since MGs respond to rostral inputs and LGs to caudal ones, tailflips always remove the animal from the source of stimulus. Consistent with the difference in form of MG and LG flips, the MGs excite MoGs in every abdominal segment, whereas the LGs excite MoGs only in more rostral segments (circuit of center top) (based on Wine and Krasne, 1972; Mittenthal and Wine, 1973; and taken from Wine and Krasne, 1982).

From: Krasne & Wine (1984) in Neural Mechanisms of Startle Behavior, RC Eaton (Ed.)

Chemical Synapses

Hallmarks:
- vesicles
- diversity in ligands that activate
- specific structures common to chem sy
- diversity in morph-ology
- *etc.*



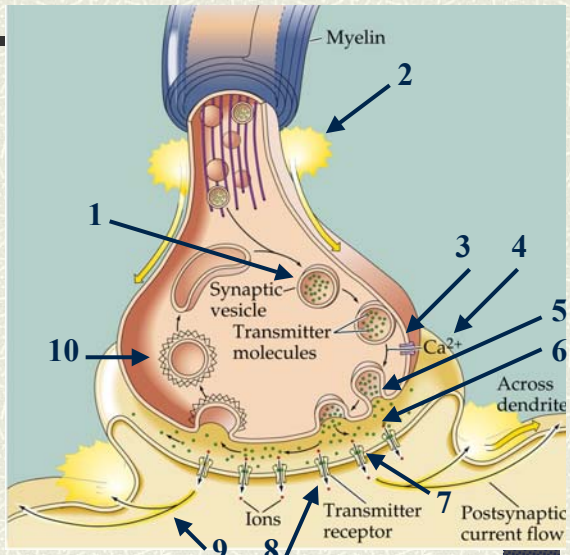
Pre- synaptic events

- AP in presynaptic neuron
- Depolarization opens Ca^{2+} channels
- Increase in $[Ca^{2+}]$ locally
- Increase probability of vesicle fusion
- Increased rate of NT release
- Increased [NT] in synaptic gap

Post-synaptic events

- NT molecules bind to postsynaptic receptors
- Increased probability of open state of channel
- Increased g_i
- Production of synaptic current, PSP
- NT removed

Sequence of events involved in transmission at a typical chemical synapse



Purves *et al*. 2001(Fig 5-3)

Structure of chemical synapse

- "Motor Unit"
- factors which alter number of muscle fibres innervated by a single neuron

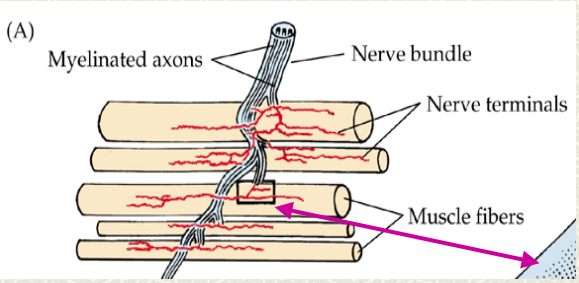


Fig 9.4

cont…Structure of chemical synapse

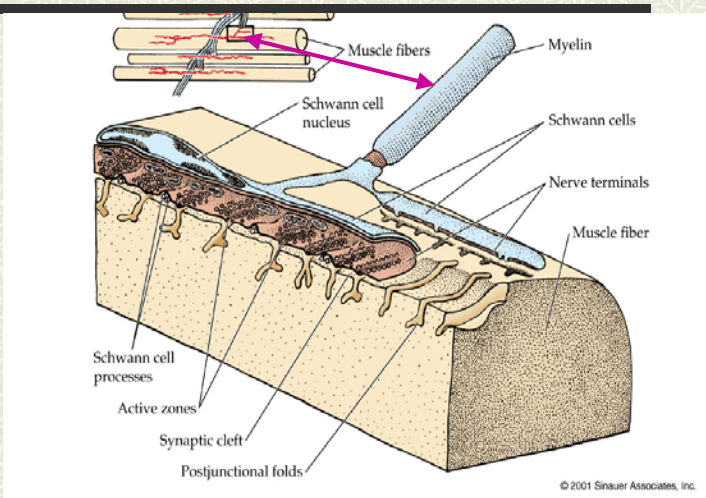


Fig 9.4

Synaptic potentials

nAChR

Safety factor

Effect of curare

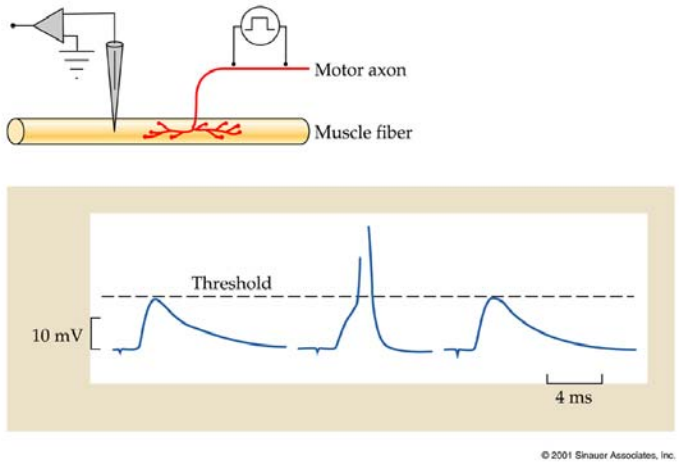


Fig 9.5

Passive spread of synaptic potentials

- Recall passive properties of axon
- Not a good conductor
- Requires regenerative response for depolarization at distance from synapse

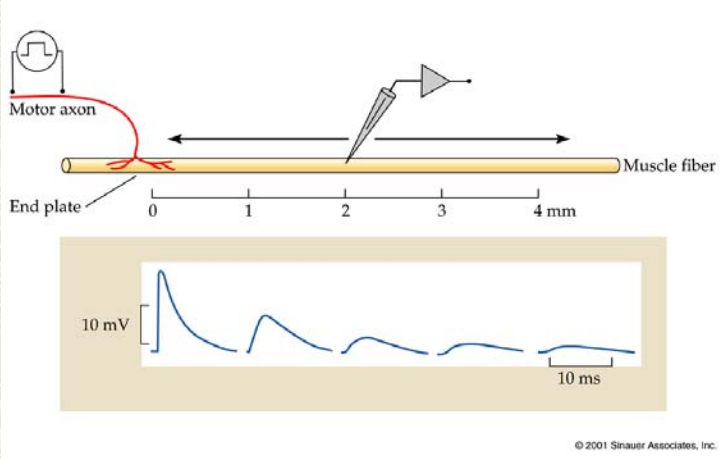


Fig 9.6

Ionophoresis of ACh on muscle fibre

- Region of greatest sensitivity
- Mimic natural event by application of exogenous cmpd
- criteria to establish substance as a NT

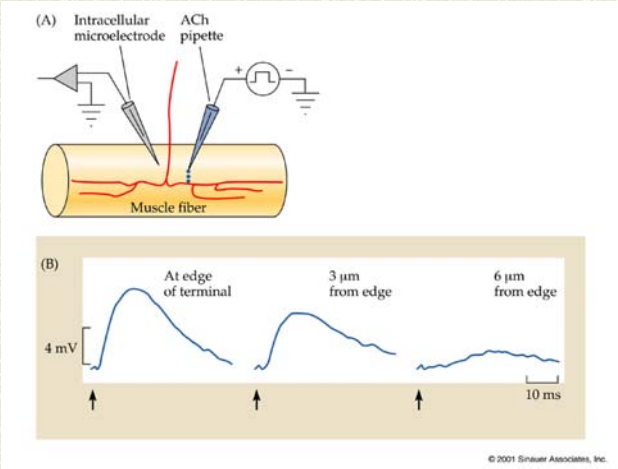
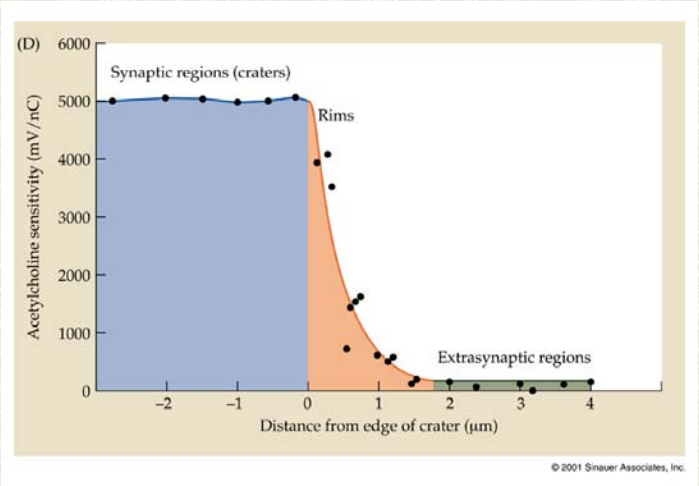
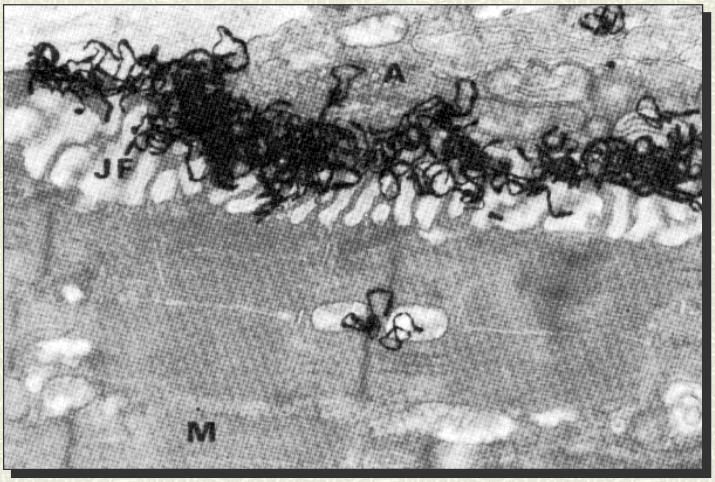


Fig 9.7

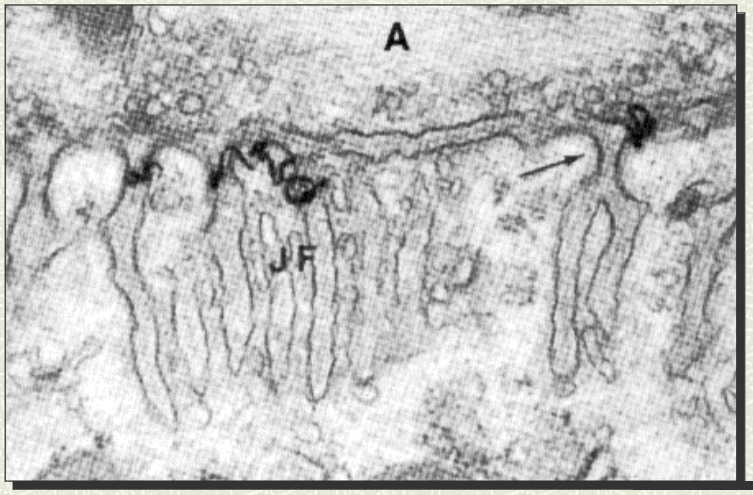
Location of ACh receptors



Location of ACh receptors revealed by labelled α-bungarotoxin

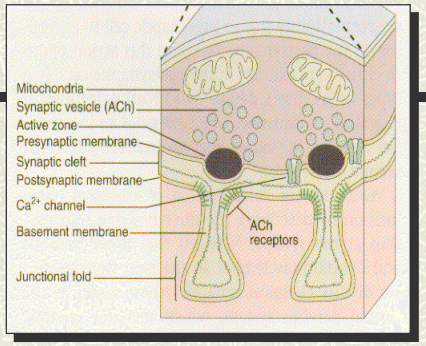


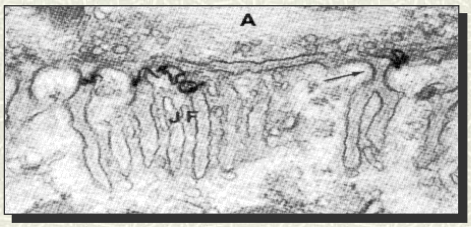
ACh receptors at peak of folds close to presynaptic membrane



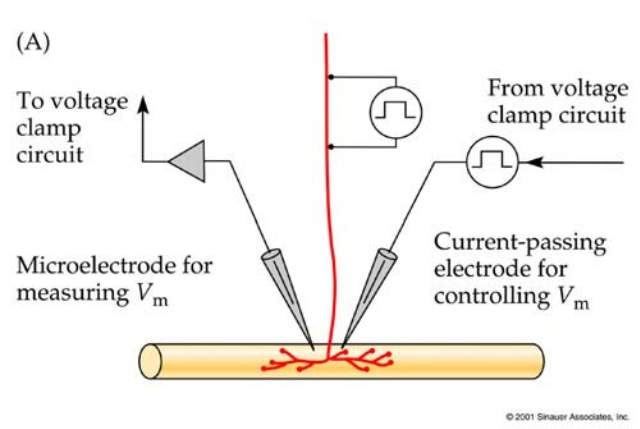
Junctional folds at the end plate

- High conc. of Na^+ channels
- (also Ca^{2+} channels?)
- conducting path to T-tubule system/SR

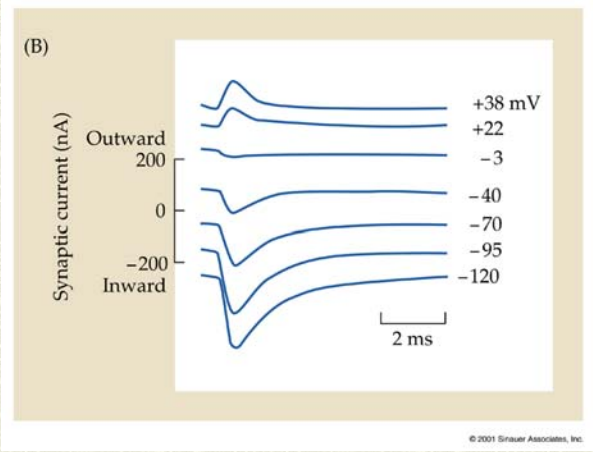




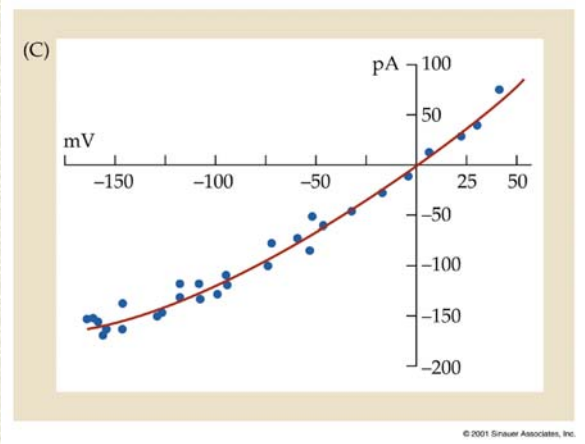
Determining the reversal potential for synaptic response



cont…Determining the reversal potential for synaptic response

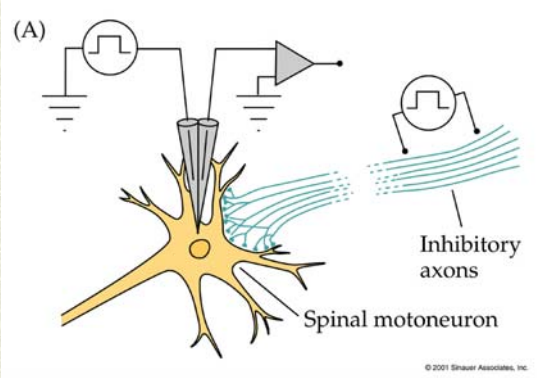


cont…Determining the reversal potential for synaptic response



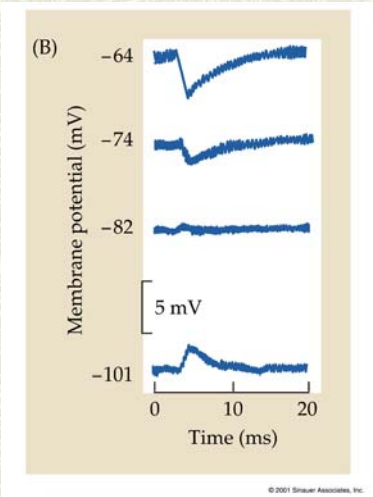
Recording inhibitory synaptic potentials

- Current injection for "current clamp"
- Record membrane potential post synaptic

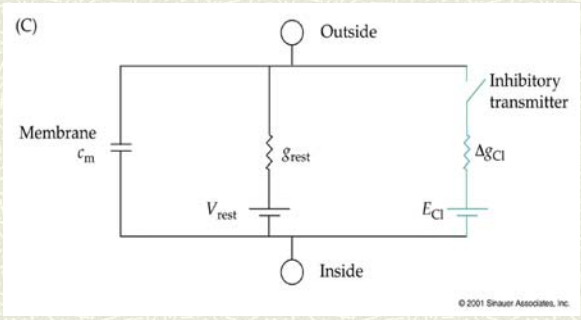


Inhibitory synaptic potentials

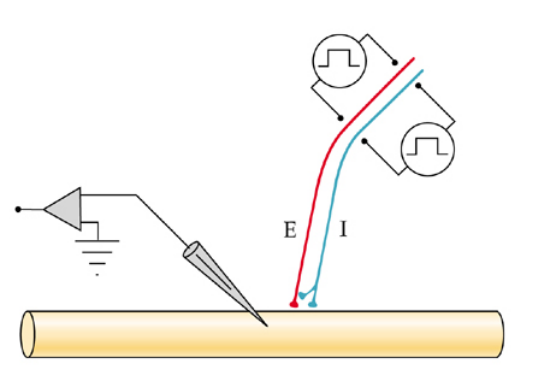
- Set membrane potential by injecting current
- Stimulate presynaptic
- Record response in motor neuron cell body
- Reversal potential



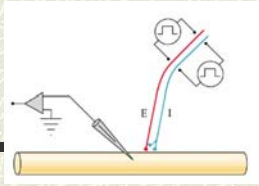
Electrical model

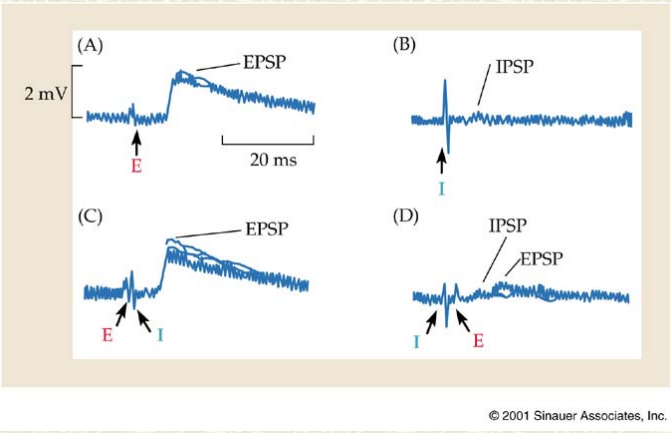


Crayfish NMJ – presynaptic inhibition

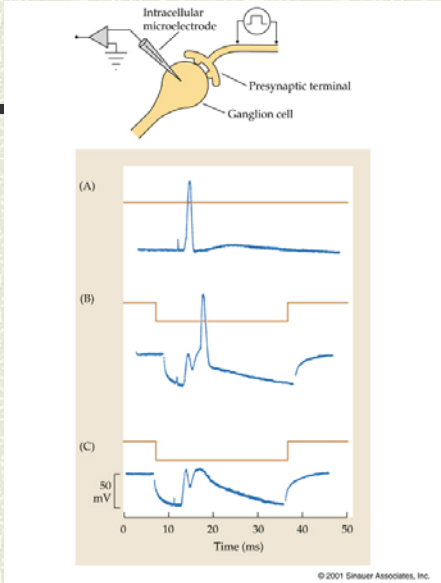


Timing of inputs determines efficacy





Discussion Figure



Next…..more on synapses/receptors