Formation of the NMJ

Chapter Eight
Receptor Clustering in the CNS

What factors regulate receptor clustering in the CNS?

Is it Agrin like motor neurons?

Gephryrin is involved in the clustering of GABA receptors

Gephryrin localized with GABA receptor clusters.

Inhibition of gephyrin translation with antisense oligonucleotide, prevent GABA receptor clustering
CNS Receptor Clustering

Other signaling molecules:

- Narp – concentrated at excitatory synapses
  - Mutant Narp → reduced clustering of GluR
- Ephrin B/EphB
  - Knockout leads to no clustering of AMPAR
- Neurotrophin-Trk signaling
  - Exposed to BDNF → clustering doubles
- Neurotransmitter itself induces clusters
EphrinB needed for AMPAR clustering

(Adapted from Dalva et al., 2000; Herkemeyer et al., 2003)
Receptor Clustering

Summary:

• Several candidate signals are involved in receptor clustering:
  – Agrin, EphrinB, neurotransmitter itself

• Released by both excitatory and inhibitory nerve terminals

• Depends on the:
  – Target tissue
  – Neurotransmitter receptor type
Internal Membrane Proteins

Tons of membrane proteins hold the receptors together internally:

• Gephyrin
  – Binds tubulin

• PSD-95 and 93
  – Receptors will be internalized without these

• GABA-RAP
  – Specific for GABA Receptors
Block Gephyrin – stop clustering

Gephyrin brings Tubulin together
Forming a scaffolding
Scaffolding holds receptors together
Regulation of Receptor Expression and Synthesis

- Synapse formation not only result in significant rearrangement of neurotransmitter receptors (clustering) but also induce significant changes in receptor expression.

- In the NMJ, receptor clustering at the synapse site involve insertion of new receptors at the contact site.
Regulation of Receptor Expression and Synthesis

- In the NMJ, insertion of new nAChRs occurs within hours of innervation.
- Labeling of old with rhodamine-conjugated α-Btx.
- New receptors with fluorescein-conjugated anti-nAChR.
- Majority of receptors at the NMJ are newly inserted receptors (fluorescein-labeled).

(Applied from Rule et al., 1985)
Regulation of Receptor Expression and Synthesis

- Innervation also regulates the subunit composition of newly inserted receptors.
- Extrasynaptic AChRs consist of 5 different subunits: $2\alpha, \beta, \gamma, \delta$.
- Following synaptic contact, the $\gamma$ subunit is substituted by the $\varepsilon$ subunit.
- The newly inserted receptors consist of $2\alpha, \beta, \gamma, \varepsilon$. 
Regulation of Receptor Expression and Synthesis

The extrasynaptic (immature) and synaptic (mature) nAChR have different electrical properties.

- nAChRs containing $2\alpha,\beta,\gamma,\delta$ subunits have longer open periods (<10 ms vs. 1 ms) and smaller conductance (30 pS vs. 50 pS) than nAChRs containing $2\alpha,\beta,\gamma,\epsilon$ subunits.
Changing subunits over time

(Adapted from Gu and Hall, 1988 and Martinou et al., 1991)
Does presynaptic terminal provide a signal to increase receptor synthesis following synapse formation?

Inhibition of presynaptic action potentials or elimination of cholinergic transmission increases nAChR expression.

It appears that in muscle cells electrical activity regulates receptor synthesis by regulating calcium ions and PKC.
Inhibiting transmission **induces** receptor expression

Seems that neurotransmitter activity works to keep receptors at the synapse. Rather than all over the entire muscle tissue (B)
ARIA is a Transynaptic Regulator of Transcription

- If electrical activity decreases synthesis of nAChRs how does synaptic contact stimulate receptor synthesis at the motor nerve terminal?

- ARIA, or ACh Receptor Inducing Activity, is a 42 kD protein isolated from the chick CNS that increases synthesis of the ε subunit of the nAChR

- ARIA regulates ε subunit transcription
ARIA is a Transynaptic Regulator of Transcription

- ARIA heterozygotes mutant mice express less ARIA protein and a significant reduction in nAChR clustering. Notice that ARIA mutation results in less synaptic activity at the NMJ.

- ARIA mRNA is localized in the muscle fibers to the synapse.

- ARIA protein is released into the NMJ where it concentrates in the presynaptic site of the basal lamina.
ARIA is a Transsynaptic Regulator of Transcription

Application of an ARIA containing medium to cultured myotubes induces a significant increase in the muscle response to ACh

Corfas and Fischbach, 1993
ARIA is a Transsynaptic Regulator of Transcription

ARIA belongs to the neuregulin family of trophic factors. This family of trophic factors activate the EGF receptor tyrosine kinase ErbB.
Synaptic Transmission

- Synaptic contact between the axon terminal and the target induces rapid changes in synaptic transmission.

- Notice that in the Xenopus NMJ contact between the axon growth and a myoball increases synaptic transmission 5 min after contact.
Maturation of Synaptic Transmission

- Although synaptic connections can induce significant changes in synaptic transmission, synaptic properties are adjusted gradually during development.

- The duration of synaptic potentials decreases during development. In the lateral superior olive (LSO) there is a significant reduction in EPSP duration.
Maturation of Synaptic Transmission

Different subunits are used in the immature receptor vs. the adult receptor.

This sort of switch has been seen for every neurotransmitter.
Maturation of Synaptic Transmission

- Changes in the biophysical properties of NMDA glutamate receptors during development

- In rat hippocampal neurons, there is a significant reduction in the duration and decay time of NMDA PSP. This will have important consequences for the temporal and spatial integration of synaptic inputs
Synaptic Flexibility

• Fully mature synapses have flexible responses to neurotransmission
• “Short term plasticity”
• Facilitation
  – 2nd action potential is larger than 1st
• Depression
  – 2nd action potential is smaller than 1st
• Purpose is to respond appropriately to trains of signal – repeated firings
Plasticity changes with maturation

Depression

Facilitation

(From Reyes and Sakmann, 1999; Jossli et al., 2002)
Reasons for Changes in Plasticity

1. Action potentials last longer in immature synapses
   - Are open longer

2. Pools of available vesicles increases with development
   - More vesicles are available

3. Post-synaptic receptors are increasing and clustering over development
   - Quicker, more accurate response
Expression of Synaptic Inhibition

Expression of inhibitory transmission is important for development of neuronal circuits (Not only expression of excitatory neurotransmission)

Inhibitory synapses are synapses that use the neurotransmitters GABA or glycine to **down regulate** excitatory stimulation of the nervous system
Expression of Synaptic Inhibition

During brain development there are dramatic changes in the expression and distribution of GABA receptors.

- **Alpha 1 receptor type**
  - Left and purple

- **Alpha 5 receptor type**
  - Right and red
In the adult nervous system, **inhibitory synapses hyperpolarize** the membrane potential because they allow **influx of Cl\(^-\) ion** into the cell. In this case Cl\(^-\) influx is determined by the high Cl\(^-\) concentration in the extracellular space.

During early embryonic development stimulation of **inhibitory synapses induces depolarization**.
During early embryonic development, stimulation of inhibitory synapses causes membrane depolarization and calcium influx.
Experiment – 8.33

• Ca++ entry is measured
  – Because Ca++ will enter when membrane has been depolarized

• Ap5/CNQX – antagonist against glutamate
  – Glutamate is excitatory – should hyperpolarize

• BIC – antagonist against GABA
  – GABA is inhibitory – should depolarize

• Works in adult neurons, opposite effect in immature neurons
Expression of Synaptic Inhibition

At early stages of development stimulation of inhibitory synapses cause depolarization because there is a significant decrease in the Cl⁻ concentration gradient (more Cl⁻ is accumulated in the intracellular space). Under this conditions stimulation of inhibitory synapses cause Cl⁻ efflux from the cell (depolarization).

(Adepted from Ban-Ari et al., 1985; Colrain and van den Pol, 1999; Raikova et al., 2003; Saini et al., 2006)
Expression of Synaptic Inhibition

- At early stages of development stimulation of inhibitory synapses cause depolarization because there is a significant decrease in the Cl\(^{-}\) concentration gradient (more Cl\(^{-}\) is accumulated in the intracellular space). Under this conditions stimulation of inhibitory synapses cause Cl\(^{-}\) efflux from the cell.
Summary

• During development inhibitory signals aren’t really inhibiting action potentials
• In fact they are exciting them!
  – Exact opposite of expected
• Reason is because intracellular chloride ions are elevated in immature neurons/synapses
• As the concentration of Cl- changes the neurons mature – respond correctly
Electrical Differentiation During Development

- Neurons communicate with each other via the generation of electrical signals called action potentials.

- Action potential generation depends on **passive** (resting membrane potential, input resistance, membrane time constant) and **active** (ion channel expression) properties of the membrane.
During development there is a significant shift of the resting membrane potential toward more hyperpolarized potentials. This is partly due to the expression of energy-dependent ionic transporters and better regulation of extracellular K⁺ concentration.

What is the functional role of a more hyperpolarized resting membrane potential?
During development there is also a significant reduction in input resistance and membrane time constant. Input resistance decreases because more channels are added per unit of membrane. These parameters will determine how much the membrane voltage will change and how rapidly for a given current pulse.
Electrical Differentiation During Development

- Changes in the number and nature of ion channels in the plasma membrane regulate the action potential.

- In *Xenopus* Rohon-Bear neurons, the earlier, calcium dependent, long lasting action potential is substituted by a short duration, sodium-dependent action potential.

- These changes are mainly due to an increase in sodium and potassium ion channels gene expression.
Neurons express a multitude of ion channels. Expression of a particular set of ion channels will determine the shape of the action potential and repetitive firing.

During maturation of rat Purkinje neurons there is a significant change in the expression of large-conductance, calcium dependent potassium channels.
Electrical Differentiation During Development

These changes in the action potential properties are mainly due to an increase in sodium and potassium channel density.

These changes can be reproduced in vitro suggesting that increased channel expression at this early stage of development is independent of cell-cell interactions.
Regulation of ion channel expression is an autonomous process at early stages of development. However, as neurons make connections, cell-cell interaction become more important in regulating ion channel expression.
Regulation of Ion Expression by Cell-Cell Interactions

- In chick ciliary ganglion (CG) cell, expression of large conductance, calcium-dependent potassium channels depends on interaction of CG neurons with target tissue in the eye (Iris).

- Culture of CG neurons with an iris extract can induce channel expression *in vitro*.
The stimulating effect of iris extract on channel expression in CG neurons is mediated by a soluble factor that has been identified as a TGFβ molecule.
Calcium ions have a dual role in cell physiology: as a depolarizing factor and a second messenger molecule that can regulate a variety of cellular processes.

Expression of voltage-gated, calcium channels in chick lumbar motoneurons is developmentally regulated.
In developing chick lumbar motoneurons, there is an increase in high voltage calcium currents (L/N) and a reduction in the expression of low voltage-activated calcium channels (T).
Any Questions?

Exam Two – April 11th

Study Chapters 5 through 8
Electrical Differentiation During Development

Electrical properties of Rohon-Bear neurons at various stages of development

From Spitzer, 1979
Electrical Differentiation During Development

- Developing neurons express a variety of potassium channels
- Expression of potassium channels have a significant effect on the maturation of the action potential
In chick lumbar motoneurons, expression of large-conductance, calcium-dependent potassium channels is regulated by interaction with skeletal muscle and ongoing electrical activity in the spinal cord.
Calcium ions may also regulate the expression of other ionic conductances.

Xenopus spinal cord neurons cultured in calcium free medium express a slowly activating potassium conductance.
Regulation of Calcium Channel Expression

- Early expression of T-type calcium currents regulates acquisition of a GABA phenotype in spinal neurons.