Different modes of expression of AMPA and NMDA receptors in hippocampal synapses

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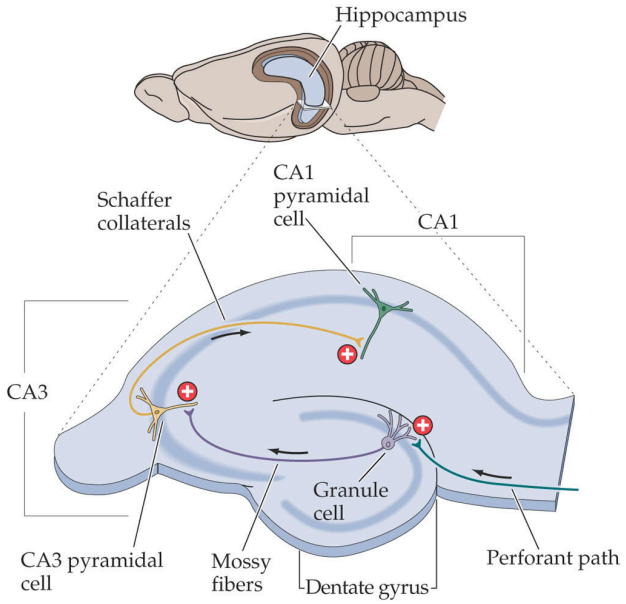
Eric Rinvik and Ole P. Ottersen (1999)

Glutamatergic Fibers in the Hippocampus

- Mossy Fibers – dentate gyrus granule cell axons projecting to CA3 pyramidal cells
- Schaffer collaterals – CA3 pyramidal cell axons projecting to CA1 pyramidal cells
- Excitatory synapses



Major Receptor types

- AMPA, NMDA
- Both are ionotropic glutamate receptors
- NMDA receptors required for long term potentiation (LTP)

What we know from previous experiments….

- Glutamatergic synapses are asymmetric
- AMPA and NMDA receptors are concentrated in postsynaptic density (PSD)
- Ratio of receptor types varies greatly (zero to infinite values)

Why is this important?

- Ratio has physiological significance
- Synapses in which the ratio is near zero are considered “silent”
- Synapses with very high ratios are incompatible with NMDA receptor-dependent LTP
- Ratio indicates the extent of plasticity in a synapse

What do they want to find out?

- What determines ratio?
- Hypothesis: Synaptic size is an important factor in determining the ratio of AMPA and NMDA receptors at the synapse.
- Study focused on Schaffer collateral – commissural (SCC) synapses

SCC Synapses

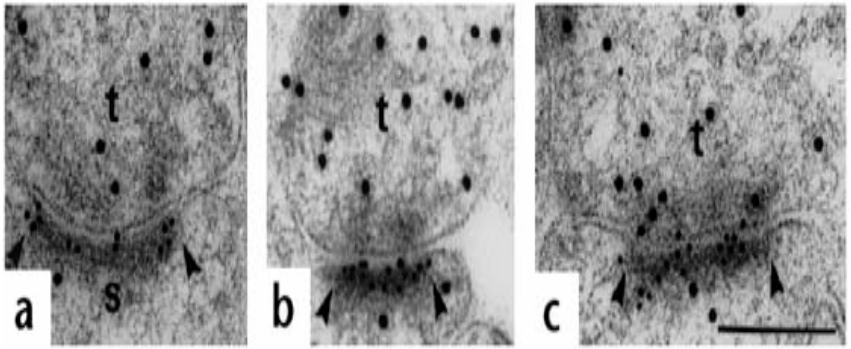
- Wide variation in size among these synapses
- Sustain NMDA receptor-dependent LTP
- Some SCC synapses contain few or no AMPA receptors
- AMPA receptor number increases with synapse size

Methods

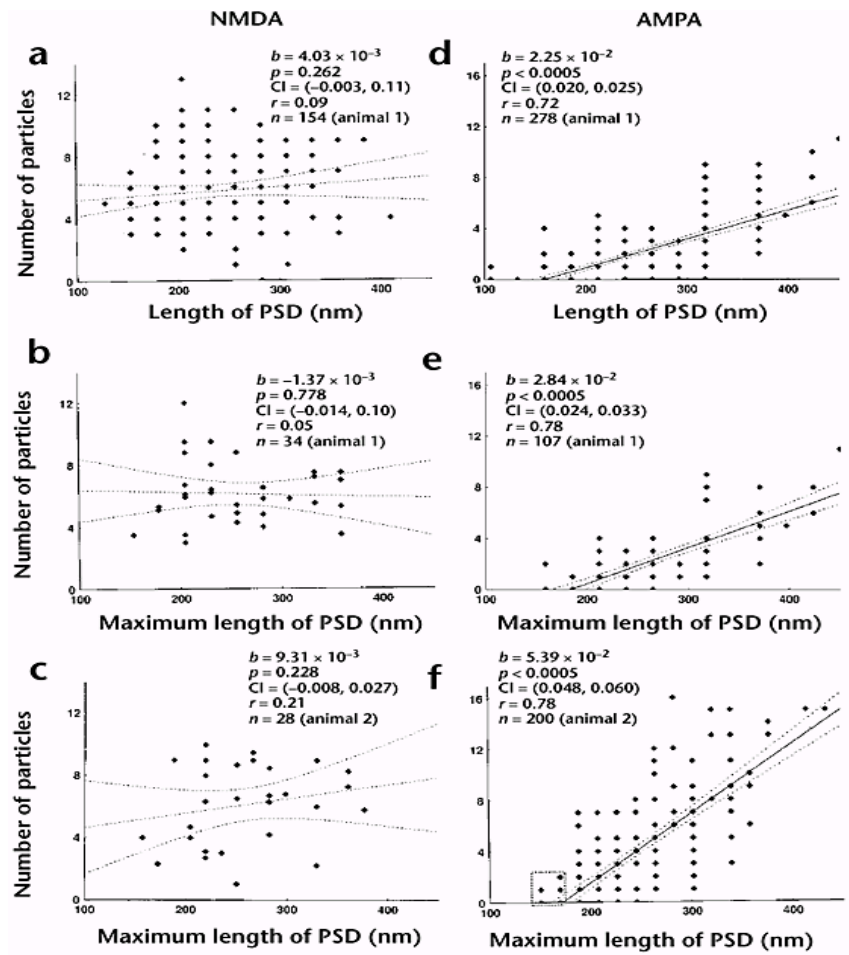
- Subjects: Adult Rats
- Small tissue blocks removed from CA1 region were embedded, sliced into 50-70 nm sections, and processed for immunogold cytochemistry
- Sections were labeled using antibodies to glutamate, AMPA receptor subunits (GluR1,2,3 and 4) and NMDA receptor subunits (NMDAR1 and NMDAR2A/B)
- Receptor density was determined by counting the number of gold particles per synapse
- Section were analyzed using electron micrography

Results and Figures

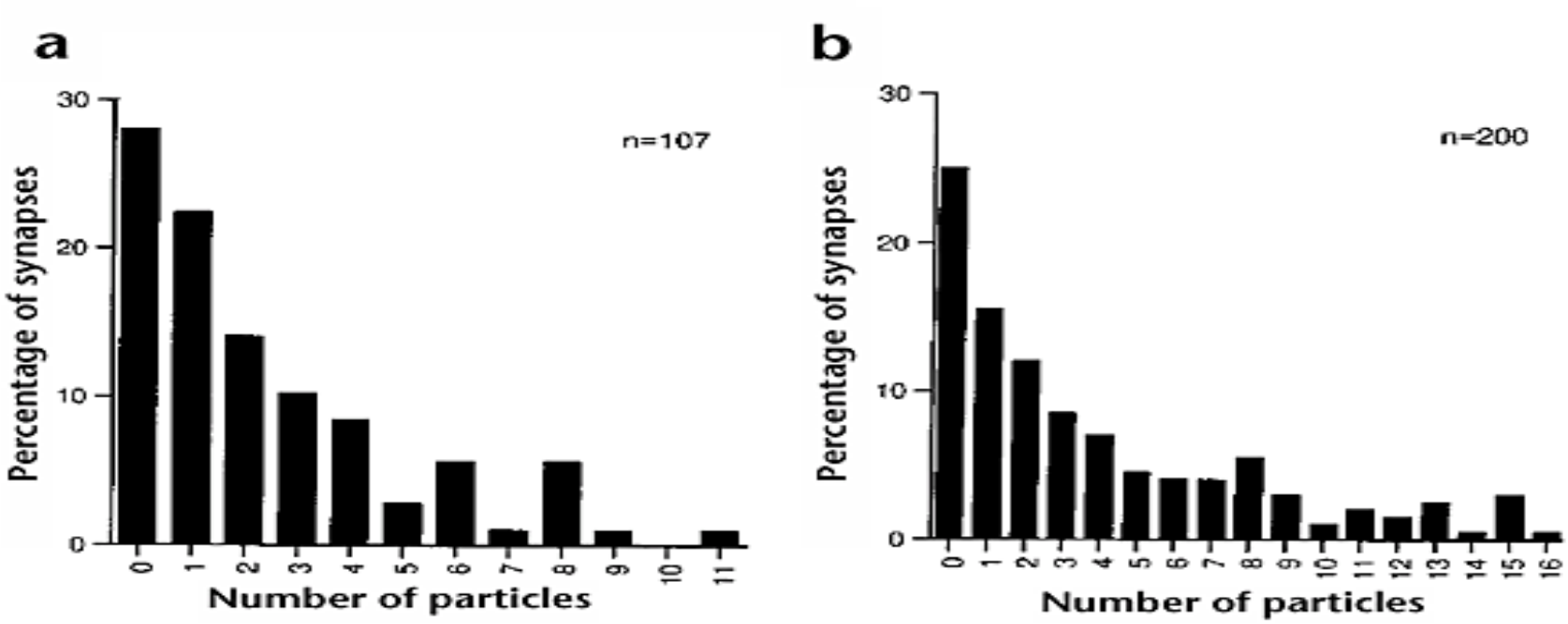
- **SCC Synapses**
- Double labeling - SCC synapses were positively labeled for glutamate and NMDA receptors
- In sections labeled with NMDA receptor antibodies, there was no significant relationship between number of gold particles per PSD and PSD length.



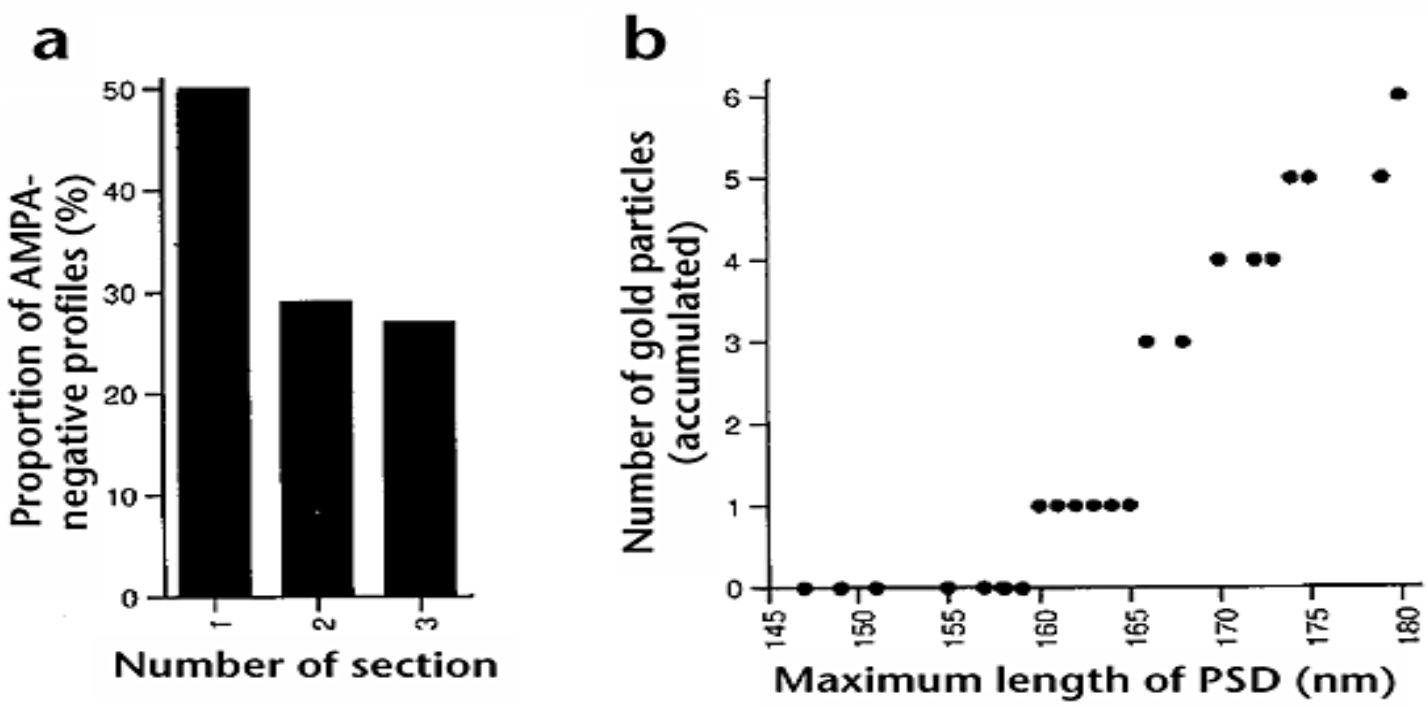
- Sections labeled for AMPA receptors showed a significant relationship between number of gold particles and PSD length (r = 0.78, p<0.0005)



- Double-labeling showed that 25% of NMDA immunopositive synapses were immunonegative for AMPA receptors



- AMPA immunonegative synapses were small (< ~180-nm)



Mossy fiber synapses

- All were AMPA and NMDA immunopositive
- Correlation between number AMPA labeling gold particles and synapse size was much weaker ($r = 0.42$)
- AMPA receptor density was more than twice that found in SCC synapses
- Fewer NMDA receptors than SCC synapses

What do these results mean?

- NMDA and AMPA receptors are co localized at approximately 75% of SCC synapses
- AMPA receptors are not present in SCC synapses smaller than 180-nm

- NMDA and AMPA receptors have different expression patterns in SCC synapses

  - Number of NMDA receptors is independent of synapse size

  - Number of AMPA receptors is a linear function of synapse size

Was the hypothesis correct?

- Hypothesis: Synaptic size is an important factor in determining the ratio of AMPA and NMDA receptors at the synapse.
- Were they right?

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- Were they right?
- Yes!

  The ratio between AMPA and NMDA receptors depends critically on PSD diameter

Conclusions

- The ratio of the receptors (AMPA:NMDA) increases linearly with PSD diameter
- SCC synapse size varies over a wide range. The physiological consequence is a large variability in synaptic strength
- Synapse function determines expression
- NMDA receptor number showed less variability, which is consistent with the fact that LTP requires a minimum range of NMDA receptor size
- Thus, all SCC synapses are potential loci for NMDA-receptor-dependent plasticity

- Mossy fibers do not show same patterning of AMPA receptors and they have less NMDA receptors
- Consistent with previous findings that mossy fiber synapses do not exhibit NMDA-receptor-dependent LTP

Other interesting data….

- Other studies have shown that the number of AMPA immunonegative CA1 asymmetric synapses decreases between 2 and 5 weeks postnatally
- Number of silent synapses may be regulated during development by changes in synaptic activity

Future experiments?

- Is the PSD enlarged in potentiated synapses?
- How are AMPA receptors recruited in silent synapses?