Enhancement of synaptic transmission by cyclic AMP modulation of presynaptic I_h channels

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Background

- I_h channels discovered in 1976 (Noma A. and Irisawa H.)
- Voltage-gated channels, regulated by cyclic nucleotides
- Activated by hyperpolarizing voltage to near most cells resting potential (up to -60mV)
- Increased expression may lead to epilepsy (Bender R. et al., 2003)

I_h Channel Diversity

Function:

- Contributes to cells resting potential
- Generates spontaneous pacemaker activity in heart and CNS
- Membrane resistance and dendritic integration
- Regulate synaptic transmission

I_h Channel Diversity

Localization:

- SAN cells and Purkinje fibers in heart, thalamocortical relay neurons, inferior olive neurons in brainstem, hippocampal stratum oriens interneurons
- Cerebellar Purkinje neurons, hippocampal stratum lucidum interneurons, neurons of respiratory brainstem nucleus
- Photoreceptors, hippocampal CA1 pyramidal neurons, cardiac ventricular myocytes

Primary pacemaker current

Not primary pacemaker current, maintains range for Na+ driven spontaneous firing

Regulates response to excitatory or inhibitory inputs

I_h Channel Diversity

Activation time:

- Activation is slow in heart and thalamic relay neurons (sec)
- Activation is fast in hippocampal CA1 neurons (30-60 ms)

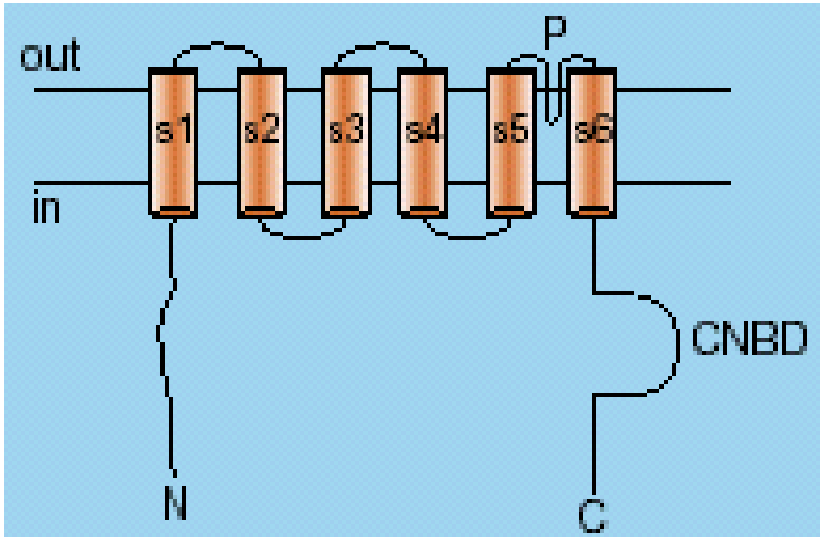
cAMP Regulation via Neurotransmitters:

- Increased cAMP causes more complete and faster I_h activation [Serotonin]
- Decreased cAMP causes less complete and slower I_h activation

Where does diversity come from?

Genetics

- 4 Mammalian isoforms HCN1-4 (High homology except in CNBD domain)
- HCN belong to voltage-gated K^+ channel family



(Siegelbaum S. 2000, Fig. 1a)

Proteins

6 Transmembrane segments
Positively charged S4 voltage sensor
Pore-forming P region
Cyclic nucleotide binding domain at C-terminus

Serotonin Background

- Increases the number of vesicles available for release
- 2 Second messenger systems: PLC/Adenylyl cyclase
- Increased cAMP → Increases Serotonin action

  Excitor → Glutamate
- Decreased cAMP → Decreases Serotonin action

  Inhibitor → GABA

Goal of Study

- Demonstrate that cAMP levels modulate axonal I_h channels
  - Through activation of the serotonin receptor

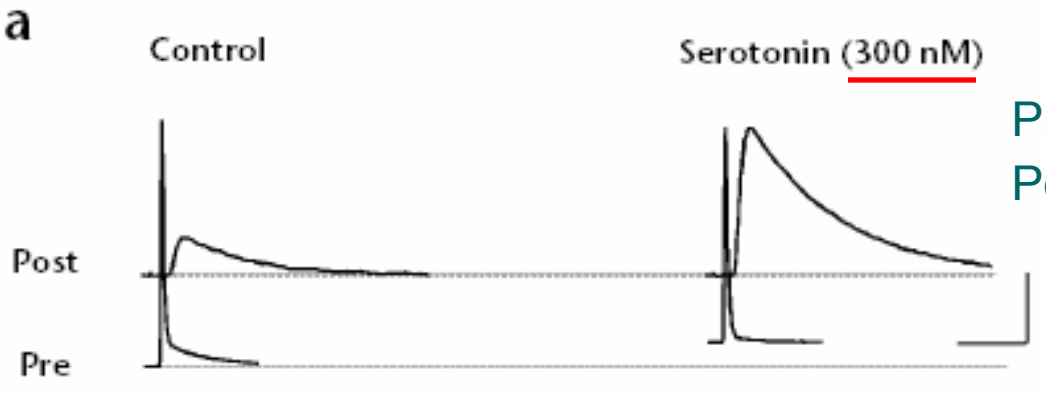
Also:

- Identify a new mechanism for cAMP and I_h to regulate synaptic plasticity
- Demonstrated that cAMP targets presynaptic I_h channels

Figure 1a. Serotonin Enhances Synaptic Transmission

Method: Recorded AP and muscle EJP before and after serotonin/forskolin was applied

- Each trace is average of all EJP/AP for 1 min at 2 Hz



Pre → Action Potentials
Post → muscle EJP

Results: Amplitude increased 310% in EJP

- Depolarized potential by 10 mV

Figure 1b. Adenylyl Cyclase Activation Enhances Synaptic Transmission

- Forskolin → Activates adenylyl cyclase

Results: Increased EJP amplitude by 120%

- Depolarized membrane by 7 mV

- 8-Br-cAMP → Membrane- permeable cAMP analog
- Enhanced EJP amplitude 80 +/-12%

Figure 1b. Adenylyl Cyclase Activation Enhances Synaptic Transmission

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8-Br-cAMP → Membrane- permeable cAMP analog
Enhanced EJP amplitude 80 +/-12%

Figure 1c Enhanced Synaptic Transmission - Summary

- 8-Br-cAMP → Membrane- permeable cAMP analog
- Enhanced EJP amplitude 80 +/-12%

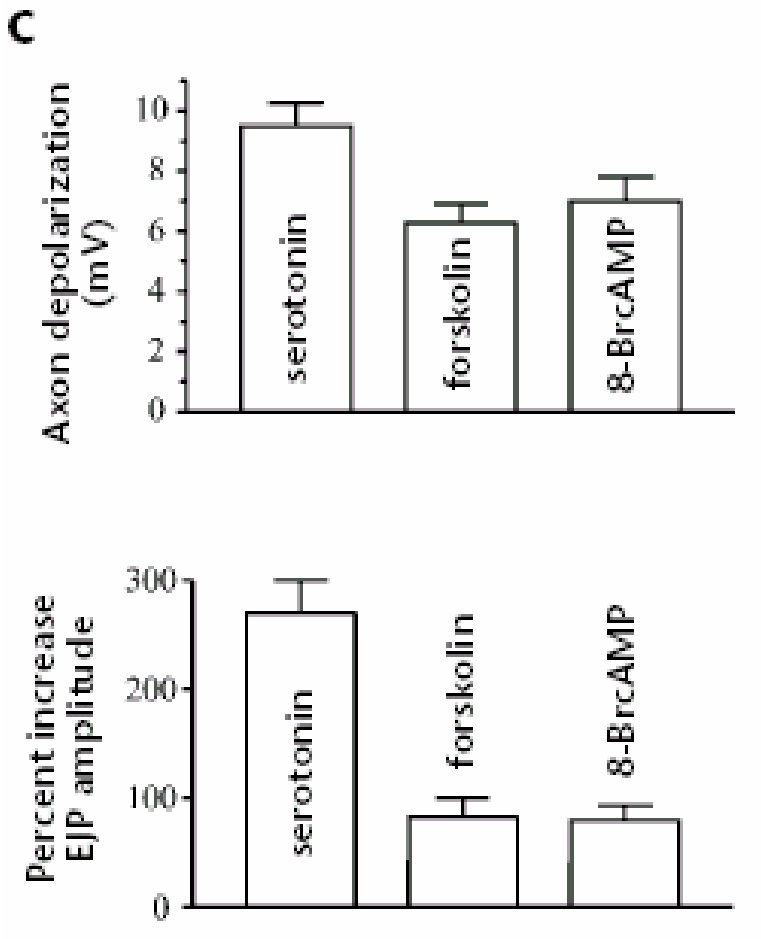
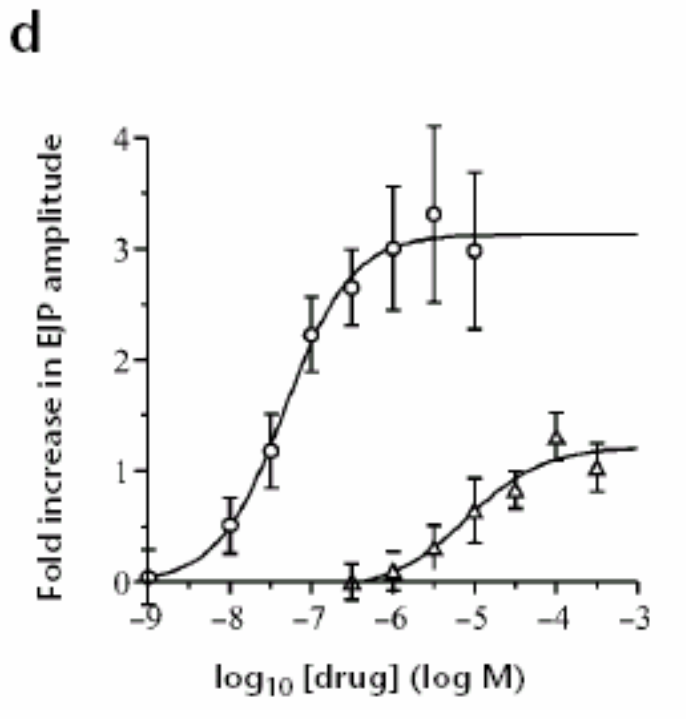


Figure 1d Concentration-Response Curves

- Serotonin increases cAMP levels

Results: Activation of adenylyl cyclase alone does not account for increase in EJP amplitude with serotonin



O → Serotonin

Δ → Forskolin

Figure 1e Role of Endogenous cAMP

Methods:

Applied 300 nM serotonin → Increased EJP 304 +/- 46%

After EJP amplitude returned to normal, applied $1\mu M$ IBMX → Increased EJP 30 +/- 19%

- IBMX Prevents breakdown of cAMP

If Mechanism: Serotonin Receptor Activated → cAMP → should see enhanced EJP amplitude

Figure 1e Role of Endogenous cAMP

Results:

- Applied serotonin → Increase EJP amplitude 457 +/- 122%

Possible errors:

- IBMX is a nonselective adenosine-receptor antagonist (Adenosine receptor inhibition may cause potentiation) [control experiments]

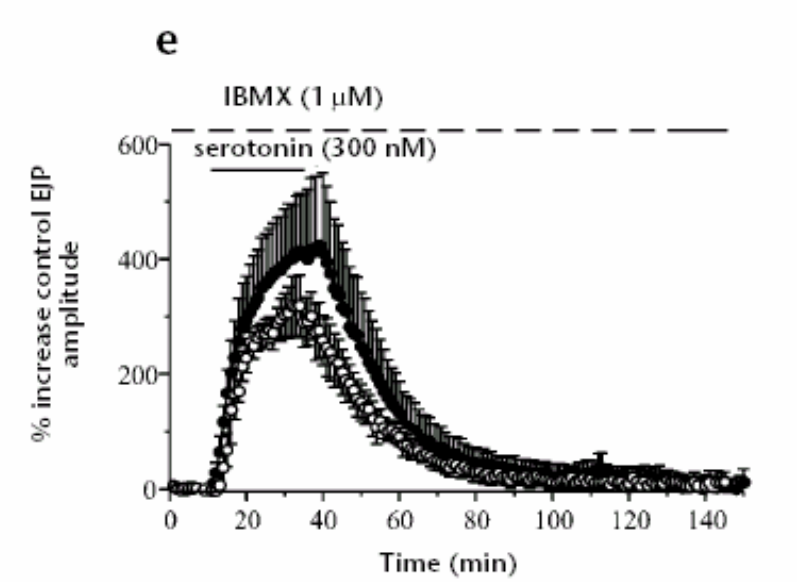
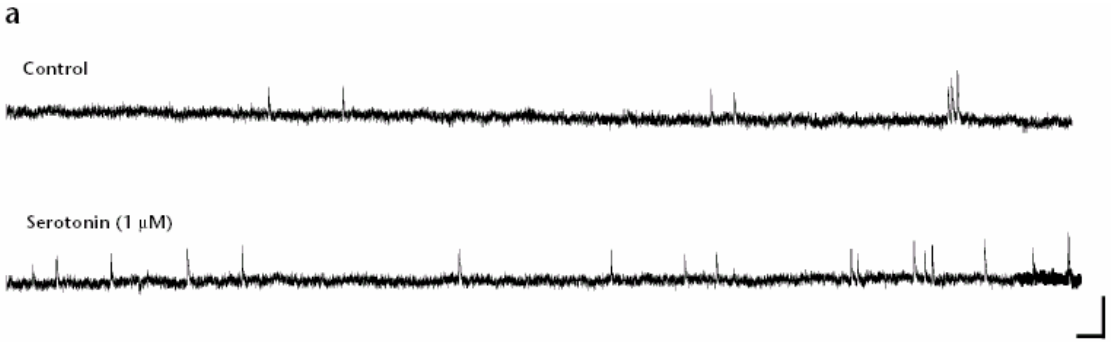


Figure 2a Presynaptic Activation

Methods:

- Recorded mEJP in Normal Van Harrevald's solution with 1 μM TTX → Freq=0.31 +/- 0.1 Hz, Amp=254 +/-36 μV
- Incubate in 1 μM serotonin/30 μM forskolin

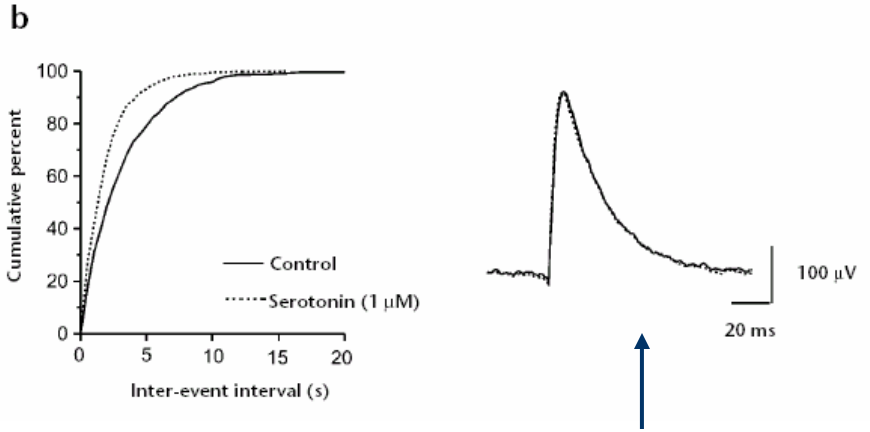


- Serotonin induced increase in frequency 64 +/-11%
- No change in mEJP amplitude

Figure 2b Presynaptic Activation

- Forskolin increased frequency 50% of the time by 42 +/- 33%, no change in amplitude

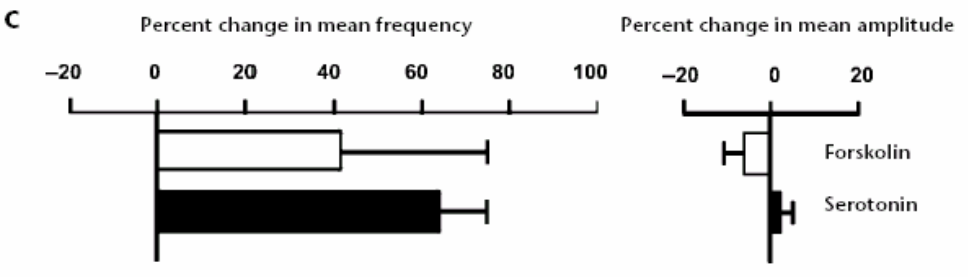
Higher event frequency in serotonin



mEJP with and without serotonin show no change in amplitude

Figure 2c Presynaptic Activation

- Serotonin and forskolin increased mEJP frequency
- No change in mEJP amplitude in serotonin or forskolin



? Increased mEJP frequency supports presynaptic activation

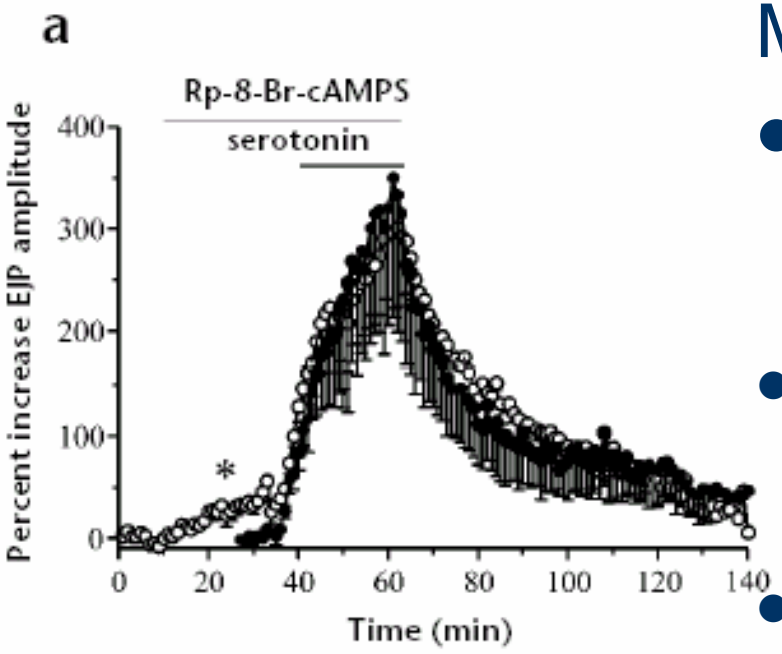
Figure 3a Eliminating PKA

PKA may become activated downstream of cAMP

Methods:

- Applied serotonin (100 nM, 25 min) → EJP amplitude increase 144 +/- 24%
- Applied serotonin/8-Br-cAMP to 30μM H-7 (inhibits PKA, PKC, PKG) → no amplitude increase

Figure 3a Eliminating PKA



Methods:

- Rp-8-Br-cAMP: highly specific, cell-permeable PKA inhibitor, cAMP analog
- Incubate with Rp-8-Br-cAMP → EJP amplitude increase 40 +/- 13%
- Incubate with Rp-8-Br-cAMP and serotonin → EJP amplitude increase 292 +/- 68% (Serotonin alone → 324 +/- 125%)

Figure 3b Eliminating PKA

- Rp-8-Br-cAMP EJP amplitude increase similar to 8-Br-cAMP EJP amplitude increase
- EJP amplitude increased even when PKA was inhibited, suggesting a different target for cAMP

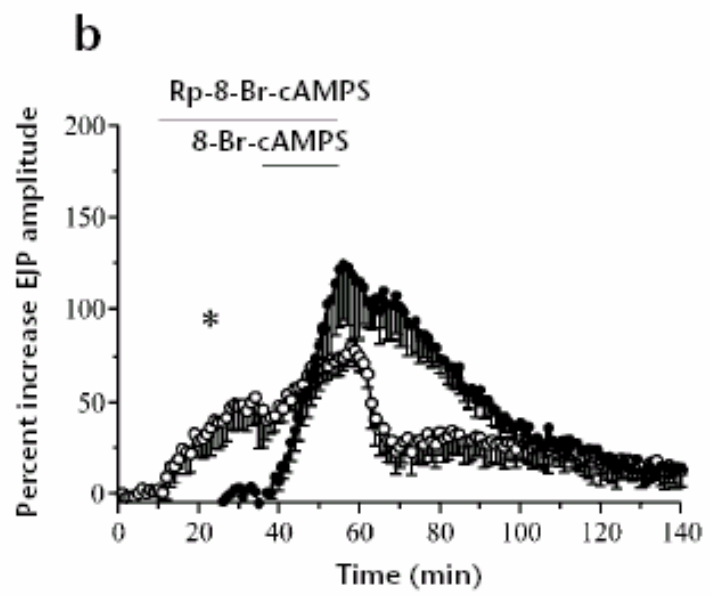


Figure 4a Presence of I_h Channel in Axon

Presynaptic Ih channels may be cAMP targets

Methods:

- Inject hyperpolarizing current pulses into axon
  - Should see 'depolarizing sag' back to resting potential when I_h channels are active
  - Ending the pulse should produce after depolarization potential, overshoot of resting potential
  - ADP initiated firing of AP
- Applied Cs^+ (I_h blocker) → Resting membrane potential hyperpolarized 4mV, ADP amplitude decreased

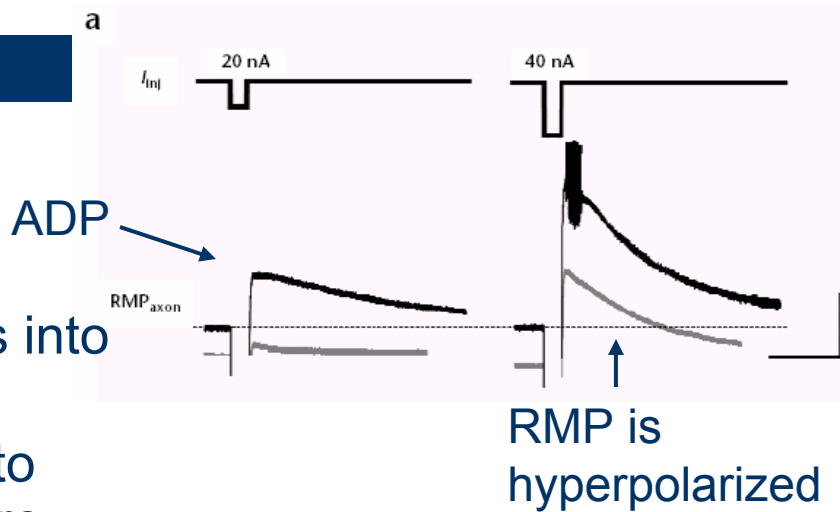


Figure 4b I_h involvement in RMP

ZD7288: I_h blocker

- ADP blocked by Cs^+ and ZD7288 (up tp -30nA)
- Loss of ADP by I_h blockers suggest I_h plays a role in Resting Membrane Potential

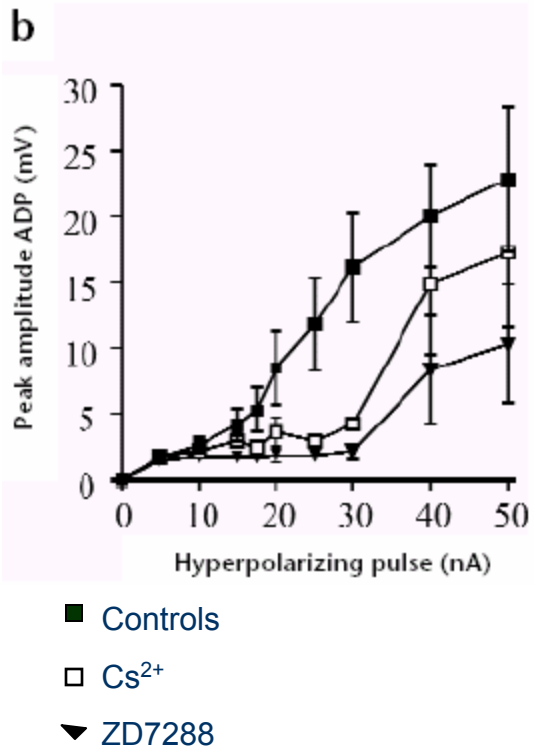


Figure 5a I_h Channel Modulation

Block depolarization of axon by serotonin and forskolin:

- Applying serotonin depolarized membrane 10 mV
- Serotonin is washed out and membrane potential returns to normal
- Applying Cs^+ blocks depolarization
- Cs^+ is washed out and BA^{2+} incubation (K^+ channel blocker), serotonin is applied with unaffected RMP or depolarization

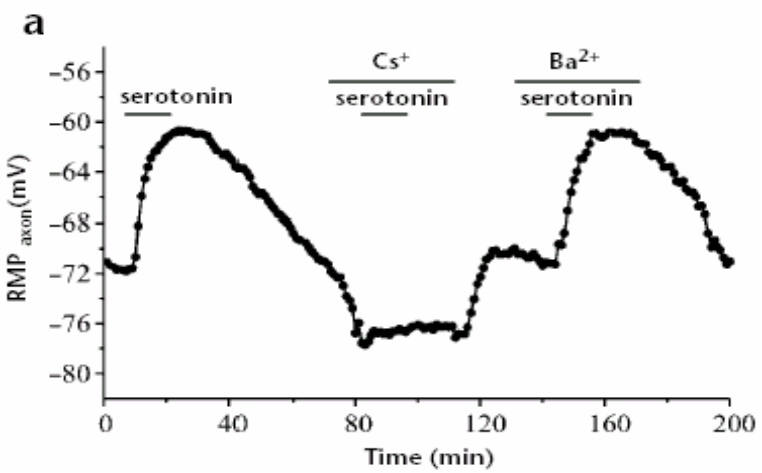


Figure 5b Concentration Inhibition Curves

- ZD7288 reduced depolarization of axon from serotonin
  - Similar to its action on I_h channels

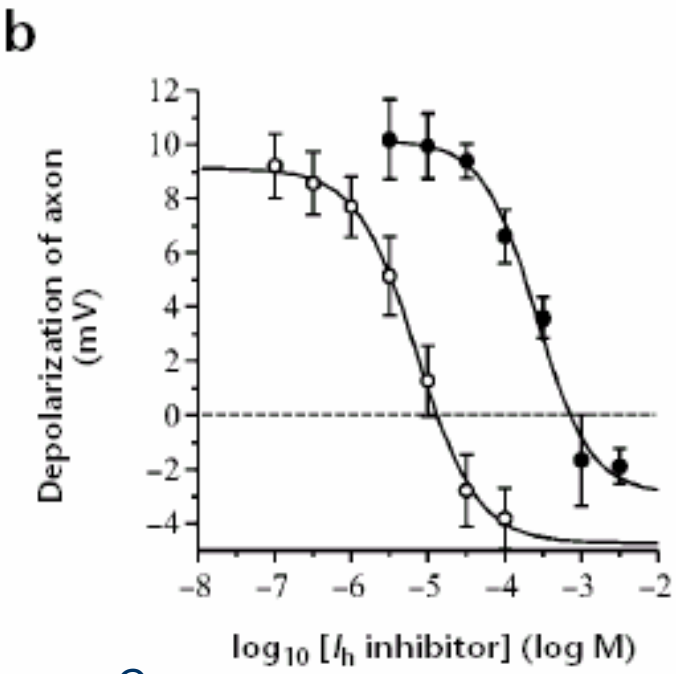


Figure 5c,d I_h Channel Modulation by cAMP

- Forskolin → Activates adenylyl cyclase
- Forskolin can be blocked by Cs^+
- Forskolin can be blocked by ZD7288

➢ Conclusion: cAMP acts on ? I_h channels in axons during synaptic enhancement

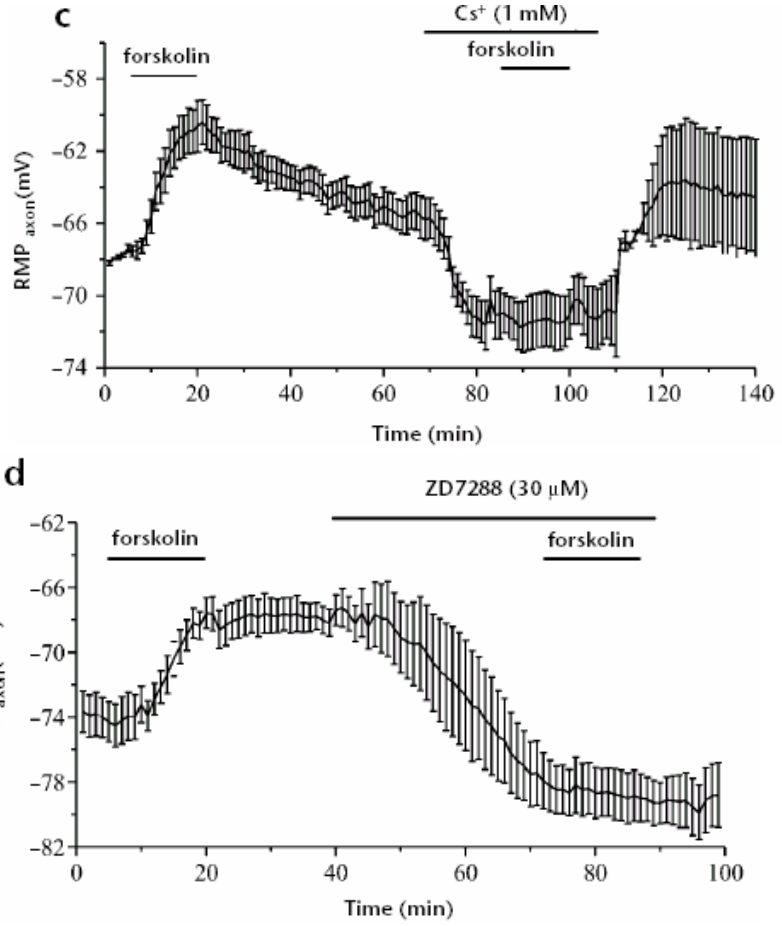


Figure 6a Synaptic Enhancement from cAMP Modulation of I_h

Serotonin: (EJP Amplitude)

- Alone → 2.9 +/- 0.3 fold increase
- Ba^{2+} → slight insignificant increase
- Cs^{+} → 43 +/- 7% reduction
- ZD7288 → 42 +/- 13% reduction

Forskolin:

- Alone → 1.4 +/- 0.2 fold increase
- Ba^{2+} →
- Cs^{+} → 31 +/- 15% reduction
- ZD7288 → 67 +/- 6% reduction

8-Br-cAMP:

- Alone → 0.8 +/- 0.1 fold increase
- Ba^{2+} →
- Cs^{+} → 82 +/- 2% reduction
- ZD7288 → 70 +/- 18% reduction

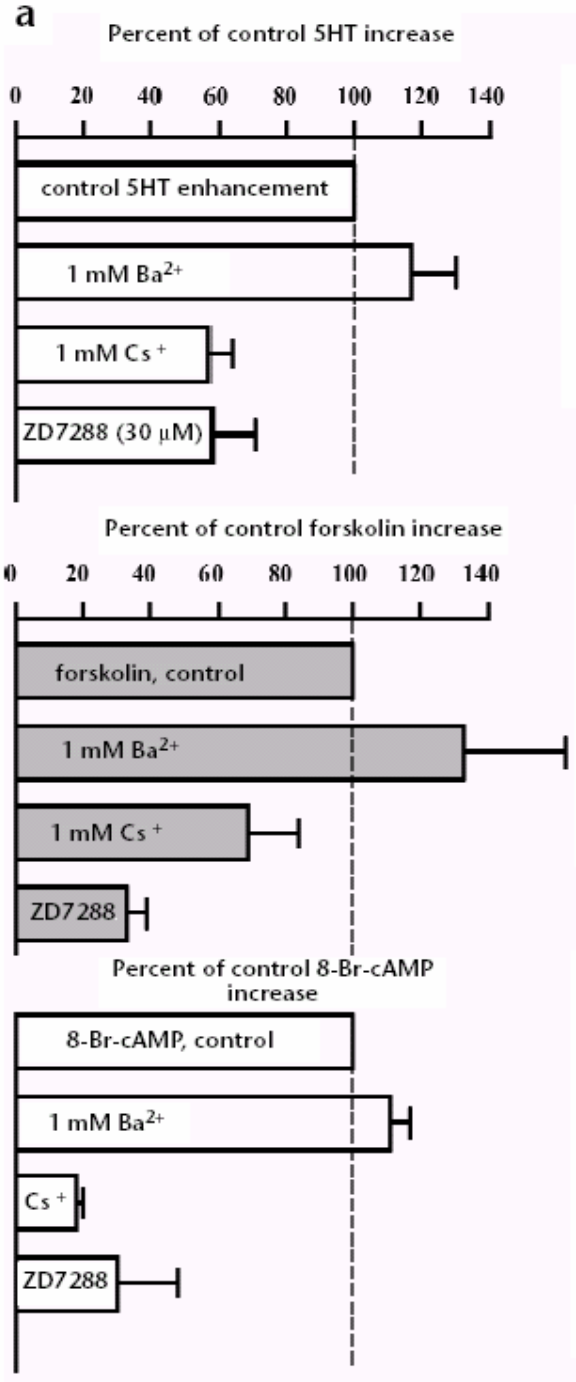
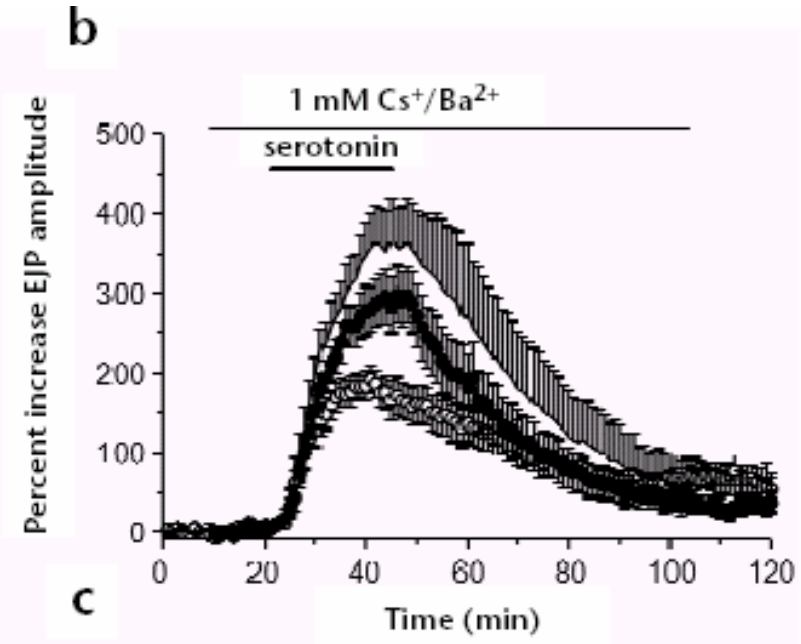
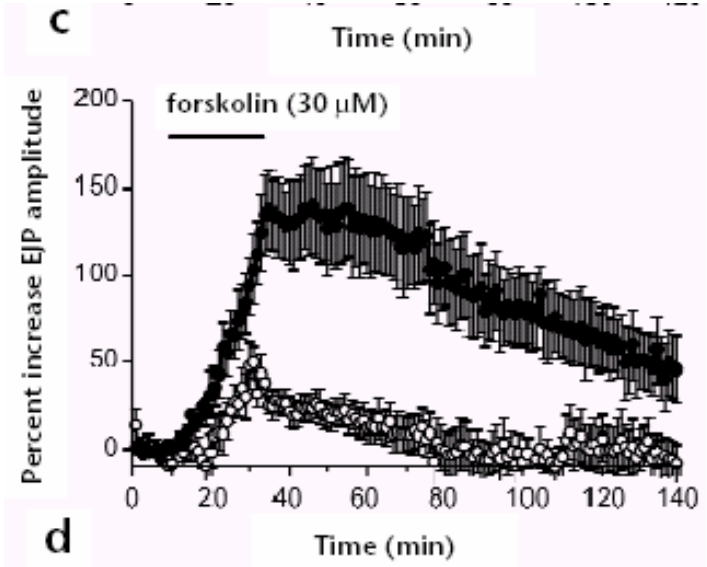


Figure 6b Data for Bar Charts



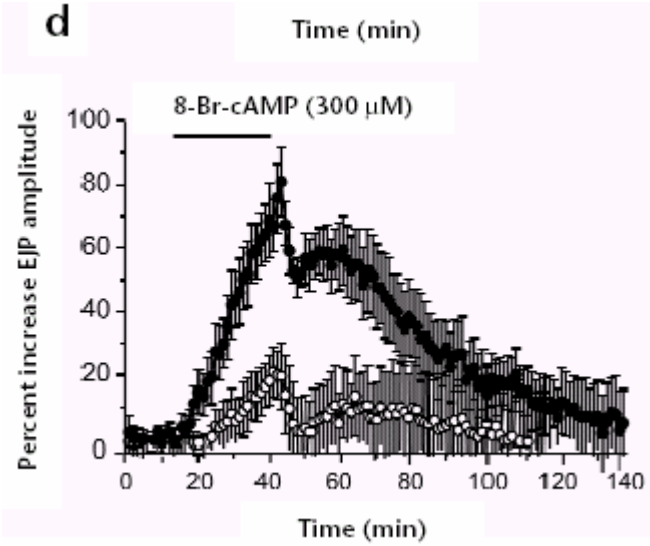
- Serotonin alone
- Cs^+
- Ba^{2+}

Figure 6c Data for Bar Charts



- Forskolin alone
- ZD7288

Figure 6d Data for Bar Charts



- 8-Br-cAMP alone
- ZD7288

Figure 7a Controls for ZD7288 and Cs^+

Serotonin and Forskolin reduction by ZD7288 and Cs^+ may be from hyperpolarization from ZD7288 and Cs^+ instead of I_h channels

Methods:

- Elevated extracellular K^+ with serotonin → ZD7288 reduction was same as normal extracellular K^+

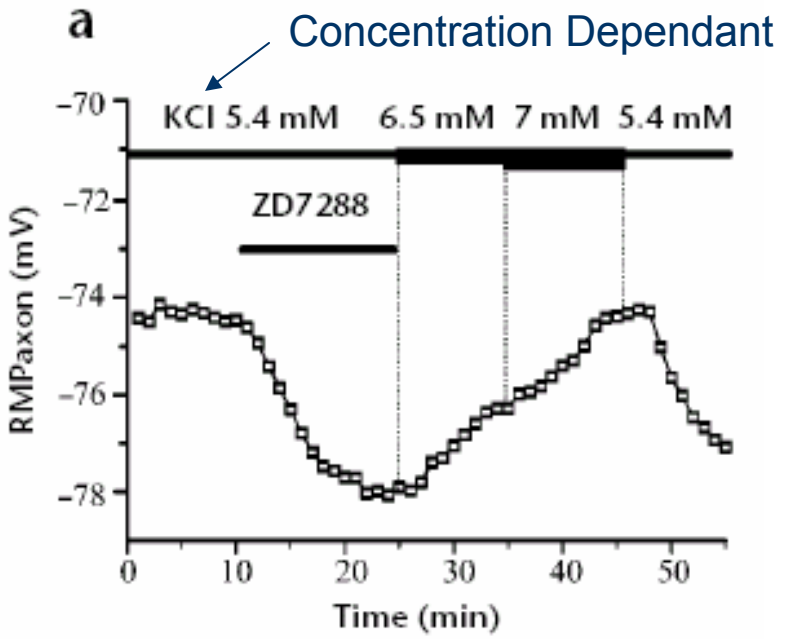


Figure 7b Controls for ZD7288 and Cs^+

- Elevated extracellular K^+ with forskolin → ZD7288 reduction was same as normal extracellular K^+

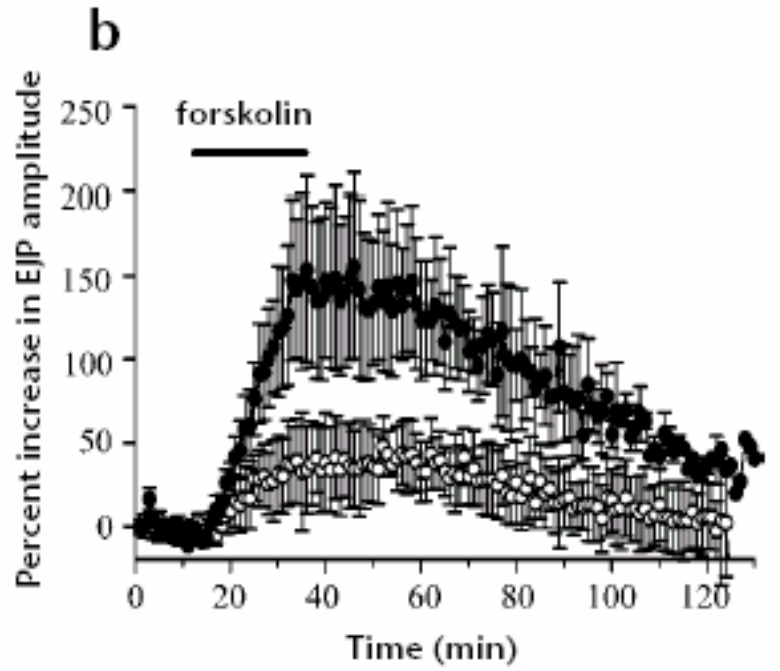
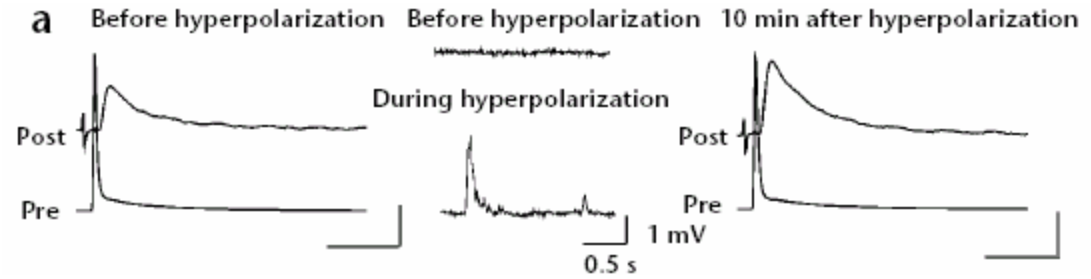


Figure 8a

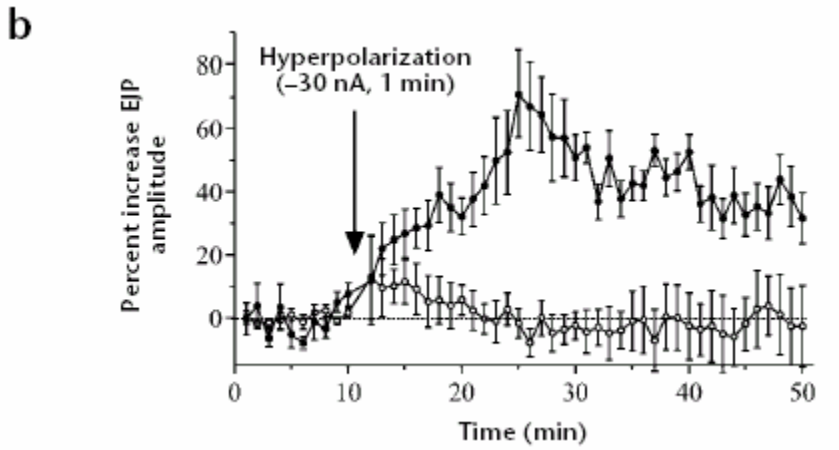


Method: Recorded AP and muscle EJP before and after hyperpolarizing current injection to activate I_h channels

- Each trace is average of all EJP/AP for 1 min at 2 Hz
- Spontaneous EJP increase during hyperpolarization

Figure 8b

- EJP before and after hyperpolarization



- I_h channels with ZD7288
- I_h channels without ZD7288

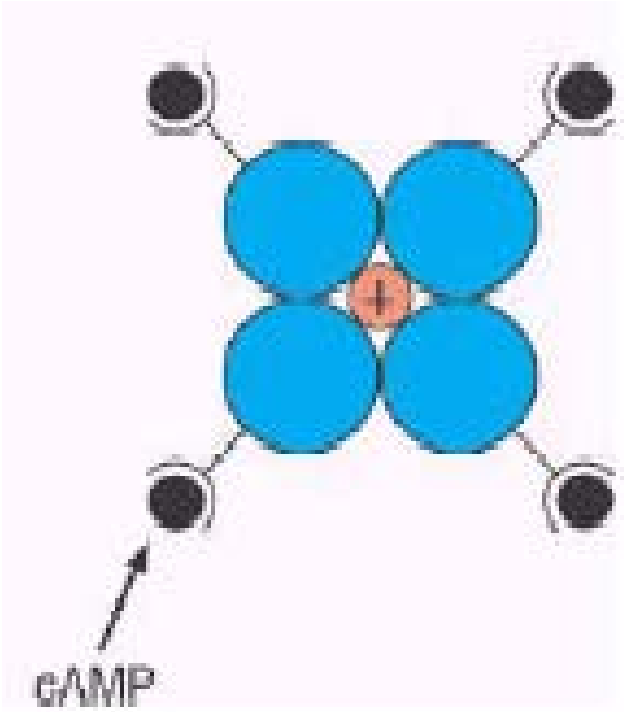
Conclusions

- Blockade of I_h with ZD-7288 or Cs+ inhibited the synaptic facilitation with serotonin
- Hyperpolarizations that activated I_h could produce facilitation in the absence of serotonin.
- Beaumont and Zucker extend role of I_h channels in axons to include altering neurotransmitter release from presynaptic terminals
- Suggest that I_h channels enhance synaptic vesicles available for release

Since the paper was published

HCN Isoform Expression:

- Tetrameric channel with 4 cyclic nucleotides bound to channel (in open state)
- cAMP binding removes inhibitory action of CNBD
- Isoforms form homomeric I_h channels in various cells resulting in different kinetics, voltage dependence, cAMP modulation
- Different isoforms expressed in same cell (HCN1+HCN2 form heteromeric channels)



(Robinson R., 2002 Fig. 3a)