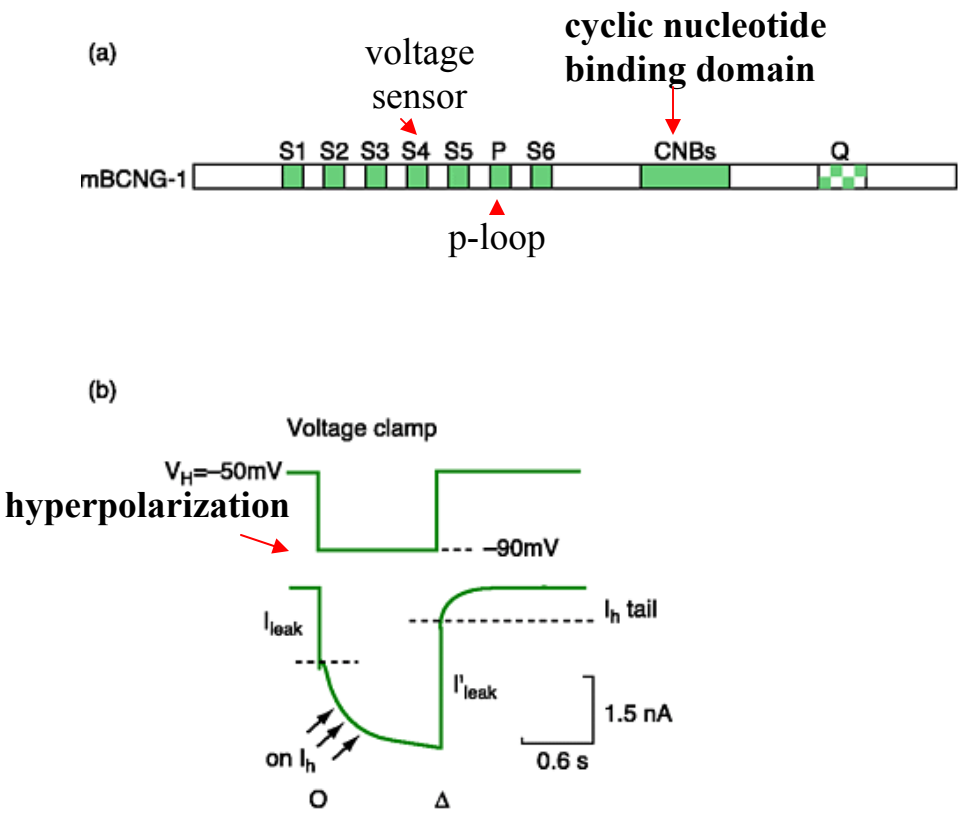
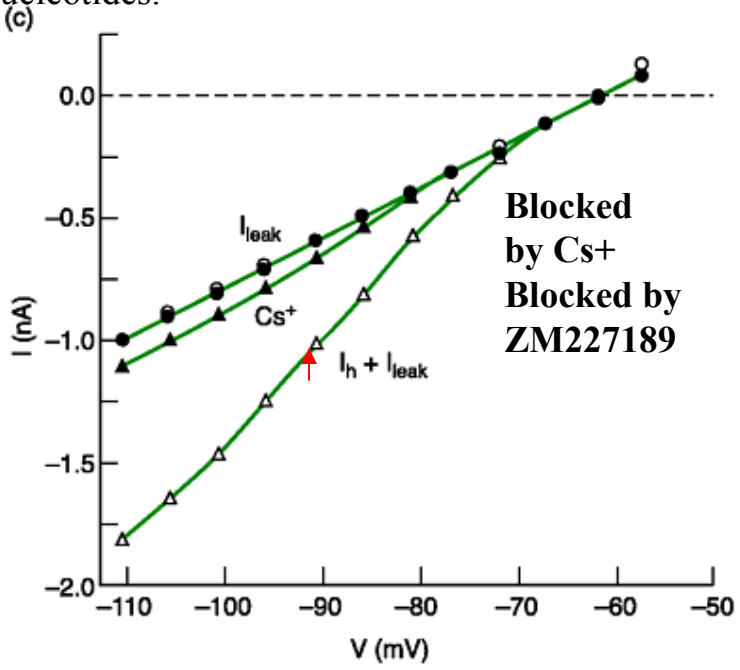
MCP's De-obfuscation (hopefully) of Beaumont & Zucker (2000)

Hyperpolarization activated cationic channel (Ih, If, Iq)

(hyperpolarization-activated, cyclic nucleotide-gated channels, HCN)



These are non-specific cation channels carrying mostly Na^+ and K^+. These channels have a cyclic nucleotide binding Domain and their activity is greatly facilitated by cyclic Nucleotides.



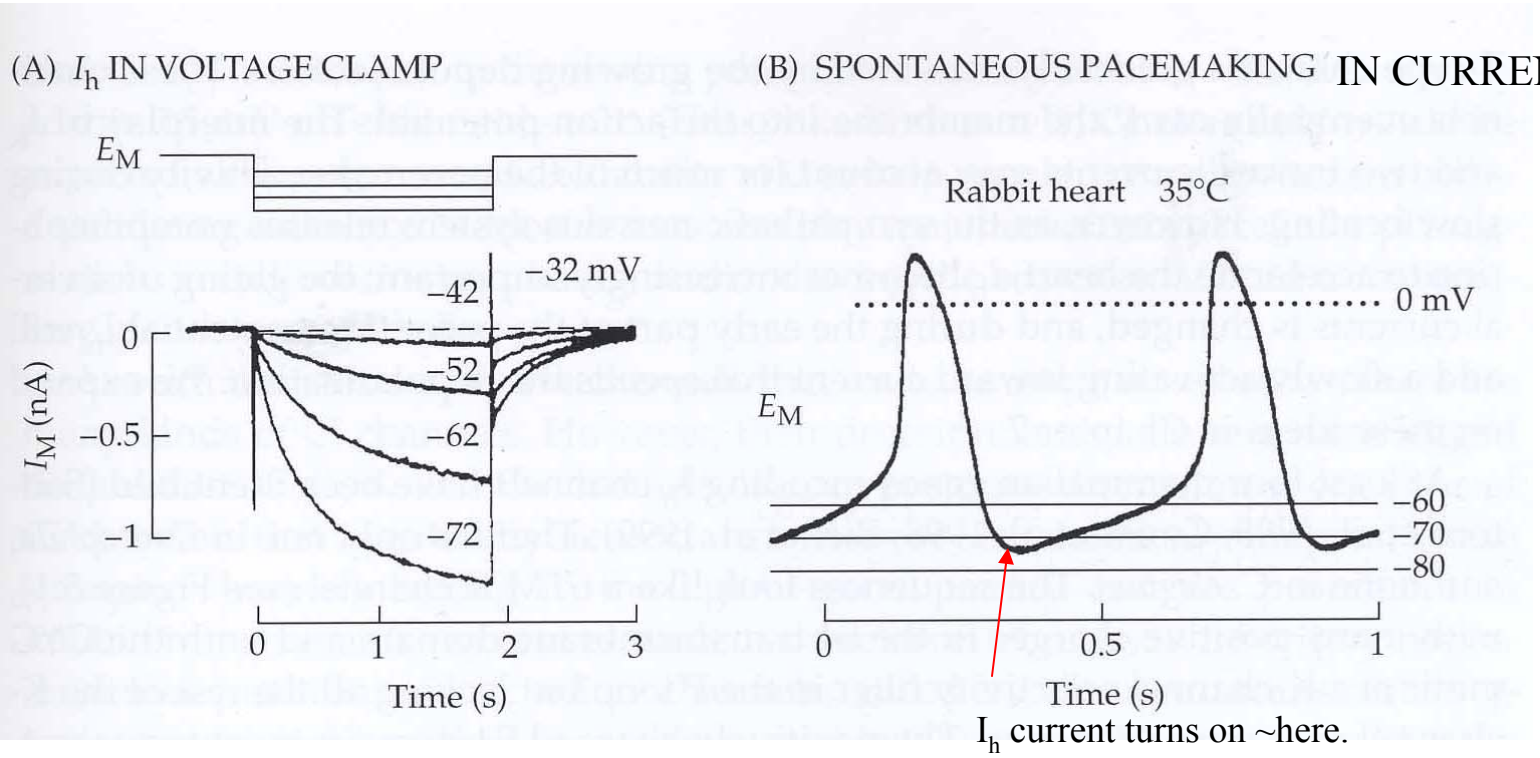
Activated by hyperpolarization -produces an inward, depolarizing current that functions in rhythmically active neurons,in the thalamus, in hear etc. to enhance transmitter release (Beaumont & Zucker, 2000).

Fig. 17.6 From HammondC. *Cell & Molecular Neurobiology* Academic Press 2001

The confusion last Wednesday was several fold:

A) Below shows the various different membrane voltages (top) at which a cell with an I_h current is clamped. The second figure (below) shows the inward (positive curren)t that flows when I_h is activated by the hyperpolarizing current steps.

B) Below is a current clamp recording with the depolarization produced as a result of the previous action potential's after hyperpolarization.

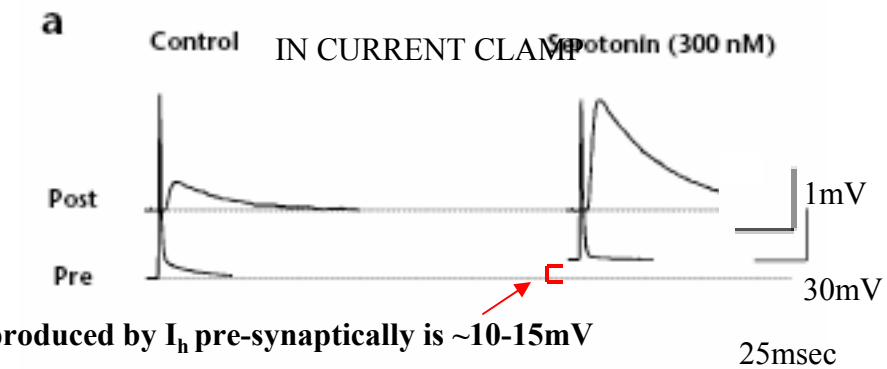


From: Hille (2001) *Ion Channels of Excitable Membranes* 3rd Ed

I_h current turns on ~here.

I_h channels are not blocked by Ba++ ions. I_h are blocked by Cs+ ions.

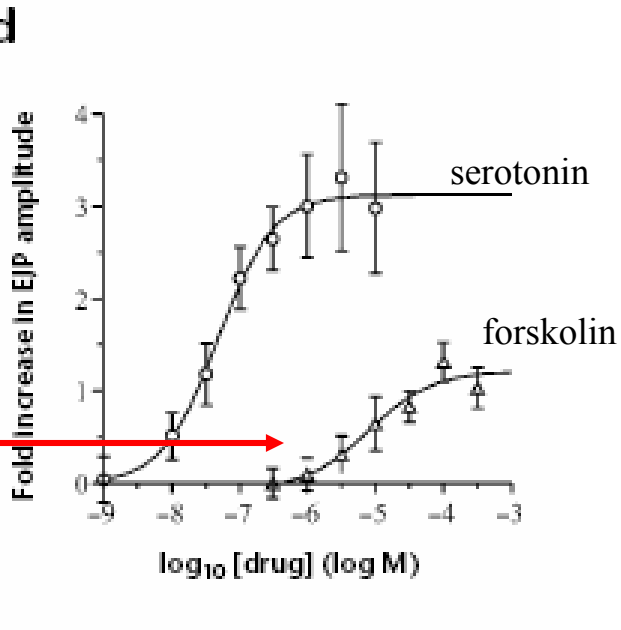
Figure 1



The depolarization produced by I_h pre-synaptically is ~10-15mV

Forskolin that directly activates adenylyl cyclase is not as effective as serotonin at increasing the post-synaptic response

Both serotonin and forskolin depolarize the pre-synaptic axon.



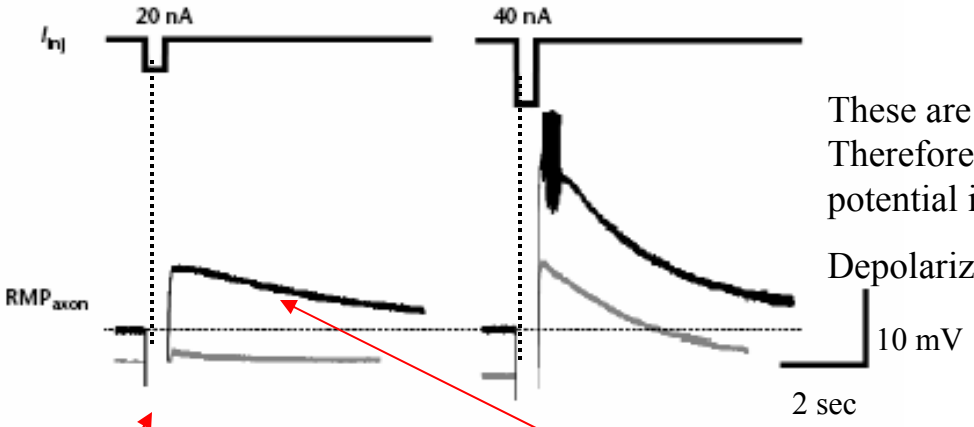
This inability of saturating amounts of forskolin to duplicate serotonin's effect indicates that serotonin is doing something **in addition to activating** adenylyl cyclase. We never know what that is.

Beaumont & Zucker, 2000

Probably the major point of confusion in this paper is that I_h is activated by hyperpolarization of the membrane but the current itself is depolarizing

Figure 1 a

A hyperpolarizing current injection is actually an **outward** current using the convention that a current is given in the direction of **positive current flow.** This trace merely marks the onset, duration, and amplitude of the current flow.



These are current clamp recordings. Therefore the change in membrane potential is being shown and it is

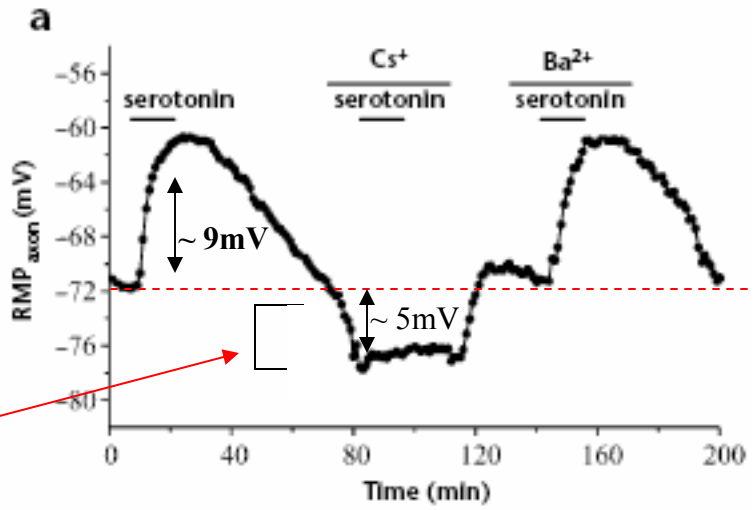
Depolarizing.

This marks the onset of I_h even though you can't see the beginning of the depolarization because the outward (hyperpolarizing current is still flowing.

This depolarizing potential called by Beaumont & Zucker the after depolarizing potential (ADP) is due to I_h. I_h is carried by Na^+ and K^+ ions. However the net effect is an inward current that results in a depolarization of the axon membrane.

Cs^+ blocks K^+ channels plus the I_h channel
Ba^{++} blocks K^+ channels but does not block I_h channels

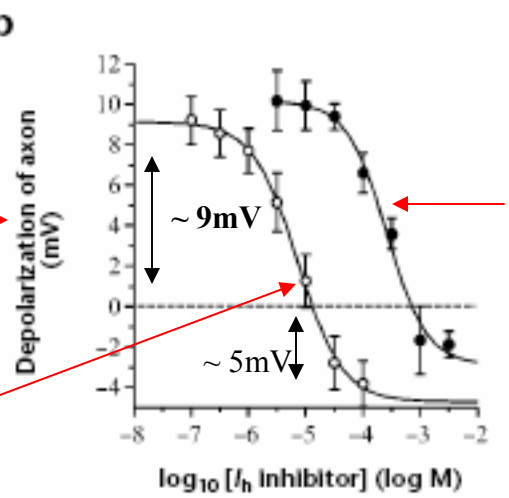
RMP means membrane "steady state"level of the membrane potential



Therefore, in the presence of Ba $^{++}$, serotonin can still increase the activation of I_h.

axon membrane potential with no drug

This hyperpolarization by CS^+ is never commented on. Cs+ blocks K+ channels and I_h. (We do not know where E_{K+} is in these axons.) The membrane potential may become hyperpolarized in the presence of Cs^+ because normally there is a small I_h serving to slightly depolarize the membrane and when I_h is blocked the effect is gone.



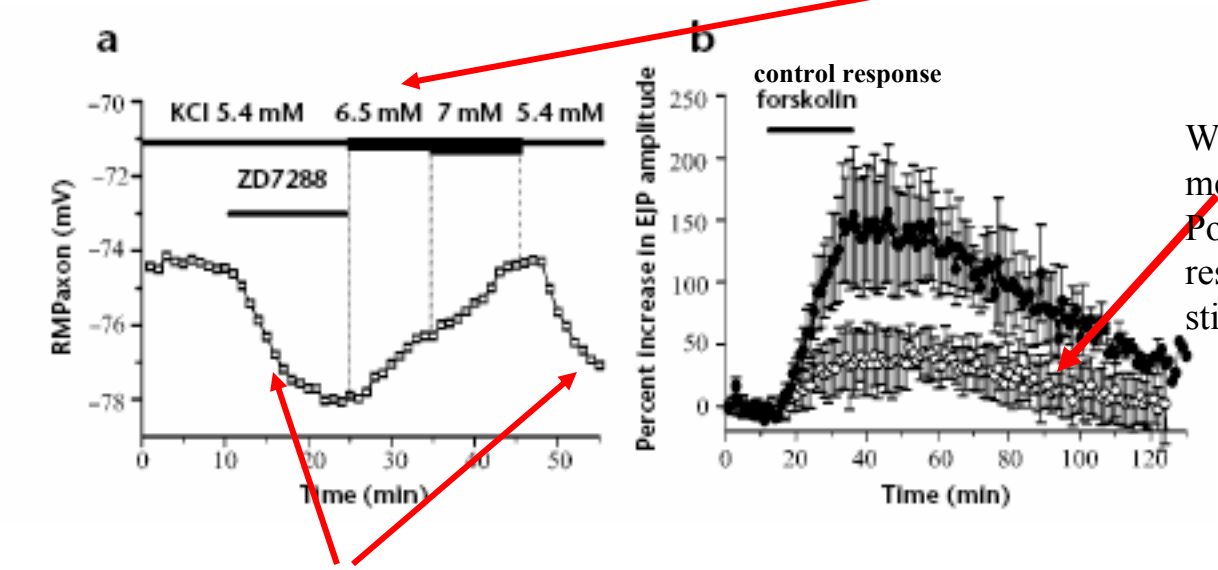
I_h channels are blocked by Cs^+

Means more positive or more negative than the resting potential

ZD7288, a specific antagonist of I_h channels. Both ZD7288 and Cs+ Move the membrane potential ~3-5mV more negative than the RMP.

BLOCKING Ih WITH ZD7288 ISSHOWN TO DEPRESS THE FORSKOLIN MEDIATED EJP 9POSTSYNAPTIC) INCREASE IN FIGURE 6

However, ZD7288 irreversibly hyperpolarizes the axon membrane probably because a small I_h is continuously shifting the membrane potential to a slightly depolarized state. The membrane potential can be shifted toward depolarized values by shifting E_{rev} K^+ to a more depolarized value using lower concentrations of $[K^+]_{out}$.



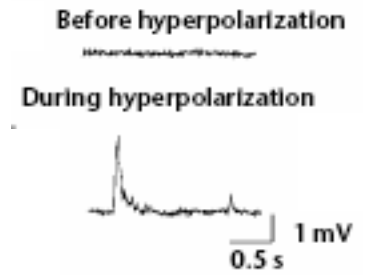
With ZD7288 but axon membrane at its normal Potential. The forskolin response of the EJP is still depressed.

Shifting to normal $[K^+]_{out}$again still shows the hyperpolarizing effect of ZD7288

Now the question becomes, can ZD7288 still inhibit the effect of direct activation of adenylyl cyclase by forskolin in increasing the post-synaptic EJP when external $[K^+]$ is increased so that ZD7288 does not hyperpolarize the axon? The answer is yes. **Therefore, adenylyl cyclase effects on EJP operate through I_h .** Also **the I_h effect on the EJP is not due to the fact that it changes the baseline resting membrane potential of the axon.**

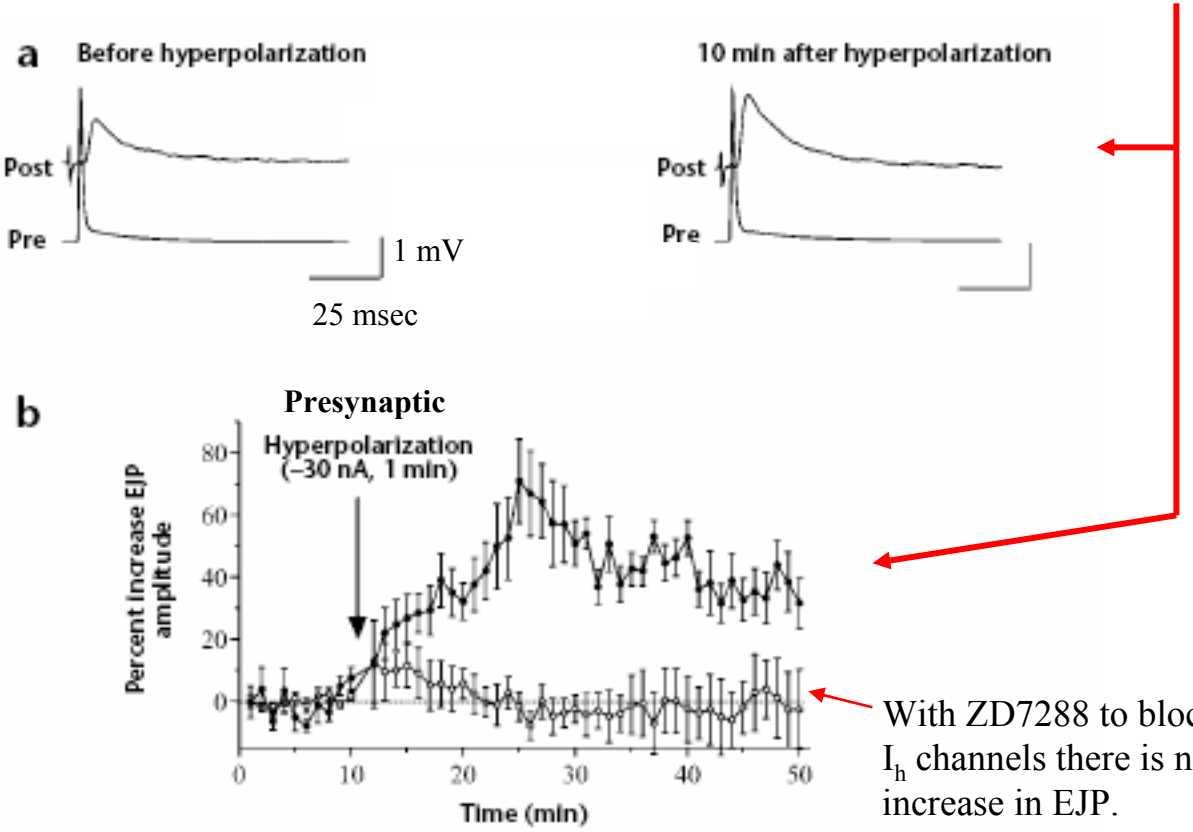
Now direct hyperpolarization of the axon membrane near the axon terminal is used to investigated whether initiation of I_h by this direct method can increase the EJP.

The increase in spontaneous EJP frequency with hyperpolarization is used to reveal that the hyperpolarizing electrode is sufficiently close to the presynaptic release site.



After the hyperpolarization the EJP produced by I_h remains elevated for a prolonged time.

Figure 8



With ZD7288 to block I_h channels there is no increase in EJP.

Conclusions:

1.There is a serotonin activated current that potentiates the EJP at the crayfish excitatory neuron-muscle synapse and also depolarizes the pre-synaptic axon membranes by ~15mV.

2. At least part of the serotonin response is mediated by cyclic AMP

3. Serotonin or drug treatments that increase cyclic AMP increase the frequency not the amplitudes of miniature EJPs but not their amplitude. Therefore, the effect of the agents appears to be on pre-synaptic release.

4. PKA, a frequent mediator, of cyclic AMP effects is not involved because a specifc antagonist of PKA was ineffective at blocking the serotonin effect. Note: the investigators had some difficulty substantiating this conclusion because the PKA blocker they used (Rp-8-Br-cAMPs) partially mimicked the effects of cyclic-AMP itself.

5. Evidence that a hyperpolarizing current applied to the axon, an outward current (by definition), then initiates a cation current. (This is the hyperpolarization activated current, I_h). The depolarization caused by this "inward" current is called by B&Z, but not many other investigators as far as I can tell, an After Depolarizing Potential (ADP).

6. Serotonin produces this same depolarizing potential in the axon. Cs+ which blocks I_h and also K^+ channels actually hyperpolarizes the axon membrane and blocks the ADP produced by serotonin. Ba^{++}, which does not block I_h, does not block the ADP produced by serotonin.

7. Their is basal level of I_h that is continually producing a small depolarization of the axon membrane potential. However This low level of depolarization which is usually blocked by the Ih blocker ZD7288 is not a significant parameter in The ZD7288 blocking of the forskolin effect on increasing the EJP.