

Instructor: Karen O'Donnell

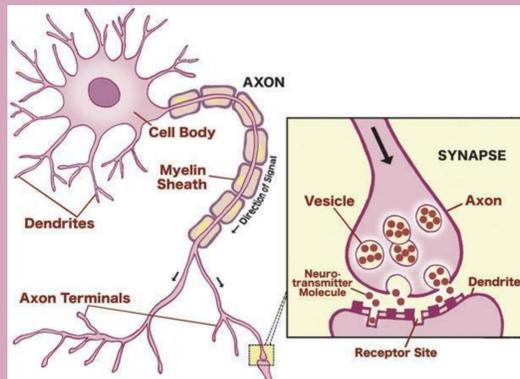
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## I. Chronic Pain and P2X4

Chronic pain, pain lasting longer than normal, can be a debilitating condition to someone that completely changes their way of life. Simple events such as a gust of wind brushing against skin, opening a door, or even a hug can be incredibly painful. In an attempt to figure out what causes such adverse pain perception, scientists have come across a protein receptor located in the postsynaptic membrane of nerve cells. This receptor is called P2X4, and scientists have found that in someone who struggles with chronic pain large numbers of P2X4 receptors are present. Studies are being done to see if there are ways to inhibit P2X4 from opening, stopping pain signals from reaching the brain. If this can be done, perhaps the issue of chronic pain could be solved and that would be, to say the least, life changing.

## II. Neuro Pathway

When a neurotransmitter attaches to an ion channel protein's binding site, the protein opens and ions will rush into the neuron to create an electrical impulse.

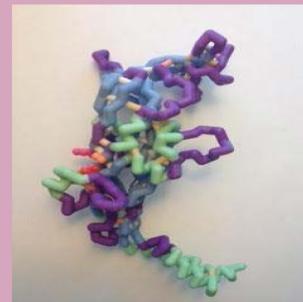


Two neurons at the synaptic cleft. neurotransmitters released from presynaptic neuron binds to proteins on the postsynaptic neuron

## III. Gateway into a Neuron

P2X4 sits in the membrane of neurons, allowing for ions to enter, causing an excitatory response in the cell. The protein consists of three subunits with three specific binding sites for a neurotransmitter to bind to. Researchers have found that in the case of P2X4, ATP is the neurotransmitter that causes the protein to open. Ethanol has become a recent molecule of interest as it has been shown to somehow alter the normal function of P2X4 and reduce the influx of incoming ions.

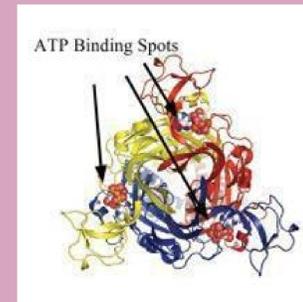
## VI. P2X4: The Model



One subunit of P2X4, derived from PDB file 4DW1

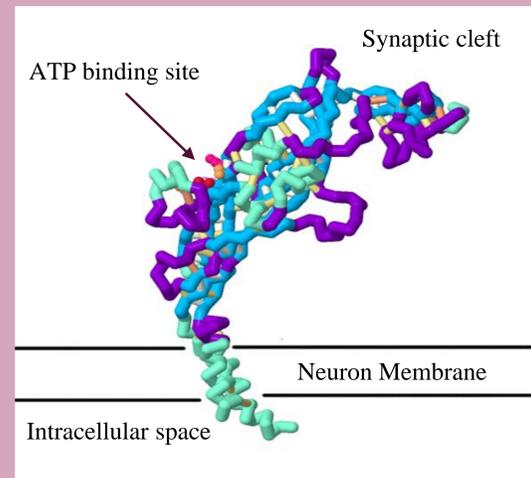


Closed P2X4 Top View



Open P2X4: after ATP binds Top View

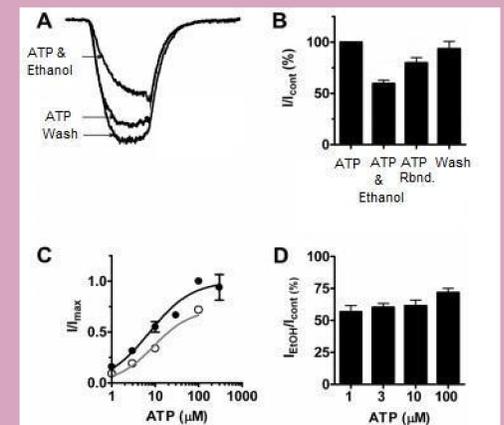
A single subunit of P2X4 resting in the cell membrane



These amino acids have been highlighted as they are part of the binding site for ATP. They are colored coral, goldenrod, deep pink, sandy brown, plum, and crimson. When ATP binds to these sites P2X4 receptors open, allowing ions into the cell. We also highlighted the disulfied bonds because they play a role in the structure of the model. The color of the disulfied bonds is light salmon.

## IV. Tracing Currents with ATP and Ethanol

To find the effects that ethanol has on the P2X4 protein receptor, experiments were done to measure the amount of current taking place in a single cell. Three washes were put over cells, one of just the neurotransmitter ATP, one of ATP plus ethanol, and another control wash.



- Three traces overlap (1. ATP (2. ATP and ethanol (3. Wash that cleanses cell of ethanol and ensures the cell does not die. Current is dramatically less in cell with ATP and ethanol.
- Data taken from several cells with the previous three traces. Current is dramatically less in cells with ATP and ethanol.
- Current measured with differing amounts of ATP. Closed dots show ATP alone, open dots show changing amounts of ATP with a fixed amount of ethanol. Ethanol is noncompetitive to ATP, and is binding somewhere else on the cell.
- Showing that with a fixed amount of ethanol and changing amounts of ATP, incoming current stays consistent. Ethanol binds somewhere independent of ATP, but still inhibits P2X4.

## V. P2X4: Moving into the Future

As a recent discovery, the interaction of ATP and the P2X4 receptor is inhibited by ethanol. P2X4 is key in neuronal communication and the perception of pain. With this discovery, scientists can find out more about the structure of P2X4 and learn other ways to inhibit the protein- perhaps solving the issue of chronic pain.

### Citations

Communication Between Neurons. Digital image. *The Urban Child's Institute*. N.p., n.d. Web. 3 Mar. 2015. <<http://www.urbanchildinstitute.org/why-0-3/baby-and-brain>>.  
P2X4 Receptor Open. Modified Web. 5 Mar. 2015. <<http://www.nature.com/nature/journal/v485/n7397/images/nature11010-f1.2.jpg>>.  
Daryl L. Davies. 2011. Ethanol Is a Fast Channel Inhibitor of P2X4 Receptors. *The Journal of Pharmacology and Experimental Therapeutics*. Vol. 337, No. 1: 173  
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