**Non-Selective Cation Channels Annotated Bibliography**

Peer Reviewed Journal Sources

Brunet, L. J., Gold, G. H., & Ngai, J. (1996). General anosmia caused by a targeted disruption of the mouse olfactory cyclic nucleotide-gated cation channel. *Neuron*, *17*(4), 681–693. [https://doi.org/10.1016/s0896-6273(00)80200-7](https://doi.org/10.1016/s0896-6273%2800%2980200-7)

Cyclic AMP (cAMP) and inositol trisphosphate (IP3) serve as secondary messengers in the olfactory signaling cascade. This paper aims to uncover the role of cAMP in the signaling cascade utilizing a cyclic nucleotide gated channel (CNG) knockout mouse model. In a normally functioning olfactory system, an odorant binds to a G protein-coupled receptor which results in G-protein (Golf) activation and subsequent adenylyl cyclase III activation. Adenylyl cyclase III activity induces increased cAMP levels within the cytosol. cAMP is then free to bind to a CNG allowing for both sodium and calcium influx into the cell and depolarization. Thus, the use of a CNG KO mouse model elucidates the role of cAMP in olfactory signal transduction. Electroolfactogram recordings showed a complete absence of olfactory responses, regardless of the odor presented, within the CNG KO model.

Firestein, S., Zufall, F., Shepherd, GM. Single odor-sensitive channels in olfactory receptor neurons are also gated by cyclic nucleotides. *Journal of Neuroscience, 11 (11),* 3565-3572*.* <https://doi.org/10.1523/JNEUROSCI.11-11-03565.1991>

Cyclic nucleotide gated channels are found in low density within the dendritic membrane of olfactory sensory neurons. A cell-attached configuration, where a tight giga-ohm seal is maintained between the recording pipette and the cell, was used to determine CNG channel roles in olfactory transduction. A cell-attached patch clamp technique was beneficial to measure a single channel located under the pipette tip area without changing cytosolic content. 150ms odorant pulses produced activity in the majority of membrane patches. Once odorant evoked activity was established in a given membrane patch, IBMX was added to the bath. IBMX increases intracellular cAMP concentrations by blocking secondary messenger inactivation. Similar levels of activity to that in response to odorant exposure were evoked in the patch of membrane following IBMX introduction indicating that the channels located on the dendritic membrane of OSNs were CNG.

Menini, A. (1999). Calcium signalling and regulation in olfactory neurons. *Current Opinion in Neurobiology, 9 (4),* 419-426*.* [https://doi.org/10.1016/S0959-4388(99)80063-4](https://doi.org/10.1016/S0959-4388%2899%2980063-4)

Olfactory receptors are localized within olfactory sensory neuron cilia. Olfactory cilia contains a high concentration of calcium ions and calcium-activated chloride channels. Secondary messenger activity results in the influx of calcium and sodium into the olfactory cilia and subsequent calcium-activated chloride channel opening. Calcium-activated chloride channels then allow an efflux of chloride ions and further depolarization of the cell. This review provides an analysis of the experiments performed prior to 1999 to understand calcium’s role in olfactory sensory neuron excitation.

Okada, Y., Miyamoto, T., & Toda, K. (2003). Dopamine modulates a voltage-gated calcium channel in rat olfactory receptor neurons. *Brain research*, *968*(2), 248–255. [https://doi.org/10.1016/s0006-8993(03)02267-4](https://doi.org/10.1016/s0006-8993%2803%2902267-4)

 Dopamine receptors have been found within rat olfactory sensory neuron terminals and

cell bodies suggesting a possible modulatory role of dopamine in the olfactory signaling cascade. Using whole-cell patch clamp and a 70ms ramp protocol, barium currents from -50mV to +50mV were readily apparent. However, the addition of nifedipine, an L-type calcium channel blocker, significantly reduced barium current indicating that L-type calcium channels were responsible for the calcium current in rat olfactory sensory neurons. Dopamine exposure also significantly reduced calcium current. Therefore, it appears that dopamine may modulate L-type calcium channel activity within rat OSNs.

Pietra, G., Dibattista, M., Menini, A., Reisert, J., & Boccaccio, A. (2016). The Ca2+-activated Cl- channel TMEM16B regulates action potential firing and axonal targeting in olfactory sensory neurons. *The Journal of general physiology*, *148*(4), 293–311. <https://doi.org/10.1085/jgp.201611622>

TMEM16B is a prominent calcium-activated chloride channel within the cilia of OSNs and has been found to account for 90% of transduction current. TMEM16B knockout mice display altered olfactory behavior compared to WT mice. Interestingly, a buried cookie assay performed showed the KO mice had an inability to recognize new odors without a change in the ability to respond to a given odor. KO mice had significantly lower transduction currents than wildtype mice stimulated with IBMX, a useful alternative to odor stimulation. The addition of NFA, a chloride channel blocker, was not able to further reduce transduction current in the KO mice indicating that the smaller current seen was a result of the CNG current and not the calcium-activated chloride channel.

Pifferi, S., Boccaccio, A., Menini,A. (2006). Cyclic nucleotide-gated ion channels in sensory transduction. *FEBS letters*, 580(12), 2853-2859. <https://doi.org/10.1016/j.febslet.2006.03.086>

Cyclic nucleotide-gated channels (CNGs) are prominent within both the olfactory and visual sensory systems. CNGs are activated through the binding of cyclic nucleotides such as cAMP or cGMP and are weakly modulated by membrane voltage. However, CNG structure is similar to that of voltage gated channels with four subunits each consisting of six transmembrane domains. CNGs can be negatively regulated by a calcium and calmodulin complex resulting in OSN adaptation.

Simms, B. A., & Zamponi, G. W. (2014). Neuronal voltage-gated calcium channels: structure, function, and dysfunction. *Neuron*, *82*(1), 24–45. <https://doi.org/10.1016/j.neuron.2014.03.016>

Voltage-gated calcium channels are located within most excitable cells and play prominent roles in several physiological functions such as excitation-contraction coupling, growth regulation, cardiac, neuronal pacemaker activities and stimulation-secretion coupling. High voltage- gated calcium channels open following large changes in membrane potential while low voltage-gated calcium channels do not require large membrane potential alterations. Within the low and high voltage-gated calcium channel families are several subtypes of voltage-gated calcium channels including L-type, P/Q-type, N-type and T-type. High voltage-gated calcium channels consist of pore-forming alpha unit and corresponding ancillary beta subunits. However, low voltage-gated channels are lacking ancillary beta subunits.

Wetzel, C.H., Spehr, M., Hatt, H. (2001). Phosphorylation of voltage-gated ion channels in rat olfactory receptor neurons. *European Journal of Neuroscience, 14 (7),* 1056-1064*.* [**https://doi.org/10.1046/j.0953-816x.2001.01722.x**](https://doi.org/10.1046/j.0953-816x.2001.01722.x)

PKA, protein kinase A, is a family of enzymes that is activated by high levels of cAMP. While cAMP activates a CNG channel and calcium and sodium influx into the cell to encourage depolarization, PKA is subsequently activated and may have a modulatory role within the olfactory transduction pathway at the level of the CNG channel. Odorant adaptation occurs when odorant perception is shifted and olfactory responses are lessened in response to a repeatedly exposed odor. This study aims to identify if PKA may modulate CNG channel activity and induce odor adaptation. Protein kinase A inhibitor exposure resulted in decreased CNG channel activity exemplifying that PKA may have a negative regulatory effect on CNG activity even in high concentrations of cAMP and be responsible for odor adaptation.

Zak, J.D., Grimaud, J., Li, R., Lin, C., Murthy, V.N. (2018). Calcium-activated chloride channels clamp odor-evoked spike activity in olfactory receptor neurons. *Scientific Reports, 8.*

 Ano2 is a calcium-activated chloride channel also known as TMEM16B. This paper analyzes the role of the same channel as the paper by Peitra *et al*. *In vivo* imaging was used to view activity of olfactory receptor axon projections to the olfactory bulb. Mice with the channel KO performed significantly worse compared to controls on an open field olfactory behavior test in which latency to find a localized odor source was measured. Interestingly, when odor evoked responses were mapped, Ano2 KO mice exhibited increased calcium signals and signals from the olfactory sensory neurons to the glomeruli were enhanced. This paper was interesting as it shows findings that are not in support of that found by Pietra *et al.*

Zufall, F., Firestein, S., & Shepherd, G. M. (1991). Analysis of single cyclic nucleotide-gated channels in olfactory receptor cells. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *11*(11), 3573–3580. <https://doi.org/10.1523/JNEUROSCI.11-11-03573.1991>

 This is the accompanying research article to the Firestein, Zufall and Shepherd research article referenced above. Olfactory sensory neurons were isolated from salamanders and used to compare olfactory channel sensitivity to that of what was known of photoreceptors. Photoreceptors are insensitive to cAMP but respond to cGMP. Zufall, Firestein and Shepherd

utilized inside-out patch clamp techniques to evaluate cyclic nucleotide gated channel activity

within OSNs in response to cAMP. Inside-out patch clamp techniques involve the attachment

of a small piece of membrane to the recording pipette tip and the exposure of the cytosolic

surface of the membrane. This patch clamp arrangement is advantageous when one wants to

analyze how cytosolic events affect excitability. Due to cyclic nucleotides binding on the

cytosolic side, bath solutions containing cAMP and cGMP can be readily used and swapped.

OSNs respond to both cAMP and cGMP and do not experience channel desensitization

following increased cAMP concentrations.

URLs

Calcium Channel Blockers: <https://www.mayoclinic.org/diseases-conditions/high-blood-pressure/in-depth/calcium-channel-blockers/art-20047605>

Calcium channel blockers are often clinically used to lower blood pressure by inducing blood vessel relaxation and sometimes a slowing of heart rate. This webpage presented by MayoClinic provides an overview of the clinical uses of calcium channel blockers and the side effects associated with each.

Cayman Chemical – Non-specific inhibitor of cAMP and cGMP phosphodiesterases

Cayman Chemical is a laboratory supply company where IBMX can be purchased. This link is a purchasing link for IBMX and includes a functional overview of IBMX, pricing, technical information and a safety data sheet.

Electrophysiology – The Patch Clamp Technique: <http://www.personal.psu.edu/sma3/pdf/electro.pdf>

This weblink was provided by a professor at Pennslyvania State University and outlines the functional definitions of the various types of patch clamp electrophysiology. It also provides other useful links for understanding how to evaluate patch clamp data.

GPCR: <https://www.nature.com/scitable/topicpage/gpcr-14047471/>

G protein-coupled receptors (GPCR) are receptors located on the plasma membrane that interact with G-proteins. The mammalian odorant receptor genes are the largest group of GPCRs. This resource outlines the molecular function of GPCRs and the activation of secondary messengers.

Guide to Pharmacology: <https://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=689>

This resource gives an overview of ion channels as a whole and allows selective filtration of specific ion channel types. Once a channel is selected, it allows the choosing of a channel subtype and viewing of respective nomenclature, genes, gating inhibitors and channel blockers specific to the subtype.

How to…Whole Cell Patch Clamp: <https://www.axolbio.com/page/whole-cell-patch-clamp-protocol>

This website provides a comprehensive protocol for whole-cell patch clamp techniques. This resource provided necessary background to understand many of the primary literature’s methodology.

Human Physiology - cAMP Second Messenger: <https://www.youtube.com/watch?v=GskbODSxAU8>

This is a short 2-minute animation that outlines the olfactory signaling cascade and focuses on the molecular function of cAMP.

Kegg Olfactory transduction – Homo sapiens (human): <https://www.genome.jp/kegg-bin/show_pathway?hsa04740#:~:text=Within%20the%20compact%20cilia%20of,be%20transmitted%20to%20the%20brain.>

This is a pictogram of the olfactory transduction pathway within humans. It illustrates the pathway that occurs during initial odor presentation and during recovery and adaptation.

Scientifica – Using cell-attached patch clamp to monitor neuronal activity: <https://www.scientifica.uk.com/neurowire/using-cell-attached-patch-clamp-to-monitor-neuronal-activity>

This resource was provided by Dr. Vincenzo Marra and details cell-attached patch clamp techniques. Answers to common questions are outlined and pictures are provided to visualize sources of resistance within the patch clamp circuit.

Wikipedia – IBMX: <https://en.wikipedia.org/wiki/IBMX>

This Wikipedia article briefly summarizes the functional definition of IBMX and links additional scientific resources pertaining to phosphodiesterases.

Wikipedia – L-type calcium channel: <https://en.wikipedia.org/wiki/L-type_calcium_channel>

This webpage provides an overview of L-type calcium channels and their functional and structural significances.

Wikipedia – Protein kinase A: <https://en.wikipedia.org/wiki/Protein_kinase_A>

 This Wikipedia article explains the molecular role of protein kinase A, an enzyme dependent on cAMP.