
Central unanswered question in olfaction research is the role of inhibition presynaptic to the CNS in the coding of odorant stimuli. It is important to understand the functional organization of inhibition to glomeruli in order understand how odorant responses are modulated based on multiple inputs to one glomerulus or among neighboring glomeruli. Presynaptic inhibition between olfactory sensory neurons (OSNs) converging on the same glomerulus is termed “intraglomerular” inhibition, while presynaptic inhibition between OSNs converging on different neighboring glomeruli is termed “interglomerular” (lateral) inhibition.

McGann et al. present a study in which they attempt to answer these questions by using a technique to image the release of vesicles containing neurotransmitter at the synapse between OSNs and a glomerulus. They use synapto-pHluorin (spH), a protein composed of a pH-sensitive GFP variant and VAMP-2a, to report synaptic vesicle fusion. They create transgenic mice that express spH in all mature OSNs. This way, when OSNs are activated by either electrical stimulation or odorant presence, input to glomeruli can be visualized by an increase in fluorescence. This method allows for chronic in vivo imaging of glomerular activation.

First they use in vitro slice preparations from these mice to characterize the effects of presynaptic intra- and interglomerular inhibition. In order to investigate intraglomerular inhibition, they locate two OSNs that project to the same glomerulus and test paired-pulse stimulation responses using concentric bipolar electrodes. They find significant paired-pulse depression (PPD) when the OSN axons are successively stimulated. This depression is highest at an inter-stimulus interval of 100ms. This PPD was reduced by blockers of ionotropic glutamate receptors and GABA<sub>B</sub> receptors.

Next they look at interglomerular inhibition by stimulating one axon that converges on a glomerulus, then stimulating another axon that converges on a different glomerulus. PPD was only seen for glomeruli that were less than 400um apart. This inhibition was similarly eliminated by blocking ionotropic glutamate and GABA<sub>B</sub> receptors.

They then ask how these types of inhibition affect odorant representation in vivo. They present odors to anesthetized mice and image fluorescence in the olfactory bulb through thinned bone. In order to look at interglomerular inhibition, they focus on two neighboring glomeruli that are activated by different odorants. They present a mixture of these two odorants and find that neither glomerulus is inhibited by the activation of the other. The responses of one glomerulus to just its preferred odorant and to a mixture of that odorant and another are identical, even when the two glomeruli are less than 400um apart. Therefore lateral interglomerular inhibition does not affect the sensory input to a glomerulus.

To investigate intraglomerular inhibition in vivo, they compare responses of one glomerulus to an odorant in the presence and absence of intraglomerular inhibition. They remove this inhibition by blocking GABA<sub>B</sub> receptors, since they discovered in their previous experiments that GABA<sub>B</sub> receptors were responsible for intraglomerular inhibition. In the presence of CGP35348, the magnitude of response increased but the spatial maps of response remained the same.

The authors conclude that interglomerular inhibition has no effect on odorant representation and that intraglomerular inhibition modulates the amplitude of the odorant representation, but does
not affect the representation (spatial map) itself. They reason that the role of feedback presynaptic inhibition might be to prevent saturation of the glomerulus, something that could easily happen since there are many OSNs converging on one glomerulus, each with a very high release probability. This prevention of saturation in turn could reduce responses during sustained sniffing and enhance the responsiveness of mitral and tufted cells.

The topic of this paper is of great interest to the field because it addresses the question of how odor information is modified by inhibitory circuits surrounding the first synapse in olfactory processing. One of the most important contributions of this study is the demonstration that transgenic spH mice can be used to measure presynaptic inhibition, something that was not previously possible. Despite the complex circuitry of this inhibition, the only role that the authors find is control of the strength of olfactory input to the CNS. This is surprising to anyone who appreciates the intricacy of the feedback circuitry at the level of the OSN-glomerulus synapse, and therefore requires further investigation before publication in *Neuron*.

**Major issues requiring revision:**

1. These results seem to be specific to the particular method used in this paper to quantify glomerular response levels. Another group, Vučinić et al. (2005), has reported different results using presynaptic calcium imaging as their readout of activity. They see that blocking GABA<sub>B</sub> receptor-mediated presynaptic inhibition in vivo caused small changes in maps of OSN input to glomeruli. Possible disadvantages of spH must be considered, like slow kinetics due to the slow process of endocytosis. This could create an illusion of increased signal in vivo during odorant stimulation due to summation of the spH signal. Perhaps this contributes to the observation that intraglomerular inhibition results in modification of the strength of the signal in vivo.

2. There is much discussion in this paper of what causes the complex kinetics of the fluorescent signal changes upon activation of OSN axons. Figure 1 is almost entirely devoted to looking at this question, and ultimately shows that pH changes in the synaptic cleft due to neurotransmitter release and uptake are responsible for these intricacies in the kinetics of the response. It is unclear why this topic is given so much attention in the beginning of the paper, as efforts to reduce these effects are not taken during the experiments. This could instead be supplementary data or simply mentioned in the text to explain why they chose to use peak spH amplitude to measure the presynaptic modulation of neurotransmitter release from OSNs, which is perhaps why they felt compelled to include the analysis in the paper in the first place.

**Minor issues requiring revision:**

1. A possible explanation for why they saw lateral inhibition in vitro (in the form of paired-pulse depression) and not in vivo is mentioned in the discussion. The authors propose that lateral inhibition is more diffuse because it is indirect excitatory input. They suggest that lateral inhibition plays more of a role in affecting odorant representation at the level of output from the glomerulus. This still does not explain why they saw paired-pulse depression when they stimulated one axon that converged on a glomerulus and then stimulated another axon that converged on a different glomerulus.

2. It must be considered that the in vivo experiments performed in this paper are done on anesthetized mice while inhalation of the odors is being controlled by an artificial sniffing protocol. The role of presynaptic inhibition in odorant representation may be completely different during normal sampling of odorants.
3. It could be helpful to include a circuitry diagram of the connections surrounding glomeruli. Proposing a model to explain the results in this study would aid in visualizing the connections and roles of interneurons in this circuit.