THE NOVEL ROLE OF GLIA IN ODOURANT DETECTION AND OLFACTORY ADAPTATION

BSE656 – ENDTERM PAPER REVIEW – TEAM 1

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Introduction

The olfactory system is one of the primary senses. It is the pathway that enables the perception of smell on the detection of volatile chemical compounds. It served an essential evolutionary purpose in organisms and was one of the earliest developed senses as indicated by its prevalence and importance in primitive (lower) organisms.

A variety of Olfactory Receptor Neurons (ORNs) are responsible for detecting chemosensory inputs. (Su et al., 2009, p. 48) The olfactory system receives a myriad of sensory input due to the vast possibility of diversity in chemical compounds. Thus, it utilises combinatorial coding and adaptation among the many methods to analyse the inputs effectively. The existence of glial cells had been performed earlier. However, they were previously thought to have been limited to structural and supportive roles. Recent development has shown that they may have more critical functions that may affect sensation and perception. In the reported paper, the authors have described their findings of the role of glial cells in olfactory perception.

Background

Glia has been widely studied as a non-neuronal supporting structure with functions such as maintaining homeostasis, immune function, extending trophic, structural, and metabolic support, neurodegeneration, and many other diverse operations specifically performed by the subtypes of glia. Recent studies have shown the interaction of glia, and the neuronal component plays an active role in the structural development of brain and function, memory formation, identity, as well as the role of glia in somatosensory processing, which is also apparent from the breakdown of function in the presence of defected glia (Abdo et al., 2019, p. 698; Bacaj et al., 2008, p. 745; Kol et al., 2020, p. 1231; Gibson et al., 2018; Adamsky et al., 2018; Yadav et al., 2019, p. 5128). There is extreme heterogeneity of structure and function of glia, as a result of which the molecular basis of neuronal-glial interactions and defined mapping of a glial subtype with physiological role remains to be completely identified. Glia is also present in the olfactory system of various species. Still, their interaction with the sensory machinery, driving olfactory adaptation and detection, has not yet been seen, especially in the peripheral sensory system. (Munger et al., 2009, p. 132)

The glia-neuronal interactions and the basic olfactory system are conserved across species to some extent. *Caenorhabditis elegans* is one such model organism for studying neural connections, glia interaction, structure, and function, as well as olfaction (Singhvi & Shaham, 2019, p. 167). Most of the neural connections in C. elegans have been identified, and glial as well as neuronal subtypes have been classified in previous studies. (Cook et al., 2019, p. 64; Yadav et al., 2019, p. 5128) It is possible to use C. elegans as a model for glia studies as it shares physiological traits with vertebrate glia in the molecular and functional aspects. Several studies have shown the function and impact of defects in C. elegans glia on behaviour across sensory modalities, along with neural circuit assembly and even neuronal degeneration (Singhvi et al., 2016, p. 946). It has been shown that there are odorant selective genes and neurons in C. elegans which detect volatile chemical compounds that may act as an attractant at a low concentration and as a repellent at a high concentration (Bargmann et al., 1993, p. 524).

ASH sensory neuron is a polymodal, largely nociceptor which has a role in the avoidance of aversive volatile chemical compounds among other stimuli, observed by intracellular Calcium responses to stimuli. Adaptation has also been observed in the ASH sensory neuron with a focus on GPC-1 (Hilliard et al., 2004, p. 71).

Olfactory adaptation is the mechanism that results in reduced stimulus-specific responses upon prolonged exposure (Colbert & Bargmann, 1995, p. 804). This study aims to show that Amphid Sheath Glial Cell (AMsh), which functions as a

sheath to various sensory neurons of which ASH neurons are one, also plays a role in chemosensory response to aversive stimuli and olfactory adaptation.

Problem Solved

In most species, the sensory neurons are often accompanied by glial cells. Previously most research was carried out on the sensory neurons themselves, and they received much of the spotlight. The role of other neural mechanisms in olfactory adaptation apart from those associated with ORNs and OSM-9 protein had not yet been identified (Zufall, 2000, p. 476). It is only recently that it was realised that the glial cells might also have a role to play in sensation. However, it has been challenging to figure out the exact role that these glial cells play, for they show extraordinary heterogeneity.

The authors of the paper investigated the amphid sheath (AMsh), a glial cell that is present around the sensory olfactory neuron in C. elegans. It was found that AMsh has chemoreceptors that can detect odour molecules. The genes specific to these chemoreceptors were identified. The role of these glial cells in chemotaxis and olfactory adaptation was demonstrated.

How does the AMsh glia regulate adaptation? The authors have hypothesised a new bi-receptor model where the glia and olfactory neurons work in unison to mediate olfactory adaptation, via a calcium-dependent process, associated with AWC and ASH neurons and the OSM-9 protein.

Results and Implications

When the nose of C. elegans was perfused with high concentrations of the aversive odour - isoamyl alcohol (IAA), a large increase in the calcium concentration in the AMsh glia was observed by using GCaMP 5.0 reporter (genetically encoded Calcium indicator). Laser ablation of the ASH neurons or the ASH activation by capsaicin did not affect AMsh glia's response to IAA. Besides, single-cell embryonic culture displayed similar responses to IAA and 1-octanol. Thus, the author concluded that the AMsh Glia's response to aversive odours is independent of the ASH neurons.

By screening the odour receptor pairs using RNAi feeding protocol, it became possible to predict the GPCR involved in the detection of IAA in both ASH and AMsh. When srh-79 was reduced globally or specifically in the glia, the calcium response was significantly reduced. Calcium responses were also found to be low in the case of the mutant strain srh-79(kan-16) whose functionality could be restored by over-expressing SRH-79. Thus, it was concluded that SRH-79 was the GPCR responsible for IAA detection in the glia, and similarly, the GPCR SRH-61 was found responsible for IAA detection in ASH.

To singularly observe the effects of AMsh glia, it was activated by a blue light targeting CoChR specific to AMsh glia. Already activated AMsh glia showed a late response upon exposure to

IAA and 1-octanol. This nevertheless suggested that pre-activation of AMsh glia habituates C. elegans of aversive odorants. After ablation of ASH neurons when the AMsh glia was activated with blue light, it did not affect the avoidance behaviour towards IAA and 1-octanol, implying that the AMsh glia works by suppressing the ASH neuron. The locomotive aspects of the avoidance behaviour such as head withdrawals and omega turns were only affected when strong intensities of lights were used explaining reversal latency being the only affected upon exposure to aversive odorants.

SRH-79, an IAA receptor, is found in AMsh glia and not in ASH neurons. *srh-79* mutant worms showed reduced adaptive behaviour towards IAA but not 1-octanol. In wild worms, response latencies corresponding to 1-octanol were affected on prior exposure of IAA. When the worms were exposed to IAA wild worms were able to adapt to the aversive IAA but not the *srh-79* mutant worms which exhibited small range for navigation. In *srh-79* mutant worms, the adaptation for the calcium responses resulting from IAA exposure was found to be reduced but not for 1-octanol. *itr-1*, *gpa-3* and *osm-9* are RNAi specific to AMsh glia. They were found to reduce the behavioural adaptation when the worms were repetitively exposed to IAA or 1-octanol. All the above-mentioned observations point towards the notion that the AMsh glia plays a significant role in adapting to aversive smells by inhibiting ASH neurons.

By using similar procedures, it was found that ODR-3 and GPA-3 were the Gα subunits involved in the IAA detection in the ASH neuron and the AMsh glia, respectively. The TRPV channel (calcium selective channel) OSM-9 was found to be involved in IAA detection downstream of the Gα subunits in both the glia and the neuron. Besides, ITR-1 was also found to be important in the glia for IAA detection. Thus, it was concluded that multiple pathways could possibly exist for detecting aversive odours in the glia.

AMsh glia's adaptation is carried out by GABAergic signalling. GABAergic means that the glia uses GABA as a neurotransmitter to convey signals. The author screened different neurotransmitters and found that the signals and suppression on ASH neurons were lost in cases where there was a loss of function mutation or when AMsh specific RNAi of unc-25. The gene unc-25 encodes for glutamic acid decarboxylase. This encoded GAD is necessary for synaptic transmission (Jin et al., 1999, p. 546). The adaptation was restored when UNC-25 was specifically restored. In the absence of the GABA signalling, optogenetic activation of AMsh and olfactory adaptation to 1-octanol did not produce any calcium transient response in ASH neurons. On exogenous GABA suppression, the 1-octanol calcium response in ASH was significantly reduced, confirming that GABA is crucial for glia signalling to ASH neurons.

Furthermore, the extracellular concentration of chloride ions is key for GABA-induced hyperpolarisation. KCC-3 is a cotransporter of KCl, which is exclusively present in glial cells. In kcc-3 mutants, optogenetic activation did not result in

calcium responses or any adaptation to 1-octanol, supporting the hypothesis.

Similar to the aforementioned approach in AMsh glia, the author found that AMsh glia mediated adaptation of 1-octanol was eliminated in ASH for lgc-38 mutant or when ASH specific lgc-38 RNAi. In these conditions, the exogenous GABA suppression was also greatly reduced, proving that the GABA receptor LGC-38 is essential in ASH neurons for AMsh glia signalled adaptation.

Critical Comments

Overall, the paper was a very balanced and informative study. It successfully conveys the idea that glial cells may have a function beyond what they are classically known for. In the process, the authors reported having found the new genes srh-79, osm-9 and gpa-3 in the AMsh glia. However, these genes are specific to one primary chemical IAA. Since the paper focused solely on IAA and 1-octanol, we need to confirm the hypothesis before extrapolating if for other sensations and with a different chemical stimulus, to generalize the function of glial cells.

The paper is focused on mechanisms of AMsh glia which affect the activities of ASH neurons in reference to olfactory aversion and adaptation. It however does not explore the possibility of mechanisms in ASH neurons affecting the processes of AMsh glia nor does it rule out the possibility for the same. This refrains us from drawing out clear mappings of cause and effect in the AMsh glia and ASH neuron pair.

The paper used methods comprehensively and has explained all relevant details about the same. The methodology utilised consisted of some of the sophisticated techniques like use of optogenetic assays, behavioural assays, calcium imaging, CRISPR/Cas9-based knockout, Feeding as well as cell specific RNAi, Laser Ablation, RT-PCR and Tissue-specific Split SfGFP.

There were multiple tests done to confirm each fact. The paper is mostly close-ended and covers all potentially immediate follow-up questions that may arise.

Future Prospects

In the case of vertebrates, at least three distinct mechanisms of olfactory adaptation have been identified in ORNs (Zufall, 2000, p. 476). Future studies should focus on investigating mechanisms related to other aspects of the neural circuit involved in olfactory processing as well specifically highlight the role of glia in detection and adaptation of olfactory inputs. Conclusions drawn from research in model organisms can provide the first insights into such studies which can be further expanded till the entire sensory processing pathway can be modelled.

The specific pathway of olfactory adaptation and detection by AMsh involving ASH neurons and GABA has to be investigated to gain insight into the exact functional role of glia in

modulation, sensing and possibly transduction. Modelling this neurotransmitter-based mechanism should be the step forward once base pathways have been investigated. This will pave the way to further research into the functional correlation of glial and neuronal subtypes based on mechanism and expand the scope of glial-neuronal interactions.

The role of AMsh glia can be extended to involve GABA inhibition across other sensory neurons (receptors) in the olfactory system as well.

ASH neurons primarily act as nociceptors, mediating avoidance behaviour to aversive stimuli apart from volatile chemical compounds too. These stimuli range from water-soluble repellents to mechanosensory inputs. The interaction and modulation behaviour of associated glial subtypes (sheathing as well as non-sheathing) including AMsh, with ASH neurons, can also be investigated in the presence of such stimuli, expanding from the conclusions and mechanisms drawn from the olfactory system.

The GABAergic system is one of the primary inhibitory neurotransmitter systems. This study opens the avenue of studying the role of GABA in the modulation of somatosensory signals in other glial-neuronal interaction systems too in the olfactory and mechanosensory systems and possibly even across other sensory modalities.

The olfactory system involves combinatorial coding for processing of input stimuli. The receptor and receptor genes were identified to be aversive to one particular odour and thus hints at the possibility of specialised receptors and genes coding for them. Future studies should attempt to investigate the presence of these receptors, as highlighted above, to gain insight into the combinatorial coding mechanism, which may or may not be uniform across glia and neurons.

Conclusion

This paper improves our understanding of olfactory reception by presenting a new two receptor sensory model of avoidance behaviour and also demonstrating the role of Glial cells in adaptation. Since odorant and receptors form a combinatorial code, it is difficult to isolate a particular receptor for an odour. The author was successfully able to find two GPCRs (SRH-79 and SRH-61) which act as receptor genes for the same odour (IAA) in AMsh glia and ASH respectively. The author further expanded on the properties of AMsh glial cells and proved that glia is essential for inhibitory adaptation and regulation in ASH neurons. This adaptation takes place by GABA signalling, which is specific to the ASH neuron. These functions of glial cells open up an entirely new interpretation of olfactory sensation, reception and adaptation. By developing on these results, and extrapolating certain functions, we can get a much better understanding of glial cells apart from their classical attributes. These findings and the evidence provided ultimately improved our understanding of sensory systems in general and olfactory adaptations in particular.

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