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Modulation in neural representation of geniculate ganglion by glial-like taste cells

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Abstract

Modulation in neural representation of geniculate ganglion by glial-like taste cells

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Recognition of the five basic tastes is processed by taste cells in a taste bud. Of these cells, glial-like type I cells which do not express taste receptors account for 80% of the taste bud. Many scientists have believed that taste cells interact with each other through neurotransmitters. However, type I cells, of three types of taste cells that do not have receptors for taste substances to respond indirectly to them, have not yet found their functions. Here, this study shows that the taste information modulated by activation of type I cells is shown in the geniculate ganglion where the cell bodies of the nerves converge by using the calcium imaging technique. First of all, I updated the previously described protocol for more stable and long-term calcium imaging of the geniculate ganglion *in vivo* by simplifying the steps of surgery. Then, by using the results of the specific purinergic receptor, P2Y2 receptor, expression in type I cells from the RNA data in our laboratory, its specific agonist Diquafosol was used to activate type I cells. 40 mM Diquafosol in physiological saline showed a positive effect on the sweet taste at the neural level. Also, as oxytocin can induce strong

activation in type I cells, oxytocin was chosen as another agonist for type I cells, but the effect was heterogeneous. These results demonstrate that the activation by the purinergic receptor from type I cells is likely to modulate taste transmission but that by the oxytocin receptor is ambiguous. I anticipate that these approaches show the possibility of a modulatory function of type I cells in a taste bud through purinergic signaling rather.

Keyword : geniculate ganglion, glial-like taste cell, diquafosol, oxytocin, in vivo, calcium imaging

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Chapter 1. Introduction

1.1. Study Background

Recognition of five basic taste sensations, sweet, bitter, salty, sour, and umami, starts from taste cells in the taste buds of the tongue. In a taste bud, three types of taste cells have different functions and structural features (Figure 2); type I cells which are called glial-like taste cells occupy most of the space in the taste bud. They express Nucleoside Triphosphate Diphosphohydrolase-2 (NTPDase2) and glutamate/aspartate transporter (GLAST) on their membrane (Roper and Chaudhari, 2017). NTPDase2 hydrolyzes ATP, which is the central neurotransmitter in taste transmission, released from type II cells for clearing the neurotransmitter to prevent desensitization from the gustatory nerve.; type II cells, also called receptor cells, express G protein-coupled receptor (GPCR) recognizing sweet, bitter, or umami taste substances and have unconventional synapses with gustatory nerves; type III cells, also known as presynaptic cells, have conventional synapses with the nerves and recognize the sour or maybe salty sensation (Roper and Chaudhari, 2017). In taste buds, many kinds of neurotransmitters were found during taste stimulation; not only ATP, but also serotonin (5-HT), norepinephrine, acetylcholine, and GABA (Huang, Maruyama and Roper, 2008; Huang, Pereira and Roper, 2011; Dando *et al.*, 2012). Therefore, there have been many suggestions about the interaction between taste cells (Yamamoto et al., 1998; Roper, 2021). A recent study showed that serotonergic

type III cells activated by optogenetically by expressing the channel rhodopsin 2 (ChR2) inhibited the response of sweet and bitter responses in chorda tympani nerve recording (Vandenbeuch, Wilson and Kinnamon, 2020). This research showed the interaction between type II And III cells, which explains the inhibitory function of type III cells by releasing GABA and 5-HT (Figure 3. Feedforward and feedback signaling in mammalian taste buds is summarized in the following schematic diagram.). Although glial-like supportive type I cells are the most numerous cells in the taste bud, few their function are known. However, because of the structural similarity to glia cells in the CNS, type I cells are thought to cooperate structurally and functionally with surrounding type II cells and afferent fibers like astrocytes. Type I cells showed mobilizing intracellular Ca^{2+} coordinated with taste signals in time and intensity, indicating that the interaction between taste cells in the taste bud (Han and Choi, 2018; Rodriguez et al., 2021). This calcium activity was eliminated in plcβ2-KO mouse that cannot release ATP from type II cell when sweet, bitter, and umami taste compounds associate with each taste receptors in previous data in our laboratory. Accordingly, this calcium activation would be likely for type I cells to participate in shaping the sensory output of taste buds via ATP signaling.

Fungiform papillae in the anterior tongue and palate are innervated by the chorda tympani nerves which constitute cell bodies in the geniculate ganglion. The geniculate ganglion is a relay station of this taste transmission from the periphery structure to the central nervous system (Figure 1). For showing the results of interaction between taste cells, recording the geniculate ganglion activity is a key method. Previous studies have used nerve recording in chorda tympani (Lundy, And and Contreras, 1999; Sollars *et al.*, 2005; Chen, Travers and Travers, 2016; Yokota and Bradley, 2017) and recently calcium imaging of the geniculate ganglion is used

as a more convenient tool for recording the activity of a single-cell body (Wu *et al.*, 2015; Zhang *et al.*, 2019; Fowler and Macpherson, 2021). The geniculate ganglion is located in the deep brain area, so the gradient refractive index (GRIN) lens can be selectively used (Barretto *et al.*, 2015). In the present study, the simplified steps for the stable preparation of the mouse sample will be introduced and several types of kinetics in the neurons were observed never seen before.

1.2. Purpose of Research

The present study aimed to determine whether the modulation of glial-like type I taste cells makes some difference in neural representation. Type I cells have shown calcium responses to taste substances not on time but at delayed time points coordinated with taste signals (Han and Choi, 2018). This makes us hypothesize that this delayed calcium response might have a role in processing the information within the taste buds. ATP could activate type I cells (Sinclair *et al.*, 2010; Rodriguez *et al.*, 2021), and recently the subtype of the specific purinergic receptor has been shown as P2Y2 receptor from our laboratory. So, on based on this result, this study used the P2Y2-specific agonist, Diquafosol (DQ), for the over-activation of type I cells. Moreover, oxytocin (OXT), which is the hormone that stimulates uterine contractions in labor and childbirth, was selected as another agonist, based on the previous study that demonstrated the oxytocin receptor (OXTR) is expressed in the type I cells (Sinclair et al., 2015, 2010). Also, our laboratory showed that OXT

induces strong calcium responses *in vivo*. Taken all together if these over-activations of type I cells modulate the taste information at the cellular level, I expect that the possibility of the recognition spectrum of chemicals in the human would be more diverse.

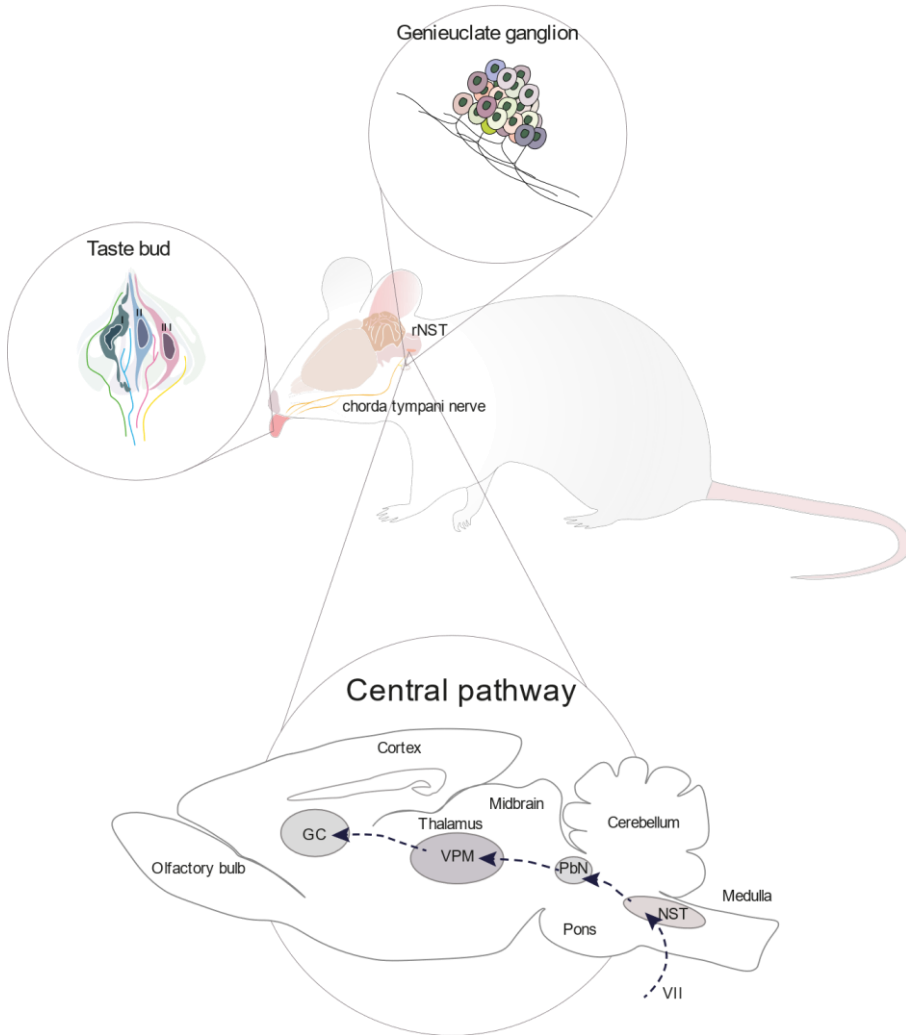

Figure 1. Illustration of taste signaling peripheral and central pathway.
In the peripheral level, taste signals start from the taste cells in the taste bud to the rostral nucleus of the solitary tract (rNST) via the geniculate ganglion. And taste responses are transmitted centrally through the parabrachial nucleus (PbN) and ventral posteromedial nucleus (VPM) in the thalamus to the primary gustatory cortex (GC) in the insula.

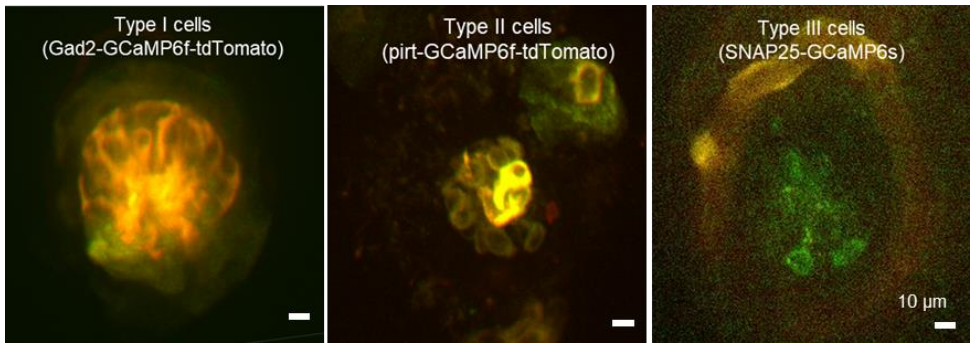

Figure 2. Fluorescent images of three types of taste cells.
Three types of taste cells imaging from different transgenic mice (type I cell: GAD2-GCaMP6f-tdTomato; type II cell: pirt-GCaMP6f-tdTomato; type III cell: SNAP25-GCaMP6s)

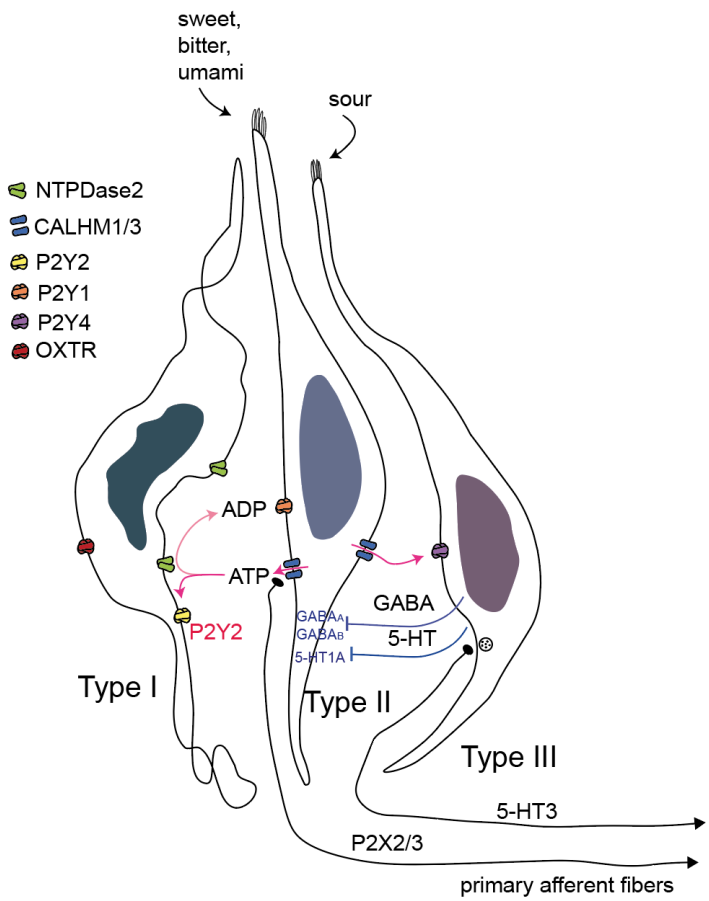

Figure 3. Feedforward and feedback signaling in mammalian taste buds is summarized in the following schematic diagram.
Type I cell express NTPDase2 on their surface that degrades ATP released during taste excitation. Type II cells express GPCR for sweet, bitter or umami taste substances. ATP excites gustatory afferent fibers, neighboring type III cells and type I cells. Type III cells release GABA when stimulated by sour tastants. GABA and 5-HT from type III cells inhibit type II cells.

Chapter 2. Results

2.1. Preparation of calcium imaging of geniculate ganglion *in vivo* system

For *in vivo* calcium imaging of the geniculate ganglion, the stable condition of the mouse sample and taste substance properly reached is needed. The transgenic mice that express the genetically encoded calcium indicators (GECIs) in the peripheral neurons, pirt-Cre; lox-GCaMP6f-tdTomato, or both in the peripheral nervous system (PNS) and the central nervous system (CNS), SNAP25-GCaMP6s, are used for the experiments (Barretto *et al.*, 2015; Fowler and Macpherson, 2021). Locally injection of retrograde transmission of the virus and systemically delivered virus (PHP.S) were used for the expression of GCaMP in the geniculate ganglion (Asencor *et al.*, 2022).

The surgery steps for reaching the geniculate ganglion are constituted generally by three steps. First, the anesthetized mouse is mounted the customized board in a supine position (Figure 4). For the stable breath of the mouse and preventing from drowning when giving the taste fluids, the tracheotomy should be done. There are two muscles to be dissected, digastric and hyoglossus muscles. Next, after removing the two muscles, the tympanic bulla appears and should be broken. Finally, inside the tympanic cavity, the interior structure is shown (Figure 5). Of them, the tensor tympani muscle should be removed which covers right over the position of the geniculate ganglion. Unlike the previous studies (Fowler and Macpherson,

2021), the present study omitted two things and simplified the total steps for surgery: the promontory of cochlea, which makes continuous fluids when broken, should not be removed (Figure 6); the temporal bone, surrounding the geniculate ganglion, should not be broken because this absence induces the fast drying of the neurons making them unstable by time. Because of these two changes, the long-term *in vivo* calcium imaging of geniculate ganglion is possible.

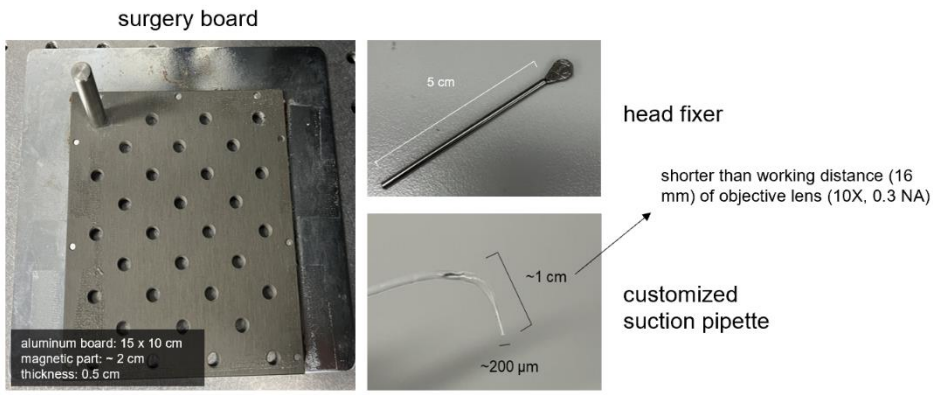

Figure 4. Customized components for the stable in vivo calcium imaging of the geniculate ganglion.
The surgery board was customized for considering the stage limitation. The head attached area of head fixer is widen compared to previous version. The customized suction pipette is used to prevent disturbing from the fluids and blood when recording.

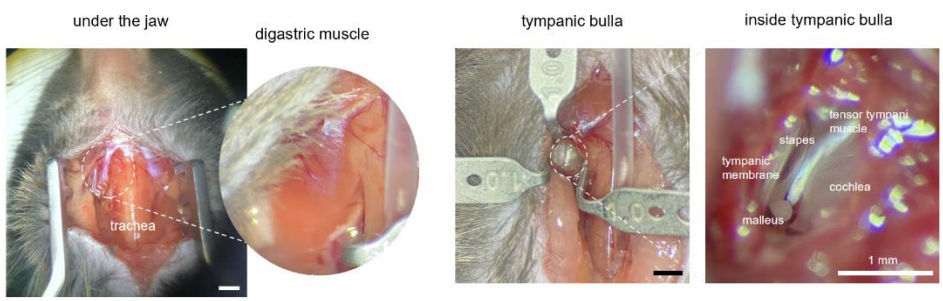

Figure 5. The surgery steps of reaching the geniculate ganglion.
After incision skin under the jaw, tracheotomy is preceded. Then, digastric and hyoglossus muscles are dissected, revealing the tympanic bulla. Inside the tympanic bulla, the interior structure is shown. Removing the tensor tympani muscle shows the geniculate ganglion surrounded by the temporal bone.

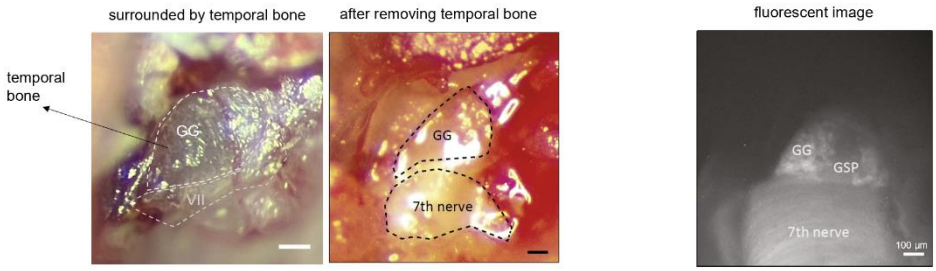

Figure 6. Modified version from previous method at the last step of exposing the geniculate ganglion.
The temporal bone surrounding the geniculate ganglion should be removed in the previous method. However, in the absence of the bone the activity of neurons is going unstable by time. Therefore, the final step of removing the temporal bone was omitted in this study, and the mineral oil was used to reduce the scattering effect from remaining bone.

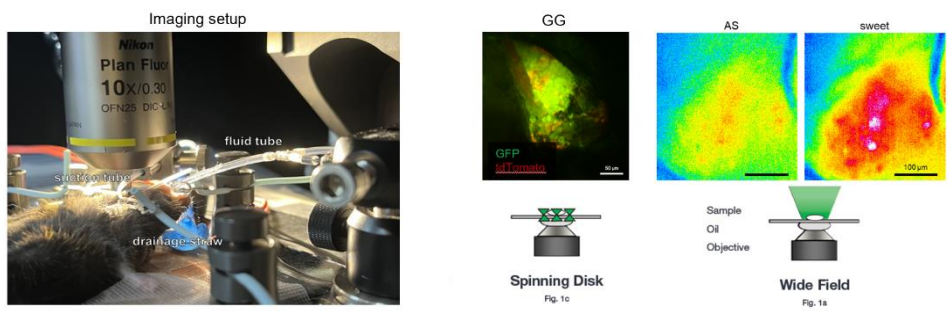

Figure 7. Imaging setup and the fluorescent image by spinning disk confocal and widefield microscope.
Imaging setup contains the fluid tube for treating the tastants and the drainage straw to remove the tastant fluids under the tongue. The fluorescent images of the geniculate ganglion (GG) were taken by spinning disk confocal microscope and widefield microscope.

2.2. Responsive types of the geniculate ganglion

After the setup for calcium imaging of the geniculate ganglion, to activate type I cells physiologically, the phasic stimulation of sweet taste, acesulfame potassium (aceK), was administered. Based on the data of calcium imaging the tongue, type I

cells response to tastants on 5-10 s delayed time. So, the stimulation of 10 s duration induces the response in type II cells and during the wash time of 20 s the response of the type II cells would be removed, and the response of type I cells begins. Using this timing, we expect that the repeatable stimulation will represent the function of type I cells over time. The average responses of ganglion neurons are not remarkably changed as the order of stimuli. However, this phasic stimulation showed the three types of response kinetics in the geniculate ganglion (Figure 8); rapid-desensitizing neurons, slow-desensitizing neurons, and delayed-responsive neurons. Rapid-desensitizing neurons show a fast rise in the stimulation and exponential decay after the peak. Unlike this type of neuron, slow-desensitizing neurons keep the calcium signals during stimulation. Delayed responsive neurons are not common to observe, but this type of neurons responds about 5-10 s after the stimulation.

Of these three types of neurons, rapid-desensitizing neurons show a slight reduction of the peak intensity as the order of stimuli (Figure 10), inferring the interaction between the type II cells and type I cells, which can be the clue of the function of type I cells.

To characterize the kinetics of neurons, the longer stimulation time (10 s → 60 s) was tried (Figure 11). This tonic stimulation experiment showed rapid-desensitizing neurons and slow-desensitizing neurons like in the phasic stimulation experiment. The mixed feature of rapid- and slow-desensitizing was also captured. This result corresponds to the previous data that the geniculate ganglion neurons constituted the two types of ionotropic purinergic P2X receptors: P2X3 homomeric and P2X2/3 heteromeric neurons (Ishida *et al.*, 2009; Vandenbeuch *et al.*, 2015). As studied before, P2X3 show the rapid-desensitizing feature and P2X2 represents the slow-desensitizing feature. Hence, the observed types of calcium responses show the

corresponding purinergic receptor expression patterns, and the rapid-desensitizing P2X3-rich neurons represent the reduced peak by the order of stimuli, inducing the modulatory effect of type I cells.

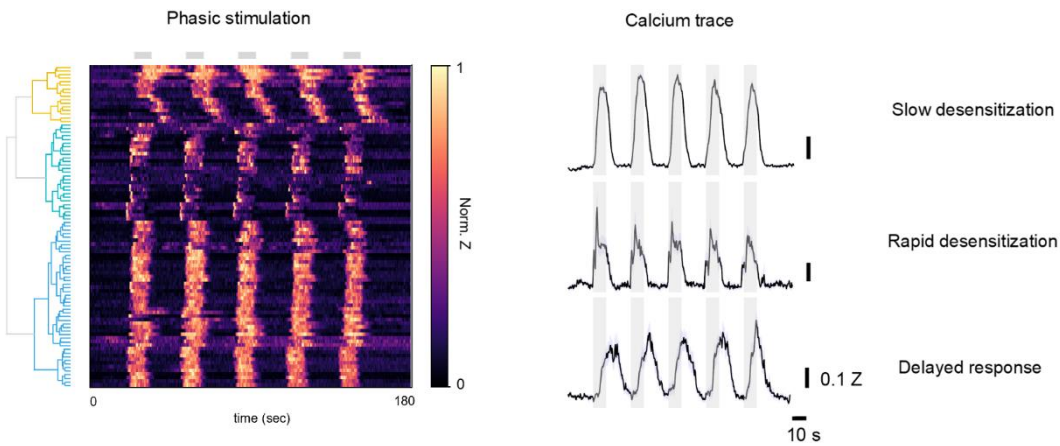

Figure 8. Phasic stimulation of aceK and calcium trace of the geniculate ganglion.
Three types of calcium trace represent during phasic stimulation. Rapid-desensitizing neurons show the reduced peak as the order of stimuli increase.

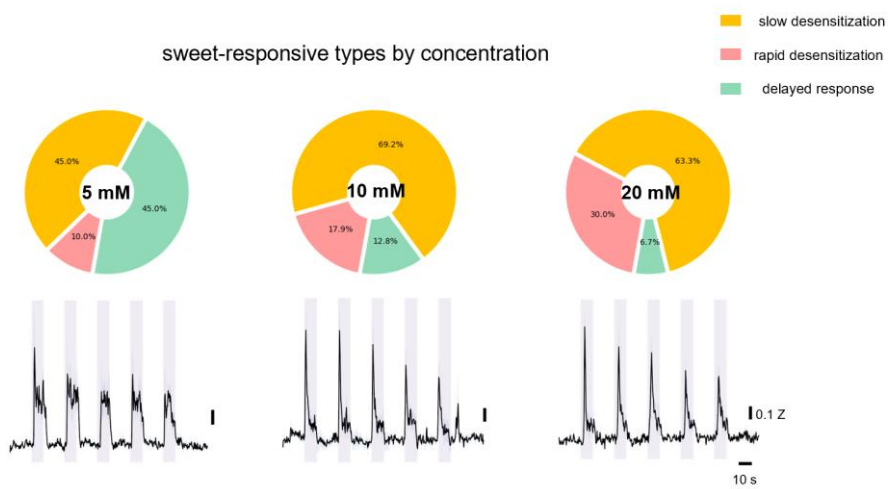

Figure 9. Rapid-desensitizing neurons by the concentration of aceK.
The ratios of the three types of neurons by concentration are represented. Most sweet neurons are slow-desensitizing neurons which are likely corresponding to P2X2-rich neurons. Rapid-desensitizing neurons which are like P2X3-rich neurons account for a small percentage of responding neurons.

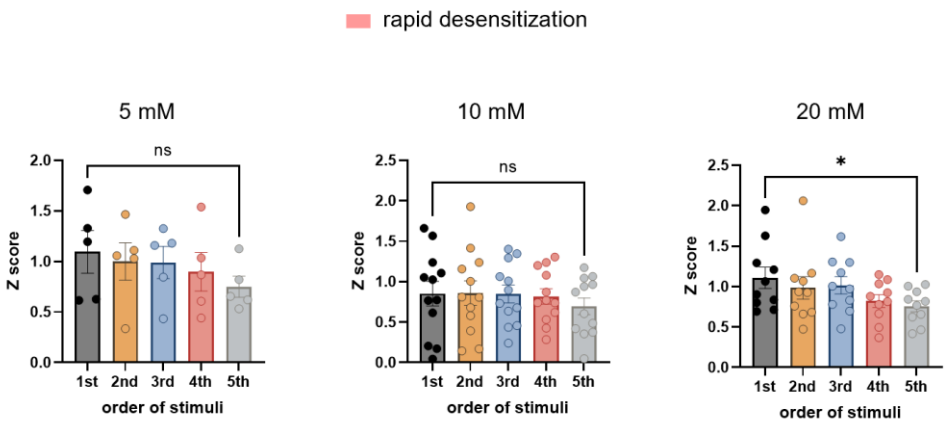

Figure 10. Rapid-desensitizing neurons show the slight reduction as the order of stimuli increases.
The tendency to reduction of the peak of calcium response in the geniculate ganglion rapid-desensitizing neurons is represented by concentration. Rapid-desensitizing neurons of 5, 10, and 20 mM aceK experiment. Paired t-test, p = 0.0827 (n=5), p = 0.3762 (n=12), and p = 0.0169 (n=10), respectively.

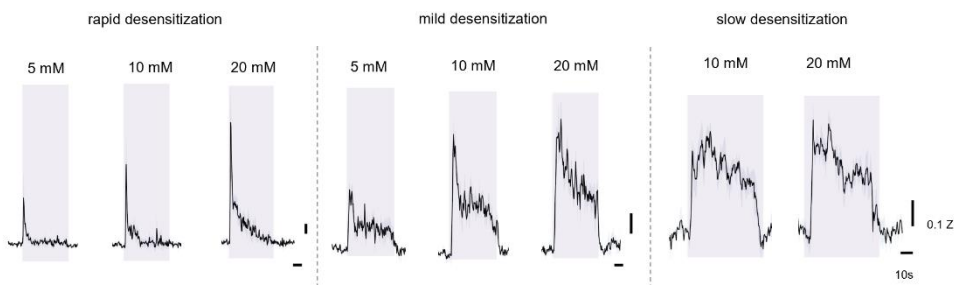

Figure 11. Tonic stimulation show the responsive types of geniculate ganglion neurons.
Tonic stimulation is 60 s duration stimuli instead of 10 s in the phasic stimulation and show the rapid-desensitizing neurons which are likely P2X3-rich expression.

2.3 Artificial activation of type I cells pharmacologically: Diquafosol

Type II cells which can recognize sweet, bitter, and umami taste sensations by GPCR

signaling release ATP via the CALHM1/3 channel to nerve fibers (Romanov *et al.*, 2018; Kashio *et al.*, 2019). This feature gives rise to purinergic signaling could be an activating candidate for type I cells. Although type I cells express NTPDase2 on their membrane, delayed calcium response might require having a threshold of response to ATP from type II cells. The time to reach the threshold for activation of type I cells is likely to be the time to start with the response when the experiments were executed in a transgenic mouse expressing ATP sensors with pirt- or Gad2-Cre dependent in our laboratory. Additionally, prior work has shown that purinoceptor antagonists dramatically inhibited the taste-evoked responses of type I cells in the slice preparation and that ATP activates them in dissociated taste buds (Rodriguez *et al.*, 2021). Based on the result of RNA expression data from our laboratory, the P2Y2 receptor is likely expressed in the type I cells. Therefore, the specific agonist of P2Y2, Diquafosol (DQ), was selected to activate the type I cells.

First of all, 5 mM DQ was treated 20 s before the tastant reached. 5 mM DQ was sufficient to induce calcium response in the type I cells as confirmed by in vivo platform in our laboratory, but in the geniculate ganglion neurons, the significant difference before and after DQ data was not shown (Figure 12).

Because 5 mM DQ did not change the neural response of tastants unlike in the taste cells, a high concentration of 40 mM DQ and longer stimulation of 120 s was tried. 40 mM DQ showed a stronger intensity and longer duration of calcium response, especially in the rapid-desensitizing neurons (Figure 13). Slow-desensitizing neurons also show a slight increase in the amplitude of the response. Another taste, bitter, not sweet, showed no difference by 40 mM DQ. Therefore, 40 mM DQ made sweet responding and rapid-desensitizing neurons more sensitive to the same stimulus (Figure 17).

Because 40 mM DQ used in the present study is dissolved in physiological saline (0.85~0.9% NaCl), the saline without DQ could have an effect of neural response. However, the saline did not increase the sweet response rather seems to slightly decrease (Figure 16).

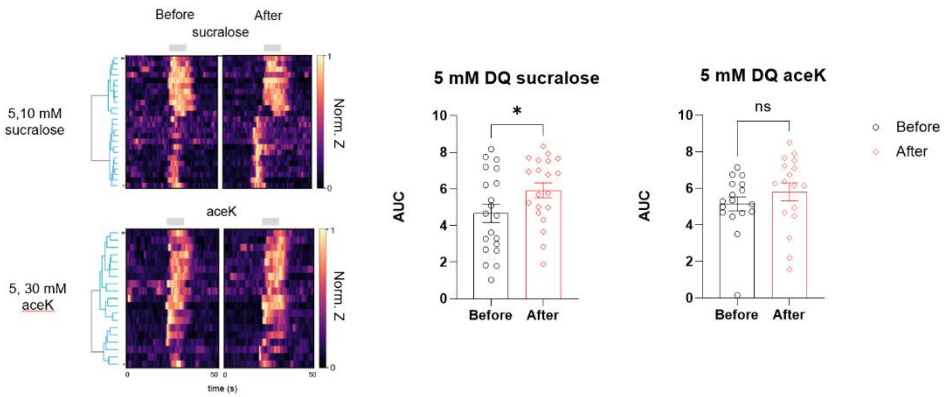

Figure 12. 5 mM DQ does not show the significant change of sweet taste.
5 mM DQ was treated before the second stimulus during 20 s. 5 mM DQ show slight increase in the sucralose but no change in aceK. 5 mM DQ of sucralose and aceK experiments. Paired t-test, p = 0.0318 (n=20) and p = 0.1158 (n=17), respectively.

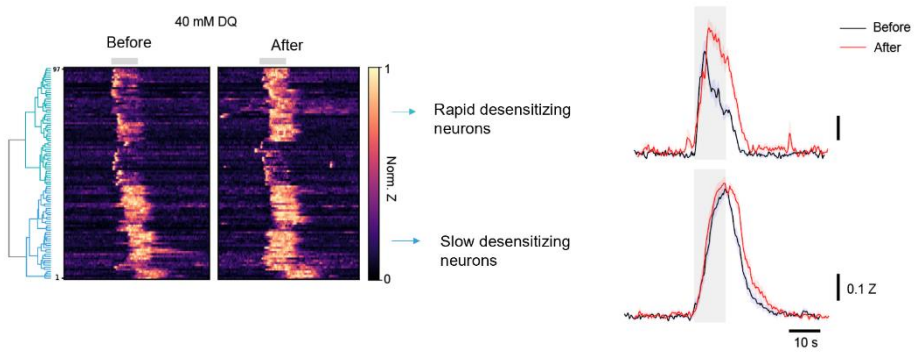

Figure 13. 40 mM DQ show the significant increase in sweet taste.
40 mM DQ was treated before the second stimulus during 120 s. The effect of 40 mM DQ was bigger in the rapid-desensitizing neurons than slow-desensitizing neurons.

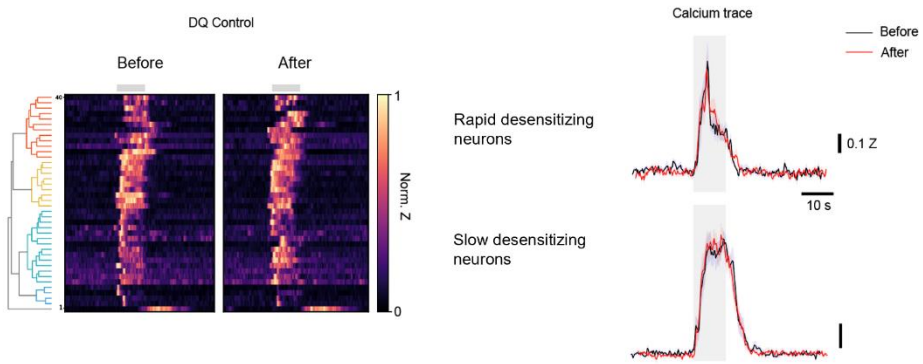

Figure 14. Control experiment show no change in sweet taste.
Control experiment show no change in both rapid-desensitizing and slow-desensitizing neurons.

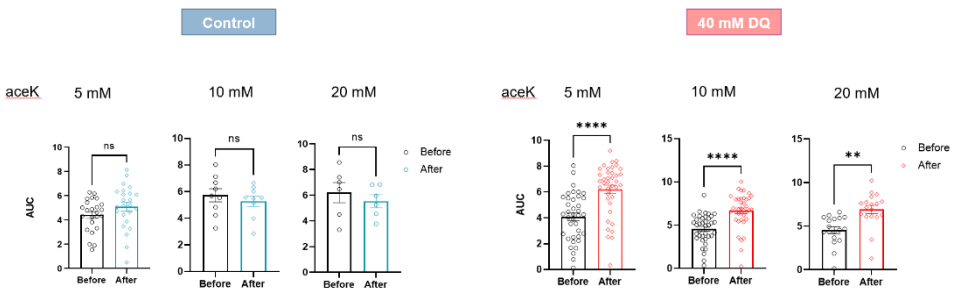

Figure 15. The increased response after treatment of 40 mM DQ.
40 mM DQ show the increased response on all of three concentration stimuli. Values are means ± SEM. Control experiments of 5, 10 and 20 mM aceK. Paired t-test, $p = 0.0639$ (n=24), $p = 0.5135$ (n=9), $p = 0.5675$ (n=6), respectively. 40 mM DQ experiments of 5, 10, and 20 mM aceK. Paired t-test, $p < 0.0001$ (n=38), $p < 0.0001$ (n=38), $p = 0.0047$ (n=18), respectively.

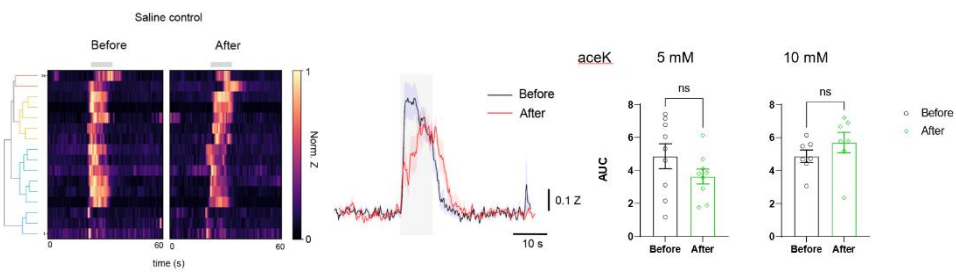

Figure 16. Saline instead of 40 mM DQ show no effect in sweet taste.
Physiological saline (0.85-0.9% NaCl) did not show the increase effect. Values are means ± SEM. 5 mM aceK Paired t-test, $p = 0.0561$ (n=9). 10 mM aceK Paired t-test, $p = 0.2847$ (n=7).

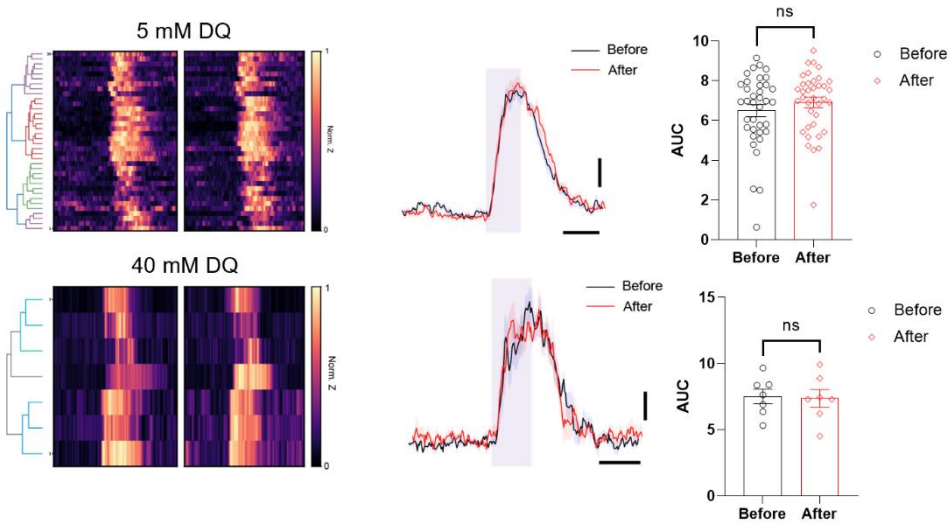

Figure 17. Bitter has no change by both 5 mM and 40 mM DQ.
5 mM DQ did not change bitter taste unlike sweet taste. Values are means ± SEM. Paired t-test, p = 0.2108 (n=37). 40 mM DQ also did not change bitter taste. Values are means ± SEM. Paired t-test, p = 0.8797 (n=7).

2.4 Artificial activation of type I cells hormonally: Oxytocin

Oxytocin (OXT) is a nonapeptide hormone known to promote lactation and parturition and a central neuropeptide that affects a range of social and other behaviors. Circular levels of OXT are related to the regulation of intake of sweet, but not all calorie-rich solutions (Sinclair *et al.*, 2010). So, in terms of the expression of OXT receptors in taste buds, the possibility that the peripheral taste system may also be a target of oxytocin signaling. Hormones have systemic effects on the gustatory system in the body. However, it is not clearly declared that taste recognition is related. Our laboratory showed the result that OXT decreases the response of taste cells to sweeteners. There is an additional result in other groups showing data using behavior

study (Sinclair *et al.*, 2015). Type I cells express the OXT receptors whose role has not been revealed. As the unique feature of type I cells, OXT was selected to activate the cells. It can induce the strong activation of type I cells, unknown its downstream. However, it can be interesting to find whether the rise of calcium signal in type I cells affects the other cells.

0.1-0.2 mg/kg OXT was intraperitoneally injected and 1 min after injection the repetitive stimuli were treated to compare the average response before and after injection because the neural responses are not always same. The results show that effect of OXT is ambiguous (Figure 18). On average, the effect of OXT seems no change on sweet taste bud the individual neurons show different result by OXT. Most of neurons have no change from OXT injection but a fraction of neurons show increase or decrease by OXT (Figure 19). This result might give additional consideration as the result above that three types of kinetics would have different effect by OXT.

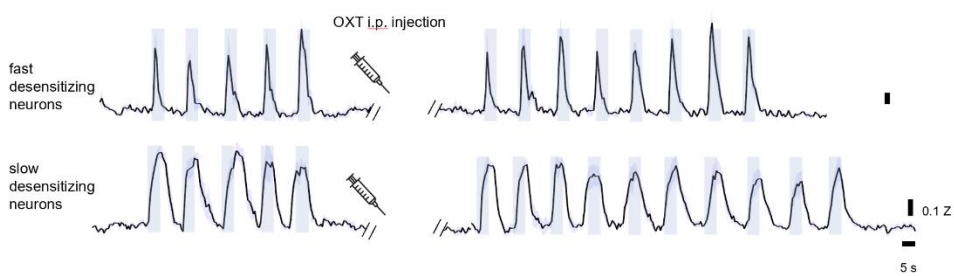

Figure 18. OXT show no difference on average in both rapid-desensitizing and slow-desensitizing neurons.
Rapid-desensitizing neurons and slow-desensitizing neurons show no change on average after injection OXT.

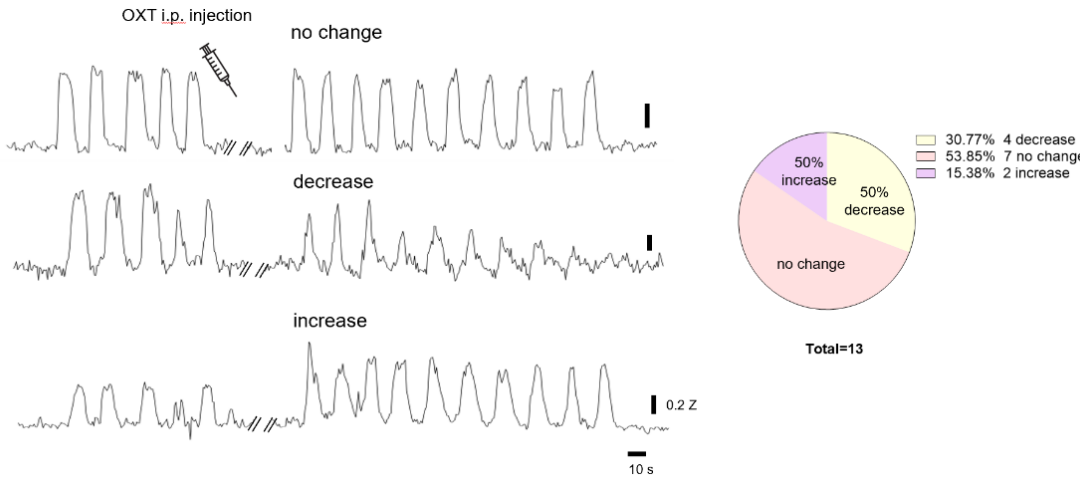

Figure 19. Individual neurons show different effect by OXT and the ratio of neurons. Individual neurons have some different result by OXT. Most of neurons show no change but some fraction of them decreases or increases by OXT.

Chapter 3. Discussion & Conclusion

There are many suggestions about the interaction between taste cells in the taste bud (Yamamoto *et al.*, 1998; Vandenbeuch, Wilson and Kinnamon, 2020). However, the function of type I cells has been very less discovered than other taste cells. The present study is the approach to the question whether the activation of type I cells modulate the results of taste information transmitted to the gustatory nerve. In a result, the over-activation of type I cell by purinergic signaling show the intensified response on sweet neurons. Here, I will discuss about this result providing the several possible explanations.

There are several cell types in geniculate ganglion neurons. Cell types can be categorized by the kinetics of concentration and time-dependent sweet responses. Gustatory neurons express P2X2/3 heteromeric ionotropic receptors or P2X3 homomeric receptors (Ishida et al., 2009). Rapid-desensitizing neurons show that the calcium signal rises fast and after reaching the peak, it decays exponentially even if the stimuli remain. This feature can be explained if these neurons express P2X3 homomeric receptors dominantly rather than P2X2/3 heteromeric receptors on the nerve endings. Slow-desensitizing neurons whose feature is the calcium response keeps plateauing during the stimuli are treated on the tongue and are likely to express more P2X2/3 heteromeric receptors than rapid-desensitizing neurons.

As geniculate ganglion neurons are categorized into three types of neurons, the effect of DQ is shown more clearly on rapid-desensitizing neurons. 40 mM DQ increases the intensity of the sweet response of rapid-desensitizing neurons and the response does not decay exponentially as their own feature. Rather, the rapid-

desensitizing feature tends to be changed into slow-desensitization. This result could be thought by some possible reasons. One thing is that the activation of type I cells by DQ directly or indirectly affects to the type II cells or nerve endings. In the case of indirect effect, responses of type I cells should interact with sweet-responsive type II cells before they give information to nerve endings. And this might be a disinhibitory function to intensify the information of the same taste signal. To test this, the activation of type I cells by purinergic signaling and its downstream should be revealed. I suggest that the isolation of type I cells *ex vivo* is followed by treatment of DQ and the components of the solution of taste cells can be analyzed to find the candidate molecules. A possible reason of direct effect can be thought that downstream of the activation of type I cells directly effect on nerve endings. There are some antagonists of desensitization of P2X3. P2X3 have the characteristics of strong acceleration of recovery (resensitization) by increases of extracellular Ca^{2+} and inhibition of recovery by extracellular Mg^{2+} (Giniatullin and Nistri, 2013). Hence, type I cells activated by DQ could have a functional effect on P2X3-rich neurons to resensitize from the desensitization state of P2X3. Additionally, 40 mM DQ is extremely high concentration to activate type I cells. This can make non-specific activation of type II or III cells by 40 mM DQ targeting P2Y1 or P2Y4 receptor, respectively (Baryshnikov, Rogachevskaja and Kolesnikov, 2003). If the purinoreceptor on type II cells were nonspecifically activated, this can induce the intensified results in sweet taste. But same results weren't observed in bitter taste, making this possibility less powerful. In summary, to be sure the effect of 40 mM DQ in this study, it should be examined how type I cells respond to 40 mM DQ and its downstream.

The remarkable feature of change by DQ was observed in the rapid-

desensitizing neurons. Desensitization refers to the decreased responsiveness that occurs with repeated or chronic exposure to agonist and is a general feature of most signaling receptors. When P2X3 homomeric receptors bind ATP, they become active and desensitize in milliseconds, but they unbind ATP and recover from desensitization in minutes (Cook and McCleskey, 1997; Giniatullin and Nistri, 2013; Li *et al.*, 2019). Channel activation frequency is restricted by this extremely lengthy recovery period, which may be a crucial defense against sensory hypersensitivity. Like in pain sensation, P2X3 expression might mean that taste sensation also should be protected by hypersensitivity. But this type of neuron is likely expressed frequently as a sweet-sensing neuron, rather than bitter, although most of the neurons are P2X2/3 heteromeric neurons that show mild desensitization by ATP. This indicates that some populations of sweet-responsive neurons have another function besides just the transmission of information about chemicals, such as recognizing the change in the concentration of compounds or components of environment. And the change of them can maybe make taste transmission sensitive or insensitive to the stimuli. Hence, characterizing of neurons by different functions would be interesting for approaching the interaction between taste cells and gustatory nerves.

OXT is a neurohormone, synthesized in the neurons of hypothalamic paraventricular nucleus (PVN) and supraoptic nucleus, inducing milk-ejection and uterine contraction and regulating social behavior, stress responses, memory, and food intake. In previous study, OXT showed the reduced intake of sweet taste sensitivity at behavior level (Sinclair *et al.*, 2015). However, the effect of OXT was not clear in this study. OXT is the agonist for activation of type I cells like DQ, but the results were not similar in two experiments. OXT showed a decrease and increase in response on aceK in some neurons, but most of the neurons were not changed.

This discrepancy could be explained by the different downstream of purinergic and OXT signaling. There were data that purinergic input and OXT evoke an increase of intracellular calcium levels in several studies (Sinclair *et al.*, no date; Rodriguez *et al.*, 2021). However, their downstream have not been discovered. Therefore, additional experience to test their downstream would be recommended like comparing the molecules in the solution of isolation of type I cells after treatment of DQ or OXT. Another explanation of the discrepant results in peripheral ganglion and behavior can be that the peripheral intraperitoneal injection could mimic the effect of central OXT via vagal nerve (Iwasaki *et al.*, 2019). The behavior test was conducted 30 min after OXT injection, but type I cells were activated within 2 min after OXT injection. As these reasons, whether the function of OXT receptors in type I cells is associated in gustatory transmission remain unknown.

For activation of type I cells, optogenetics and chemogenetics can be utilized. The previous study used the optogenetic activation of type I cells and showed that type I cells were related to sodium taste sensation (Baumer-Harrison *et al.*, 2020). Optogenetically activation is achieved by the expression of a light-sensitive ion channel, ChR2, opening the ion channel by blue light and influx of Na^+ ions. However, this study has limitations in that the GAD2-Cre mouse for targeting type I cells includes some off-targets (Larson *et al.*, 2021). Our laboratory also showed some portions of type I cells are activated when treated with NaCl *in vivo* calcium imaging. As these features, the salty sensation is unclearly explored, but it is believed “Type II-like” cells are related to salty transduction (Liman, 2020). Therefore, chemogenetics is recommended rather than optogenetic approach. The prior experiment using chemogenetic activation of type I cells are not yet executed.

Taken together, the present study showed the effect of DQ at the gustatory

neural level. P2X3-rich sweet neurons were especially affected. But OXT did not show the same influence with DQ not as expected. To address this issue, more work in the field will be necessary.

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국문초록

다섯 가지 기본 맛은 미뢰에 있는 미각세포를 통해 인지되고 슬신경절에 세포체를 구성하는 미각신경으로 신호가 전달된다. 이 중 미각수용체를 발현하지 않는 미각교세포인 유형 1 세포는 미뢰의 80% 부피를 차지한다. 많은 연구에서 미각 세포들 간에 신경전달물질을 통한 상호작용이 있을 것이라고 믿어왔다. 하지만, 맛 물질에 간접적으로 반응할 수 있는 미각교세포는 맛에 대한 반응이 어떤 기능을 가지는지 연구된 바가 없다. 본 연구에서는 칼슘 영상 기법을 이용하여 미각세포로부터 전달된 신호를 받는 슬신경절 이미징을 통해 유형 1 세포의 활성화에 의한 미각 정보의 변화를 관찰하였다. 먼저, 기존 방법보다 수술 단계를 단순화하여 생체 내 슬신경절의 안정적이고 장기적인 칼슘 이미징을 가능하게 한 이미징 셋업을 구축하였다. 그런 다음, 본 연구실의 RNA 데이터로부터 유형 1 세포에서 P2Y2 수용체 발현 결과를 기반으로 하여 특정 작용제인 Diquafosol (DQ)를 사용한 유형 1 세포 활성화를 유도하였다. 특히, 40 mM 농도의 DQ가 슬신경절의 단맛 반응을 강화시키는 현상을 보였다. 또한, 유형 1 세포의 옥시토신 수용체의 발현 결과를 이용하여 옥시토신을 또다른 작용제로 선택하였다. 본 연구실에서 옥시토신에 의해 유형 1 세포가 강하게 반응하는 것을 보였지만 DQ와 달리 신경절에서의 변화를 확인하기 힘들었다. 종합하면, 본 연구는 퓨린성 신호전달이 미뢰에서 유형 1 세포의 조절 기능의

가능성을 보여준다.

주요어 : 슬신경절, 미각교세포, 옥시토신, 디쿠아포솔, 단맛, 칼슘 이미징

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