論　文　内　容　要　約

Plastic changes in medial amygdalar neurons defined by genetic tracing of taste representation after conditioned taste aversion learning

（発生工学的トレーシングにより可視化された内側扁桃体の味覚経路ニューロンにおける味覚嫌悪学習に伴う可塑性変化）

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Introduction

Animals learn to reject a palatable, sweet tastant (conditioned stimulus [CS]) if it is associated with subsequent visceral malaise (unconditioned stimulus [US]). This phenomenon called as conditioned taste aversion (CTA) can be studied in the animal model, where an animal tasting saccharin, a novel sweet tastant (CS), and followed by intraperitoneal injection of lithium chloride that induces illness (US) can acquire one-trial learning of CTA to that particular sweet tastant.

The amygdala plays a primary role in acquisition and expression of CTA. The association of the CS and the US appears to occur in the basolateral amygdala (BLA) to get aversive to the CS. It is reported that during the CTA conditioning, subsets of neurons in the BLA respond to both the CS taste and the US. The CTA acquisition appears to be disrupted if the stimulus convergence in the basolateral amygdalar neurons is blocked.

Therefore, it is likely that the association of taste information and aversive viscerosensory information that occurs in the BLA may lead to subsequent plastic changes in downstream neurons, resulting in the acquisition of CTA memory. However, it remains largely unknown how the basolateral amygdalar neurons receiving both the CS and US induce the plastic changes in downstream neurons at a molecular, cellular or system level.

The specific aim of this study was to identify the amygdalar neurons which induce the plastic changes in activities during CTA acquisition. In this study, we examined whether subpopulations of the amygdalar neurons processing aversive bitter taste information represent the plastic changes in responses to the CS during CTA acquisition.

Materials and methods

Breeding, housing, and genotyping of transgenic mice

The transgenic mice that express the transsynaptic tracer tWGA-DsRed in mT2R5-expressing bitter taste receptor cells were used to visualize the spatial distribution in the brain of bitter taste-relying neurons labeled by tWGA-DsRed originating from bitter taste receptor cells. The mice were housed on a 12h light/dark cycle. Food and water were available ad libitum before the behavioral experiments. The animals were treated in accordance with the Guide for Animal Experiment, Hiroshima University.

The CTA procedure

Mice were deprived from water for 12 hr. Then they were adapted to the water intake schedule for 4 days. The mice were divided into three groups: “control”, “conditioning”, and “extinction”. The “control” mice were without conditioning while “conditioning”, and “extinction” were given with the conditioning schedule to acquire CTA.

For CTA conditioning, the mice were applied with two bottles filled with 0.2 % saccharin (CS) for 10 min, and then treated with an intraperitoneal injection of 0.15 M LiCl (2 % of body weight) as the US 15 min after the CS. Testing for aversion to saccharin was performed daily after the conditioning. One bottle filled with water and the other with saccharin were presented to the mice, and the AI was calculated as (water consumption)/(water + saccharin consumption) and used as an index for learned aversion to saccharin.

Sensory stimulation and detection of Zif268 expression in tWGA-DsRed-labeled neurons

To examine whether the amygdalar neurons were activated by oral application of saccharin, we detected the saccharin-induced expression of Zif-268, an immediate early gene. Mice were orally applied with saccharin.

The brain was isolated after mice were euthanized with sodium pentobarbital, and fixed with 2% formaldehyde solution (1x PBS, 2% formaldehyde). The sections are made by the film-transfer method, and fixed with 3.7% formaldehyde solution (1x PBS, 3.7% formaldehyde, 0.18% Triton X-100) for 10 min. Nonspecific binding side were blocked with 1% bovine serum albumin (BSA) for 30 min.

The sections were treated with primary antibodies against Zif268 which were pre-bound with the Alexa-647-labeled Fab fragments of

secondary antibodies in the Zenon labeling system. Location of tWGA-DsRed was determined by direct fluorescence detection. The subcellular distribution of Zif268 was visualized by detecting Alexa-647 fluorescence.

Statistical analysis

Statistical significance was determined using Chi-square test with Bonferoni-correction. P values <0.05 were considered statistically significant.

Results and discussion

Changes in the AI after CTA conditioning

After CTA conditioning, all the tested mice become aversive to saccharin and establish strong CTA. The mice that acquired CTA memory were subjected to the continued two-bottle test to monitor the daily changes in the AI. Although the time courses for the AI changes were varied among individuals, the mice had the AI declining to the value less than 50%. Those mice were used as the "extinction" group.

Location of bitter-taste relaying neurons labeled by tWGA-DsRed

We examined the spatial distribution of medial amygdalar (MeA) neurons labeled by tWGA-DsRed that originated from bitter taste receptor cells in mT2R5-WGA mice. tWGA-DsRed was mainly located in the perinuclear region of the labeled MeA neurons. The cell somata of tWGA-DsRed-labeled neurons were found in the MeA from Bregma -0.70 to Bregma -2.06.

The subsets of WGA-DsRed-lebeled neurons in the MeA were activated by the CS only after, but not before, CTA acquisition

Zif268 expression was induced by oral stimulation with the sweet tastant saccharin in more tWGA-DsRed-labeled neurons which inherently process aversive bitter taste information after acquiring the CTA memory in the "conditioning" group, compared with those in the "control" group. The ratios of neurons with Zif268-immunoreactivity were found to be 63% ("conditioning") versus 37% ("control"). The ratio of the saccharin-activated,

WGA-DsRed-labeled neurons in the “extinction” group was 65%, showing a significant difference between the “control” and “extinction” groups, but not between the “conditioning” and “extinction” groups

Comparison of Zif268 induction in tWGA-DsRed-lebeled neurons divided into the MeA subregions

To characterize the spatial distribution of the saccharin-activated neurons in the MeA, we further divided the MeA into anterodorsal medial amygdala, anteroventral medial amygdala, posterodorsal medial amygdala and posteroventral medial amygdala. However, the observed patterns in the anterior and posterior MeA were found similar to that in the whole MeA.

Conclusion

We conclude that the specific population of MeA neurons inherently process aversive bitter information may induce the plastic changes in their activities after acquiring CTA, which remain unchanged after the extinction of CTA memory.