論　文　内　容　要　旨

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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【Objective】

Conditioned taste aversion (CTA) occurs as a memory paradigm when animals learn to reject a flavorsome sweet tastant (conditioned stimulus [CS]) with the subsequent visceral malaise (unconditioned stimulus [US]). CTA is an extremely adaptive, powerful form of conditioning that species do not need a special training to obtain the restricted responses for. It can be robustly acquired only in a single trial, making animals avoid the similar tastes.

The amygdala performs a primary role while enabling animals to process emotional memory, emotional responses and decision-making, also having a hand in acquiring the CTA memory. It is reported that during the CTA conditioning, subsets of neurons in the basolateral amygdala 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 basolateral amygdala 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】

The transgenic mice that express the transsynaptic tracer WGA-DsRed in T2R-expressing bitter taste receptor cells were used to visualize the spatial distribution in the brain of bitter taste-relying neurons labeled by WGA-DsRed originating from bitter taste receptor cells. Thus, by detecting WGA-DsRed, we could define a subpopulation of neurons in the amygdala, which processed aversive bitter taste information.

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. Two bottles (one filled with water and the other with saccharin) were presented to the mice, and the aversion index was calculated as (water consumption)/(water + saccharin consumption) and used as an index for learned aversion to saccharin.

The mice were divided into three groups: “control”, “condition”, and “extinction”. The mice that acquired CTA memory were used as the “condition” group. A half of mice that acquired CTA memory were subjected to the continued two-bottle preference test to monitor the daily changes in the aversion index. When the aversion index decreased and reached the value less than 50%, those mice were defined as the “extinction” group.

To examine whether the WGA-DsRed-labeled neurons in the amygdala were activated

by oral application of saccharin used as the CS, we detected the saccharin-induced expression of Zif268, an immediate early gene, in the labeled neurons while comparing three groups of mice. Here, each group of mice got orally stimulated with saccharin, and the mouse brain was excised 45 min after the stimulation to obtain its serial coronal sections. The induction of Zif268 expression was immunohistochemically detected in the sections containing the amygdalar regions. Zif268 immunoreactivity in WGA-DsRed-labeled neurons was divided into three groups of -, +, and ++. Percentage of Zif268 immunoreactive neurons within WGA-DsRed-labeled neurons in the amygdala were compared among three groups.

【Results and discussion】

In the amygdalar regions, most of the neurons labeled by WGA-DsRed originating from bitter taste receptor cells were found in the medial amygdala from Bregma -0.70 to Bregma -2.06 while very few were observed in the basomedial amygdala or cortical amygdala. Thus, we could define the medial amygdalar neurons that process and integrate aversive bitter information by detecting the WGA-DsRed labeled neurons

The WGA-DsRed-labeled neurons which process aversive bitter taste information were more activated by the sweet tastant saccharin after acquiring the CTA memory in the "condition" group rather than the "control" group. The ratios were found to be around 70% (condition) versus 40% (control).

Interestingly, the ratio of the saccharin-activated, WGA-DsRed-labeled neurons in the "extinction" group is around 60%, showing a significant difference between the "control" and "extinction" groups, but not between the "condition" and "extinction" groups.

To characterize the spatial distribution of the saccharin-activated neurons along the anterior-posterior axis of the medial amygdala with their comparisons among three groups, we determined the percentages of saccharin-activated neurons within the WGA-DsRed-labeled neurons separately in the anterior and in the posterior regions of the medial amygdala, divided at around Bregma -1.30. However, the data showed no significant difference in the ratios of the saccharin-activated, WGA-DsRed-labeled neurons between the anterior and posterior regions in each group.

It is of note that the subsets of WGA-DsRed-labeled neurons in the medial amygdala were activated by the CS only after, but not before, CTA acquisition. Thus, our data suggest that the neurons processing aversive bitter information may induce the plastic changes in their activities after acquiring CTA, which remain being recovered after the extinction of CTA memory.