

# Brain Regions Responsible for the Expression of Conditioned Taste Aversion in Rats

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## Abstract

Conditioned taste aversion (CTA) is acquired when the ingestion of a food is followed by malaise. CTA is a kind of fear learning making animals avoid subsequent intake of the food and show aversive behavior to the taste of the food. To elucidate the brain regions responsible for the expression of CTA, our previous electrophysiological and recent *c-fos* immunohistochemical studies have been reviewed. Among a variety of brain regions including the parabrachial nucleus, amygdala, insular cortex, supramammillary nucleus, nucleus accumbens, and ventral pallidum that are involved in different phases of CTA expression, the enhanced taste sensitivity to facilitate detection of the conditioned stimulus may originate in the central nucleus of the amygdala and the hedonic shift, from positive to negative, may originate in the basolateral nucleus of the amygdala.

**Key words:** amygdala, basolateral nucleus, *c-fos*, electrophysiology, parabrachial nucleus, reward system

## Introduction

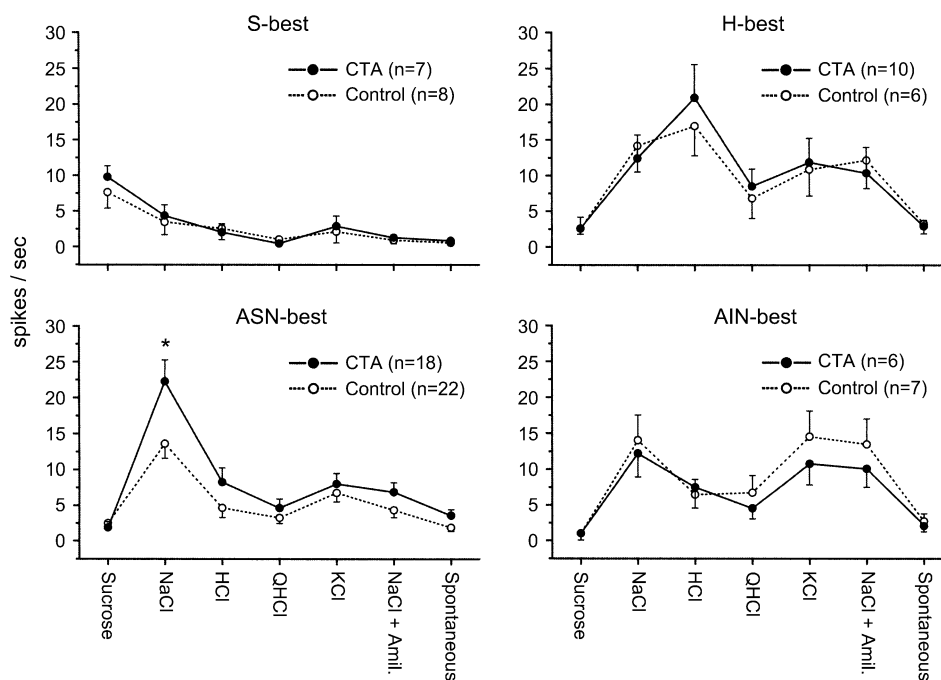
When the ingestion of a food conditioned stimulus (CS) is paired with malaise unconditioned stimulus (US) such as gastrointestinal disorders and nausea, conditioned taste aversion (CTA), an association learning between the ingested substance and internal consequences, is quickly established (for a review, Garcia et al. 1955; Bures et al. 1998). CTA is a kind of fear learning to avoid subsequent intake of the “harmful” food by exhibiting aversive behavior to the taste of the food. When saccharin is used as a CS, the sweet and palatable taste is treated as an aversive taste after CTA acquisition. The quality itself may not change, while the perceived intensity may be enhanced to facilitate detection of the harmful substance, and a hedonic shift from positive to negative occurs. Neural substrates for these alterations of sensory and hedonic aspects of the CS will be elucidated on the basis of our studies.

## Sensory aspect

In our previous study (Shimura et al. 1997), we recorded neuronal responses to taste stimuli from the pontine parabrachial nucleus (PBN) of the rat under deep urethane anesthesia. Animals were separated into 2 groups: the CTA group that had acquired a taste aversion to 0.1 M NaCl (CS) by paired presentation of intraperitoneal (i.p.) injection of LiCl (US) and the control group without CTA experience.

Taste-responsive neurons in the CTA group showed larger responses to NaCl at concentrations below 0.1 M but showed similar responses to 0.3 and 0.5 M NaCl when compared with those in the control group. Hierarchical cluster analyses revealed a strong similarity among responses to sodium salts in neurons of the CTA group compared with the control group. These results suggest that the aversive conditioning to NaCl modified PBN neurons so that the sodium taste was more salient than other tastes.

A further study using a similar experimental procedure was performed by Tokita et al. (2004). They found that the enhanced responses to the CS (0.1 M NaCl) were observed exclusively in amiloride-sensitive NaCl (ASN)-best neurons but neither in amiloride-insensitive NaCl (AIN)-best nor in any other best neurons (Figure 1). Electrical stimulation of the central nucleus of the amygdala (CeA), but not the gustatory cortex, produced an excitatory effect in significantly more neurons in the CTA group than in the control group. Decerebration after CTA acquisition abolished the increased responses to the CS in ASN-best neurons, suggesting that the influence of forebrain structures is not completely suppressed despite the deep anesthesia. The results also suggest that CTA conditioning uses an effective CeA input to modulate activity of gustatory neurons in the PBN and further that amiloride-sensitive components of



**Figure 1** A comparison of mean response profiles of best-stimulus categories of PBN neurons in CTA and control rats. Taste-responsive neurons were classified into best-stimulus categories, depending on their best sensitivity to any one of the 4 basic stimuli represented by sucrose, NaCl, HCl, and quinine hydrochloride (QHCl), such as S-best, N-best, H-best, and Q-best, respectively. N-best neurons were further classified into amiloride-sensitive and amiloride-insensitive N-best (ASN and AIN best) categories by application of an epithelial sodium transport blocker, amiloride. Each data point shows the mean  $\pm$  standard error spikes/s to each of the taste stimuli shown on abscissa. Taste responses to NaCl were significantly higher ( $*P < 0.01$ , Tukey's test) in the CTA group than in the control group in the ASN-best category, whereas taste responses in the AIN-best and other best-stimulus categories were almost identical between the groups. Therefore, aversive conditioning appears to increase the responsiveness only to a behaviorally relevant taste stimulus in a subset of neurons relevant to its taste quality, which may help the animals to avoid the ingestion of aversive, potentially harmful foods and fluids. From Tokita et al. (2004) with permission.

NaCl-best neurons play a critical role in the recognition of the distinctive taste of NaCl.

Li et al. (2005) found in the hamster PBN that more sucrose-best neurons were excited than inhibited, whereas the opposite occurred for citric acid- and quinine-best neurons in response to electrical stimulation of the CeA. These findings suggest that the descending information from the CeA modulates PBN activities toward the direction that sucrose-best neurons are excited and citric acid- and quinine-best neurons are suppressed. In accordance with this suggestion, when saccharin was used as the CS in rats, Chang and Scott (1984) reported that taste activity was enhanced after the acquisition of CTA in the subset of sweetener-sensitive neurons in the nucleus of the tractus solitarius that is also known to receive inputs from the CeA (Li et al. 2002), whereas when HCl was used as the CS, PBN neuronal response to the CS decreased in rats (Shimura et al. 2002).

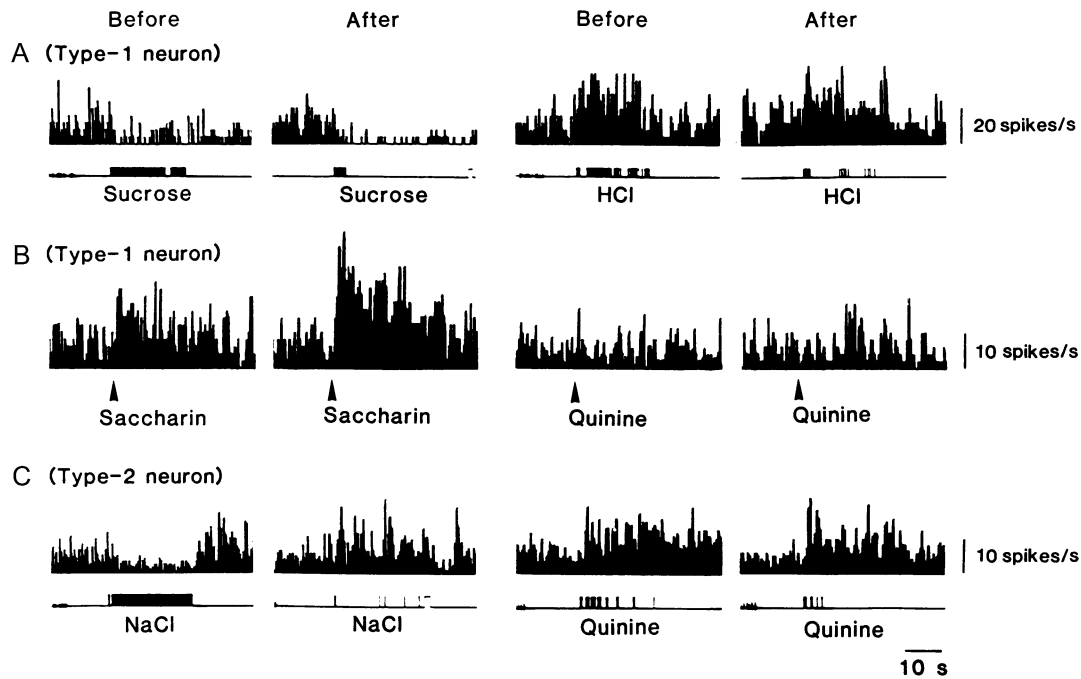
### Hedonic aspect

When CTA is acquired, a hedonic shift occurs as evidenced by a change from ingestive behavior to aversive behavior. Electrophysiological responses of neurons recorded from the gustatory cortex in awake behaving rats also change in such a way as to reflect a hedonic shift in a subset of

neurons. Yamamoto et al. (1989) classified taste-responsive neurons recorded from the rat gustatory cortex into quality type (Type-1 neuron in Figure 2) and hedonic type (Type-2 neuron in Figure 2) according to the response patterns to licking of various taste stimuli. The former neurons showed enhanced responses to a CS after acquisition of CTA, and the latter neurons exhibited alteration of the response direction, from excitatory to inhibitory or from inhibitory to excitatory, equivalent to that shown to aversive stimuli. Essentially, the same types of neurons and the altered responsiveness after CTA acquisition were observed in the amygdala in conscious rats (Yamamoto and Fujimoto 1991; Yasoshima et al. 1995).

Although the electrophysiological experiments as described above provide meaningful information, they provide limited data for understanding simultaneous overall activation of the brain. To address this issue, we have been exploring which brain regions are selectively activated by reexposure to the CS in conditioned rats by mapping immunoreactivity of *c-fos* and *zif268* as markers of neuronal activation (Yasoshima et al. 2005; Yasoshima, Scott et al. 2006; Yasoshima, Sako et al. 2006).

The major finding is that learned aversion to the CS after CTA is different from that to an innately aversive substance



**Figure 2** Responses of 2 type-1 (quality type) neurons (**A, B**) and 1 type-2 (hedonic type) neuron (**C**) before and after acquisition of CTA learning in rats. (**A**) This neuron showed more of a marked reduction of spontaneous discharge to licking of sucrose after conditioning to sucrose. (**B**) This neuron showed more of a marked excitation to licking of saccharin after conditioning to sucrose. (**C**) This neuron, which showed an inhibitory response to NaCl before conditioning, showed an excitatory response after conditioning to NaCl. Note that taste responses to stimuli other than the conditioned stimuli remained unaltered after the conditioning procedure. From Yamamoto et al. (1989) with permission.

such as quinine in terms of brain regions activated in response to these stimuli. The supramammillary nucleus (Yasoshima et al. 2005), thalamic paraventricular nucleus (Yasoshima et al. 2005), extended amygdala (Yasoshima, Scott et al. 2006), and nucleus accumbens (NAcb) (Yasoshima, Scott et al. 2006) are activated by retrieval of (or the first reexposure to) the CS after the acquisition of CTA. The former 2 regions are suggested to be involved in the expression of anxiety and psychological stress (Beck and Fibiger 1995; Ryabinin et al. 1995; Wirtshafter et al. 1998; Bubser and Deutch 1999; Spencer and Houpt 2001), and Yasoshima et al. (2005) have suggested that the supramammillary nucleus is activated by memory-elicited discomfort during retrieval of CTA. The latter 2 regions are involved in the reward system where CS information from the basolateral nucleus of the amygdala (BLA) reaches the NAcb directly or via the extended amygdala (Groenewegen et al. 1999; Shammah-Lagnado et al. 1999, 2001). The  $\gamma$ -aminobutyric acidergic (GABAergic) neurons in the NAcb send axons to the ventral pallidum (VP) as the main output target (Zahm et al. 1985), and from there, GABAergic projection to the lateral hypothalamus (LH) arises (Groenewegen et al. 1993). The blockade of GABA<sub>A</sub> receptors in the VP by bicuculline prominently increases food intake (Stratford et al. 1999) and intake of hedonically positive taste stimuli (Shimura et al. 2006). In contrast, excitotoxic lesions of the caudal aspect of the VP resulted in an aversive reaction to food (Cromwell

and Berridge 1993), and the activation of GABA<sub>A</sub> receptors in the VP by muscimol generally decreased fluid intake regardless of taste (Shimura et al. 2006).

Apart from its well-known role in reward-related behaviors as shown above, the NAcb-VP-LH circuit has also been reported to be involved in the acquisition and retrieval of CTAs (Mark et al. 1991; Turgeon and Reichstein 2002; Fenu and Di Chiara 2003; Ramírez-Lugo et al. 2006). To elucidate further the role of the VP on the expression of CTA, Inui et al. (2005) examined the effects of microinjections of a GABA<sub>A</sub> receptor antagonist, bicuculline, on the intake of CS in a retrieval test. Rats drank 5 mM saccharin or 0.3 mM quinine hydrochloride as the CS, which was followed by an i.p. injection of 0.15 M LiCl. After this pairing, vehicle or bicuculline was bilaterally infused into the VP immediately before reexposure to the CS. Microinjections of bicuculline significantly increased the intake of the saccharin CS but not the quinine CS. Whereas the control rats injected with vehicle showed aversive responses (e.g., gapes, chin rubs, head shakes, forelimb flails), the rats with infusion of bicuculline failed to show aversive responses. These results indicate that the blockade of GABA<sub>A</sub> receptors in the VP by microinjections of bicuculline disrupts the retrieval of CTA, and this may be due to elimination of aversive responses to the saccharin CS. Thus, it is suggested that the GABAergic system in the VP plays an important role in the retrieval of CTA when the CS is saccharin.

The different actions of bicuculline between aversive saccharin and quinine suggest that the brain mechanisms of learned and innate aversions are different. Quinine stimulation evoked modest *c-fos* expression in the NAcB, but saccharin stimulation after aversive conditioning induced robust *c-fos* expression in the NAcB (Yasoshima, Scott et al. 2006). The aversive behavior to quinine may primarily be established at the lower brain stem level because decerebrate animals show normal aversive oromotor and body reactions to quinine stimulation (Grill and Norgren 1978).

As suggested above, NAcB neurons may receive excitatory inputs from the BLA directly or via the extended amygdala in response to the CS after CTA acquisition. Because the innately aversive quinine does not activate the BLA (Yasoshima et al. 2005), NAcB neurons may be less excited by quinine, which is in accordance with recent electrophysiological results (Roitman et al. 2005). In addition to the reduction of aversion to the CS by the blockade of GABA<sub>A</sub> receptor in the VP (Inui et al. 2005), increasing the palatability of the sweet-tasting CS by systemic or intra-BLA administrations of midazolam, a benzodiazepine agonist, also impairs aversive behavior to the saccharin or sucrose CS after the acquisition of CTA (Parker 1995; Yasoshima and Yamamoto 2005).

Also of interest is the amygdala that is upstream of the reward system. A number of studies have dealt with the functions of the amygdaloid subnuclei in the formation of CTA. Although the studies have yielded inconsistent behavioral results, overall electrolytic or excitotoxic lesions show little, if any, involvement of the CeA in CTA (e.g., Kemble et al. 1979; Bermudez-Rattoni and McGaugh 1991; Yamamoto 1994; Yamamoto et al. 1995; Morris et al. 1999), whereas the lesions in many cases disrupted or attenuated CTA (e.g., Aggleton et al. 1981; Fitzgerald and Burton 1983; Simbayi et al. 1986; Yamamoto 1994; Yamamoto et al. 1995; Rollins et al. 2001). The BLA may play an important role in CS–US association, and also this nucleus is suggested to be involved in neophobia requisite for CTA formation (Reilly and Bornovalova 2005). Our electrophysiological, lesion-behavioral, and immunohistochemical studies, as summarized in the present article, suggest that lesions of the CeA have little effects on CTA because this subnucleus is involved with the enhanced responsiveness to the CS after CTA, and lesions of the BLA impair CTA because this subnucleus plays an important role in the acquisition and maintenance of hedonic shifts from positive to negative.

## Conclusion

This review article presents the suggestion that the enhanced activation to the CS after the acquisition of CTA originates in the CeA and the hedonic shift originates in the BLA. The cellular and subcellular basis for these essential phenomena still remain to be clarified.

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