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Molecular Signaling during Taste Aversion Learning

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Abstract

Behavioral and neural assessment tools have been used to identify cellular and molecular events that occur during taste aversion acquisition. Studies described here include an assessment of taste information processing and taste–illness association using fos-like immunoreactivity (FLI) to mark populations of cells that react strongly to the taste conditioned stimulus (CS), the illness unconditioned stimulus (US), or the pairing of CS and US. Exposure to a novel, but not a familiar, CS taste (saccharin) was found to induce robust increases in FLI in some, but not all, brain regions previously implicated in taste processing or taste aversion learning. Striking effects of taste novelty on FLI were found in central amygdala (CNA) and insular cortex (IC) but not in basolateral amygdala (BLA), pontine parabrachial nucleus (PBN), or nucleus of the solitary tract (NTS). Of those regions responding to taste novelty, only CNA showed significant elevations in FLI in response to the US, LiCl. In additional studies, FLI was examined after an effective training experience, novel CS–US pairing, and compared with an ineffective one, familiar CS–US pairing. After CS–US pairing, taste novelty modulated FLI in virtually all the regions previously implicated in conditioned taste aversion (CTA) learning, including PBN, CNA, BLA, IC, as well as NTS. Thus, a distributed and interdependent neural CTA circuit is mapped using this method, and the use of localized lesion and inactivation studies promises to further define the functional role of structures within this circuit.

Key words: amygdala, fos, insular cortex, lithium chloride, novel taste

Introduction

Taste aversion learning is a type of conditioning in which an animal learns to associate a taste (conditioned stimulus [CS]) with a drug or other treatment (unconditioned stimulus [US]) that produces nausea or illness. As a consequence of this conditioning, the taste becomes disliked and avoided (Garcia et al. 1974; Bernstein 1991). Conditioned taste aversions (CTAs) constitute a potent learning model that is associative, adaptive/defensive, and amygdala dependent (Bermúdez-Rattoni and Yamamoto 1998; Yasoshima et al. 2005; but see Reilly and Bornovalova 2005). It has a number of features that make it a promising model for neurobiological assessment. One of these features is rapid acquisition; CTAs are typically acquired after a single pairing of CS and US. Potent and durable one-trial learning provides a clear time window when underlying neuronal signaling can be identified. Another distinctive feature of CTA learning is that it can occur despite lengthy delays between exposure to CS taste and US drug (Garcia et al. 1966; Revusky and Garcia 1970). In virtually every other conditioning paradigm, close temporal proximity between presentation of CS and US are critical to effective conditioning. CTAs, however, are routinely acquired after delays of several minutes or hours between exposure to CS taste and US illness. The requirement for temporal contiguity, with an optimal range being 500 ms to 2 s, is so common a feature of associative learning paradigms that it has been used to build models of the types of cellular signaling processes that might underlie plasticity (Abrams and Kandel 1988). In this regard, taste aversion learning is clearly an anomaly.

Our laboratory has combined behavioral and neural assessment tools to identify cellular events involved in taste aversion acquisition. These studies include an assessment of taste information processing that occurs when an animal is exposed for the first time to a new taste and taste–illness associations that underlie acquisition of CTAs (Koh et al. 2003; Koh and Bernstein 2005).

Taste information processing

Robust and rapid CTA acquisition relies heavily on the novelty of the CS taste (Revusky and Bedarf 1967; Kalat and Rozin 1973; Kalat 1974). In the laboratory, one or

two safe exposures to a taste prior to conditioning can dramatically attenuate learning to that taste. Varying taste novelty provides a tool for assessment of molecular mediation of the learning because gene expression and protein synthesis critical to the learning should be modulated strongly by the novelty of the taste. Furthermore, the localization of such modulation should point to regions critically involved in taste memory.

Immediate early genes (IEGs), such as *c-fos*, couple short-term neuronal activity with changes in the level of gene transcription and are potential markers of neurons undergoing modification as a result of experience. To date, increases in fos-like immunoreactivity (FLI) have been reported in brain regions implicated in CTA learning after exposure to the US (Lamprecht and Dudai 1995; Swank 1999) and following behavioral expression of a CTA (e.g., Swank and Bernstein 1994; Houpt et al. 1996). Interference with fos expression by the central administration of fos antisense interferes with acquisition but not expression of the learning (Lamprecht and Dudai 1996).

Our laboratory examined the induction of FLI in response to taste exposure in order to mark populations of cells that react strongly to the taste but also because IEGs represent intriguing candidates for molecular mediation of CTA learning (Koh et al. 2003). The synthesis and subsequent degradation of a protein product of an IEG such as *c-fos* could be a biochemical substrate that underlies changes in conditioning strength over a range of CS–US intervals. Varying taste novelty provides a potent tool for identifying events preparatory to and supportive of learning. Thus, we hypothesize that neural responses to novel and familiar tastes differ significantly, and this determines whether the taste becomes the target of an aversion during CTA training. In particular, gene expression and protein synthesis critical to the learning should be modulated strongly by the novelty of the taste; such signals should display anatomical localization allowing convergence with signals generated by the US and should be characterized by a temporal profile allowing overlap with signals generated by the US. Furthermore, the localization of these signals should point to regions critically involved in taste memory.

Rats with and without prior experience with 0.5% saccharin solution were compared using immunostaining for the protein product of the IEG, *c-fos*, to identify patterns of neuronal activity after exposure to a novel, in contrast to a familiar, taste. Experience consisted of 6 days of preexposure for rats in the familiar group to develop a "safe" taste memory of saccharin. On the test day, familiar and novel groups were given 30 min to consume a maximum of 5 ml of the saccharin solution. They were sacrificed 2 h later, and brains were processed for FLI. Regions previously implicated in taste aversion learning were examined; these include the nucleus of the solitary tract (NTS), pontine parabrachial nucleus (PBN), amygdala, and insular (gustatory) cortex (IC).

In brief, exposure to a novel, but not a familiar, saccharin solution was found to induce robust increases in FLI in some, but not all, brain regions previously implicated in taste processing or taste aversion learning. Striking effects of taste novelty on FLI were found in the central amygdala (CNA) and IC but not the basolateral amygdala (BLA), the medial PBN, or the rostral NTS (Koh et al. 2003).

Taste and LiCl: neural responses over time

In 2 separate experiments, we performed detailed analyses of the temporal profiles of FLI activation to novel taste and to lithium chloride (LiCl). Regions that show strong neural expression to both the CS and the US are likely important to the formation of a CTA. In the taste CS experiment, thirsty rats received either 0.5% saccharin solution (familiar condition) or water (novel condition) for 30 min each day during 6 days of preexposure training. On the seventh day, all rats were given saccharin to drink with intake capped at 5 ml in 30 min. Rats were then perfused at one of the following time points: 0.5, 1, 2, 4, or 6 h after taste exposure (n = 6–7 per group). In the LiCl experiment, rats were injected with LiCl (0.15 M, 5 ml/kg) and perfused at each of the same time points as in the taste experiment (n = 5 per group). The drug dose used here is effective at inducing a CTA to novel but not familiar tastes in a single trial (Koh and Bernstein 2005). An additional group (n = 5) received saline injection (5 ml/kg) and was perfused 1 h later. In both experiments, brains were harvested and processed for FLI as previously described (Koh et al. 2003).

Figure 1 shows the patterns of FLI in CNA (A) and IC (B) following exposures to either a novel or familiar taste after various delays. For CNA, there was a significant novelty by time interaction, $F(4,51) = 6.20$, $P < 0.01$, novelty main effect, $F(4,51) = 40.75$, $P < 0.01$, and time main effect, $F(4,51) = 18.10$, $P < 0.01$. Further analysis shows that whereas novel tastes induced significant changes in FLI over time, $P < 0.01$ (1-way analysis of variance [ANOVA]), familiar tastes showed little elevation above baseline over the sampled time points. Direct comparisons between novel and familiar groups at each time point show that novel taste induced substantially more FLI than familiar taste after 1 and 2 h delays, P values < 0.05 (Tukey's HSD). Similar patterns of results were observed in the IC. There was a significant interaction, $F(4,51) = 6.13$, $P < 0.01$, novelty main effect, $F(4,51) = 36.92$, $P < 0.01$, and time main effect, $F(4,51) = 26.86$, $P < 0.01$. Significantly higher FLI expression to novel taste exposure was detected after 1 h delay, $P < 0.01$, but not at other time points although the difference approached statistical significance at 2 h ($0.08 > P > 0.07$). Analyses of FLI expression in other brain regions, including BLA, medial and lateral PBN, and rostral and intermediate NTS, failed to show significant interactions. Taken together, these results show that novel tastes increased FLI in CNA and IC and that this signal degrades over time, whereas familiar taste failed to

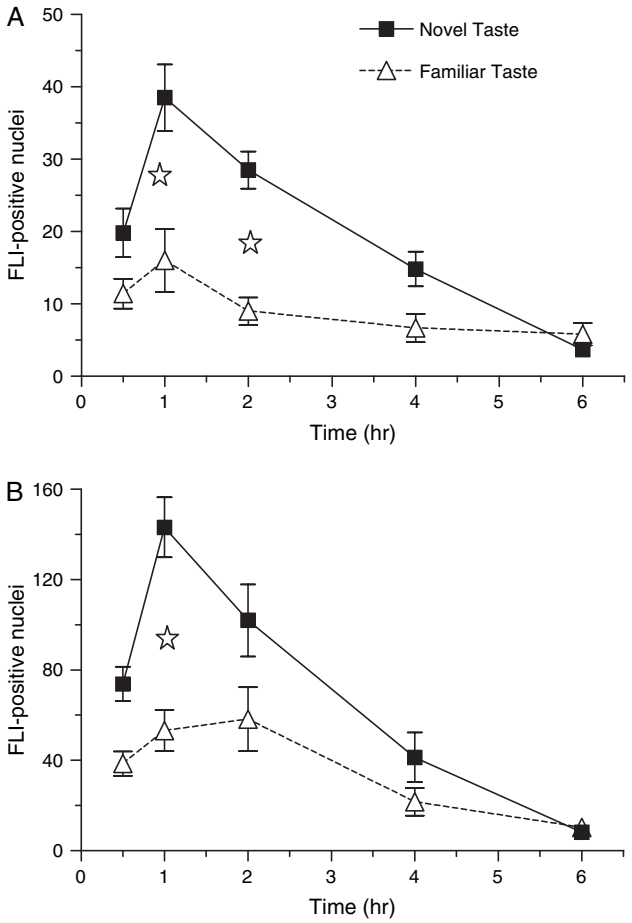

Figure 1 Mean number of FLI nuclei in CNA (upper graph, **A)** and IC (lower graph, **B)** 0.5, 1, 2, 4, or 6 h following exposure to either novel or familiar taste. Stars *P* values < 0.05 between novel and familiar groups.

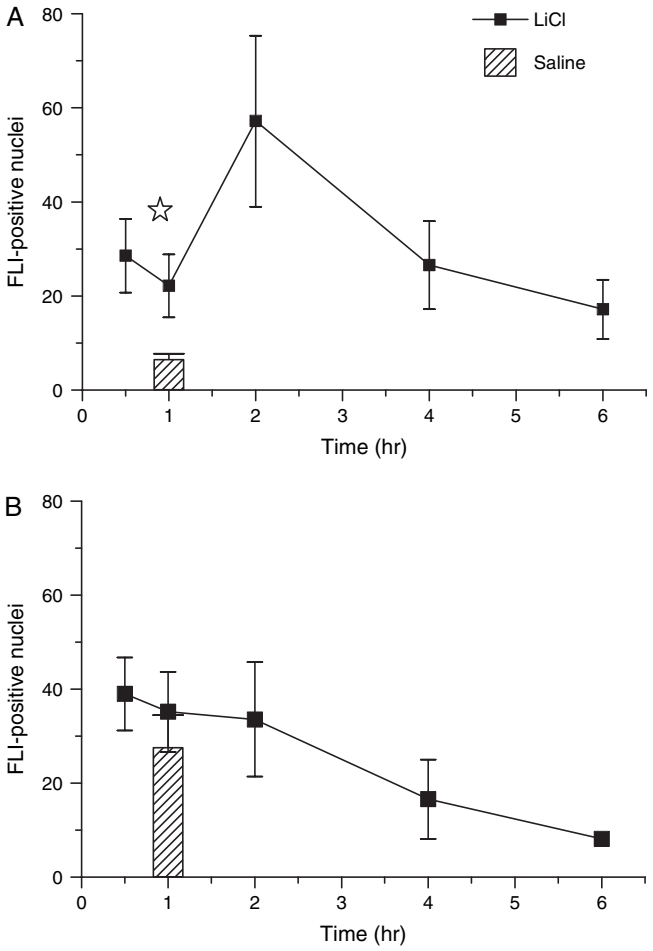

Figure 2 Mean number of FLI nuclei in CNA (upper graph, **A)** and IC (lower graph, **B)** 0.5, 1, 2, 4, or 6 h following exposure to either saline or LiCl. Star *P* values < 0.05 between saline and LiCl groups.

show any indication of FLI increases within the time course studied.

In light of the patterns of expression to novel taste, we focused on CNA and IC in the LiCl experiment. Figure 2 shows the patterns of FLI in CNA (A) and IC (B) following LiCl or saline injection. For CNA, a 1-way ANOVA revealed a significant effect among the groups, $F(5,24) = 3.08$, $P < 0.03$. Direct comparison between LiCl and saline groups at 1 h shows that LiCl induced a stronger FLI expression than saline in the CNA, $t(8) = 2.29$, $P = 0.05$, consistent with many previous reports (Yamamoto et al. 1992; Swank 1999; Spencer and Houpt 2001). In contrast, no overall group difference was detected in the IC, $P > 0.09$. Notably, the level of FLI activation induced by LiCl and saline at 1 h in the IC was comparable ($t < 1$). Hence, when these results are taken together with those of the taste experiment, the CNA appears to be the region that shows the most robust neural activations to both the CS and the US. Furthermore, the timing of increased neural activation to both stimuli overlapped considerably, which allows for molecular signals from the CS to be associated with those from the US. These results, therefore, point to the CNA as a potential site of CS–US association for the formation of a CTA.

These studies indicate that exposure to a novel taste CS induces strong FLI in CNA and IC and that elevations in FLI peak at 2 h after taste exposure and decline thereafter such that by 4 h they are no longer elevated above baseline levels. Furthermore, there is the potential for convergence in activation of the CS with the US, based on the observation that intraperitoneal LiCl elevates FLI at similar time points and in a similar location (i.e., CNA). This inference is supported by the findings that temporary inactivation of the CNA with a protein synthesis inhibitor impairs CTA acquisition (Bahar et al. 2003). In contrast, IC does not display evidence of convergence as significant elevations in FLI due to taste novelty in the gustatory region do not overlap with FLI in response to LiCl in cells responsive to visceral signals.

It may be noted that manipulations known to reduce the strength of CTA learning (e.g., taste preexposure, reduced CS intensity, and longer CS–US delays) were found to blunt

or eliminate the FLI response to CS. This provides strong support for the idea that FLI marks neural pathways critical to CS processing during CTA acquisition and, further, that key transcriptional events underlying this plasticity may involve fos expression. A causal link between elevations in fos protein and CTA acquisition has been indicated by the finding that administration of fos antisense aimed at either the CNA (Lamprecht and Dudai 1996) or the cerebral ventricle (Swank 1996) interferes with acquisition of CTA. However, those studies were unable to define whether the critical role of fos was in CS processing, US processing, or the association of the two. The present study points to a potential role for fos protein in CS processing. The patterns of results are indicative of involvement of IC and CNA in taste memory processing. They further suggest that synthesis and subsequent degradation of fos protein within these cells represents a biochemical ''trace'' of the novel taste that has the potential to bridge some CS–US intervals and play a role in mediating long-delay learning. Although only fos was assessed in the present studies, we readily acknowledge that other molecular events (not necessarily downstream of fos) are likely to be necessary for forming CTAs.

Taste–illness associations

The previous studies addressed FLI following CS alone and US alone. However, neither of these conditions is sufficient to yield CTA learning. The next set of studies addressed FLI after stimulus exposures known to be sufficient to generate strong CTA learning (i.e., CS–US pairing). Rats were exposed to one of the following conditions after which their brains were processed for FLI: novel saccharin paired with LiCl, familiar saccharin paired with LiCl, or LiCl alone. Familiarization took place as described, with 6 preexposures prior to the conditioning session. Only the novel CS–US group experienced stimulus exposure known to generate strong CTAs in a single trial. Results indicated strong and widespread induction of FLI only in animals exposed to a highly effective conditioning experience (Koh and Bernstein 2005). Differential gene expression as a function of CS taste novelty was evident throughout the CTA neural circuit, including brainstem (NTS, PBN) and forebrain (amygdala, IC), but not in areas known to be uninvolved in the learning such as the hippocampus. The striking behavioral and neural differences between groups conditioned with novel and familiar tastes provide a remarkable window on the circuitry recruited during the acquisition process.

Whereas assessment of fos expression in response to novel taste and to LiCl implicates CNA as a common site for processing stimuli critical to the learning, assessment of the same stimuli when paired together points to a more distributed circuit that engages multiple brain regions (Table 1). The specific roles of these regions to the acquisition process are a topic of investigation in our laboratory. In particular, we are currently examining how the PBN, which has been so strongly implicated in CTA acquisition through lesion studies, fits into this picture. We hypothesize that increased activation in this distributed circuit during acquisition could reflect the transmission of CS and US information to a site of convergence (e.g., CNA), the receiving of critical information from the site of convergence to facilitate CS–US association and memory consolidation, or a combination of both. Activation of IC during acquisition, for instance, has been linked to the detection of CS taste novelty and the transfer of that information to the amygdala. Appropriately, lesions of IC were found to reduce FLI expression in the amygdala to novel CS–US pairings, whereas temporary inactivation of IC during taste familiarization resulted in increased FLI expression in the amygdala to familiar CS–US pairing during CTA acquisition (Koh and Bernstein 2005). These findings suggest a high degree of interdependence among multiple brain regions for successful acquisition of a CTA.

Our studies have begun to define a distributed circuit in which neural activation is strongly modulated by taste novelty during CS–US pairing. Controls with identical stimulus exposure provide a relatively conservative comparison group. Clearly, the conditions that support CTA learning engage multiple brain sites. By combining FLI with focal and reversible lesions, we get a more nuanced view of how the circuit interacts as it processes CTA associations. Furthermore, it becomes clear that lesions in one site strongly affect activity of other structures in the circuit. By applying these combined approaches at different nodes in the CTA circuit, we expect to better define its functional anatomy.

Table 1 Summary of effects of CS taste novelty, US LiCl administration, and CS–US pairing on regional expression of FLI

	IC	BLA	CNA	HPC	lPBN	mPBN	rNTS	iNTS
CS taste (0.5% saccharin)	↑[a,b]	—[a,d]	↑[a,b]	—[d]	—[d]	—[a,d]	—[a,d]	—[d]
US LiCl (0.[a]5 M, 5 ml/kg)	—[b]	—[d]	↑[b]	N/A	N/A	N/A	N/A	N/A
CS–US pairing	↑[c]	↑[c]	↑[c]	—[c]	↑[c]	↑[c]	N/A	↑[c]

HPC, hippocampus; lPBN, lateral parabrachial nucleus; mPBN, medial parabrachial nucleus; rNTS, rostral nucleus of the solitary tract; iNTS, intermediate nucleus of the solitary tract; ↑, significantly higher levels of FLI expression relative to controls; —, no difference; and N/A, not available.
[a]Koh et al. (2003).
[b]See text for details.
[c]Koh and Bernstein (2005).
[d]Koh MT and Bernstein IL (unpublished data).

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