The highlight for October is from Dr. Ilene Bernstein of the Department of Psychology at the University of Washington in Seattle, Washington. For the past 30 years, Dr. Bernstein has provided careful and systematic analyses of a variety of phenomena related to food intake from sodium appetite to cancer anorexia and, most recently, to the neurobiology of conditioned taste aversion learning. Her initial work on cancer anorexia provided interesting and important insights into the etiology of conditioned changes in food intake and the use of aversion learning in modulating such changes. Her current work using c-Fos immunohistochemistry is no less insightful. In the present highlight, Dr. Bernstein describes her work examining the neurochemical correlates and neuroanatomical substrates of conditioned taste aversion learning. Using immunostaining for c-Fos, she and her laboratory have shown convincingly the locus of taste and toxin processing (initially in the intermediate division of the nucleus of the solitary tract) and the recruitment and involvement of forebrain areas such as the central nucleus of the amygdala and the insular cortex in aversion learning (in both safety and danger). The elegant combination of behavioral, neuroanatomical and neurochemical techniques has allowed Dr. Bernstein and her group to make significant progress in delineating the processes involved in initial taste processing and in the classification of such tastes as important stimuli consequent to toxicosis. Her summary describes these important efforts.

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My intense interest in taste aversion learning dates back several decades. In that sense there is continuity to be found in my research career. Even in the late seventies, it was the neural underpinnings of this potent form of learning that intrigued me. I was convinced that this learning model, because of its rapid acquisition and durability, would prove particularly amenable to neurobiological analysis. At that time, however, I made a major detour, and found myself studying a significant clinical problem that was impacted by taste aversion learning, namely the development of food aversions in cancer patients receiving chemotherapy. But my clinical detour has been over for some time and, during the past 10-15 years, my laboratory has been testing my conviction that the neurobiological analysis of CTA had much to tell us about neural plasticity.

Fos immunostaining as a tool in the analysis of CTA neural circuitry
Immunostaining for cFos protein can serve as a localized marker for neuronal activation in the brain. We have found this method to be an extremely useful tool in expanding our understanding of neural events underlying CTA acquisition and expression. In our laboratory this method has been successfully combined with behavioral assessment, permanent and reversible brain lesions and tract tracing to define connectivity.

Expression of the gene c-fos leads to production of cFos protein. cFos is involved in the targeted transcripion of other genes. For our purposes, the use of this method to define patterns of neuronal activity has many advantages. It marks populations of neurons activated in conscious animals by a specific, defined stimulus. This suits the CTA paradigm by taking particular advantage of the fact that strong CTAs can be acquired in a single trial. It allows individual activated neurons to be identified while being able to sample from widespread brain regions. The method can be combined with other procedures (e.g. tract tracers; immunostaining for neurochemicals) to yield critical information about activated cell populations.

Of course, fos immunostaining is not without its limitations. Neurons differ in their capacity to express fos and in their latency to do so. Strong and sustained stimulation of neurons is generally required before fos expression occurs. Thus, absence of fos expression in neurons does not necessarily mean that no neurons were activated by that specific stimulus. The nature of the signal allows for only limited temporal resolution and it can be unclear whether fos expression is a direct or indirect consequence of the stimulus.

**cFos in medial NTS: A Cellular Marker of CTA Expression**

A dramatic change in the behavioral response to a taste CS is the hallmark of a conditioned taste aversion. We asked whether we could detect changes in pattern of neural activation in response to the taste as a result of conditioning. We found elevations of cFos in brainstem neurons during CTA expression which reflect a change in neural activation to a CS taste as a result of learning. This altered response was located in the intermediate division of the nucleus of the solitary tract (mNTS). Increases in cFos were originally observed when rats were re-exposed to a LiCl-paired saccharin solution, but were later seen when other tastes were used as the CS and when other drugs were used as the US. It was not seen in “unpaired” control animals exposed to saccharin nor was it seen when the behavioral aversion had been thoroughly extinguished. Thus, the cFos response appears to be a reliable marker of the expression of a CTA. Combining a cellular marker with behavioral dependent measures can overcome some
limitations of approaches which rely on behavior alone. The cellular marker enables us to assess the effects of unilateral damage and has already yielded information about the lateralization of CTA information. Using unilateral brainstem transections and lesions, we obtained evidence for the obligate participation of an ipsilateral forebrain pathway in the conditioned cFos in NTS, suggesting that the response reflects plastic changes generated within the forebrain. Determination of Fos expression also allows for assessment of CTA learning without complete reliance on behavioral response production.

Changes in Fos expression may provide information about the nature of the plastic changes which underlie CTA learning. The Fos response observed in NTS is induced by tastes which had become aversive as a result of learning, but not by a taste, quinine, which is innately aversive. We hypothesize that the difference between quinine and conditioned saccharin is not that one is more aversive than the other, as both were rejected within an equally short time. Rather, the most salient difference between these two stimuli which elicited outwardly similar behavioral responses is that, unlike the unlearned response to quinine, the response to saccharin is learned. The unlearned rejection of quinine is evident even in the decerebrate rat while conditioned rejection of saccharin requires forebrain input and is not seen in decerebrates. Our studies of Fos induction suggest that expression of a CTA requires the involvement of a lateralized forebrain pathway which modifies the pattern of activation in NTS in response to CS re-exposure. We further hypothesize that the Fos in NTS reflects this plasticity and that the response in NTS occurs as a result of input from the forebrain, which acts to override or modify the innate/reflexive motor program which would normally be triggered by a sweet taste.

**Differential expression of cFos as a function of taste novelty.**

Taste novelty strongly modulates the speed and strength of taste aversion conditioning. To identify molecular signals responsive to novel tastes, immunostaining for Fos protein marked neurons which respond differentially to taste novelty. Novel saccharin induced larger increases in FLI than familiar saccharin. This pattern was seen in central nucleus of amygdala (CNA) and insular cortex (IC), but not in basolateral amygdala, parabrachial nucleus or NTS. Other parameters known to influence aversion learning were tested for effects on fos immunostaining. Manipulations known to reduce the strength of learning blunted the fos response, supporting the idea that fos immunostaining marks pathways critical to taste processing during acquisition, and that c-fos expression is a
key transcriptional event underlying this plasticity. However, an evaluation of the correspondence between neuronal and behavioral variables depends on the sensitivity of the assays employed --- the behavioral as well as neural. These studies extended the use of Fos immunostaining from US processing and CTA expression to CS processing. This represents a significant advance because the kinetics of synthesis and degradation of the protein provide a potential molecular trace of the taste CS that can link to a US, which is temporally remote.

**Mapping conditioned taste aversion associations using c-Fos**

Pairing a novel taste with LiCl was highly effective in producing a strong CTA in one trial. The same experience, if the taste has become familiar, yielded no evidence of learning. Thus, novel and familiar paired groups can be exposed to identical stimuli during a conditioning trial but only the novel group will be establishing an effective CS-US association. Importantly, a novel CS-US pairing induced stronger fos immunoreactivity in insular cortex (IC), amygdala, and brainstem than familiar CS-US pairing, suggesting a large circuit is recruited for acquisition. Thus, coordinate activation of key brain circuitry is displayed after the former, but not the latter, experience. Evidence of differential gene expression as a function of CS taste novelty was widespread, including brainstem (NTS, PBN) and forebrain (amygdala, IC) structures implicated in CTA learning. 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.

To better define the role of the insular cortex (IC), we combined immunostaining with lesion or reversible inactivation of IC. Lesions abolished FLI increases to novel taste pairing in amygdala, suggesting a role in novelty detection. Reversible inactivation during taste pre-exposure increased FLI to familiar taste pairing in amygdala and brainstem. The difference between temporary inactivation, which blocked establishment of 'safe' taste memory, and lesions, points to a dual role of IC in taste learning. This dual role consists of both processing taste memory as a taste goes from novel to familiar and providing ‘on-line’ comparison between incoming tastes and stored taste memories.

**Summary**

Our laboratory has employed a multipronged approach to the neurobiological characterization of taste aversion learning. This has involved behavioral assessments in conjunction with unilateral and bilateral lesions and transections, anterograde and retrograde tract-tracing studies, cFos immunohistochemistry, in situ hybridization for identification of
neurochemical phenotypes. We have also extended these studies to the mouse to exploit transgenic or knockout models. From these studies a pattern of results has been emerging that defines key features of the neural events involved in taste aversion acquisition and expression.

References


