Benzodiazepine Modulation of Gustatory Coding in the Parabrachial Nucleus

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INTRODUCTION

- Benzodiazepines (BZs) have long been known to produce hyperphagia by increasing the gustatory hedonic evaluation of foodstuffs. Behavior analyses of taste reactivity, licking microstructure, and brief access licking after BZ treatment all support this conclusion (3, 4, 6).

- Studies suggest that benzodiazepines may modify taste evaluation through hindbrain neural systems. Chronic decerebrate rats in which the forebrain is disconnected from the hindbrain exhibit enhanced gustatory responses after benzodiazepines, and direct infusion of benzodiazepines to the hindbrain fourth ventricle or parabrachial nucleus (PBN) of intact rats also produce this response (2, 7, 8, 10, 12).

- Although the behavioral evidence for BZ modulation of gustatory coding in the PBN is compelling and clear, no studies to date have evaluated electrophysiological PBN gustatory responses to benzodiazepine receptor ligands. We therefore evaluated PBN gustatory neuron responses before and after injections of chlordiazepoxide (CDP), a prototypical BZ agonist with well established gustatory behavioral effects (1, 5).

METHODS

Single unit extracellular acute recording in the PBN was performed in urethane anesthetized rats. Using tungsten microelectrodes, gustatory responsive cells were located using standard search procedures. Each taste cell was profiled 2-3 times for responses to 1.0M sucrose, 0.1M NaCl, 0.03M citric acid, 0.003M quinine hydrochloride (QHCl), and water. Tastants were delivered using a flow delivery system involving two pipettes fed by a pressurized manifold delivery system. Each tastant was applied for 20s followed by a 20s water rinse and a 60s rest interval. Water trials allowed subtraction of tactile/thermal responses.

Only a single systemic CDP injection can be made per rat. To maximize the efficiency of the preparation, two test strategies were combined. One strategy employed a "within-cell" repeated-measures test comparing taste responses of a neuron before and after CDP/saline infusion (n=23), while a second used a "between-cells" comparison (n=106). Prior to drug infusion, the first two isolated neurons were characterized for their gustatory response profile ("before CDP/saline" cells). The third cell ("within group") underwent the repeated-measures drug test: After baseline response profiling, saline/CDP (20 mg/kg) was injected and the cell was retested continuously for a maximum of 1 h (six taste batteries) or until isolation was lost prior to 1h. After testing the third neuron, additional neurons (the "after CDP/saline" group) were recruited up to 4 h after injection.

Responses were the number of spikes during the taste flow period minus the number of spontaneous spikes preceding each taste trial. Water responses were then subtracted to isolate the taste component of the response. Responses were then averaged for the repeated presentations. More than 80% of cells had at least 2 batteries of taste tests. For repeated-measures cells, the 3 batteries for the baseline phase and for the first and second 30 min periods after CDP injection were averaged. Breadth of tuning (entropy) values were evaluated using the formula ($H = -1.661 * \text{sum}[p_i(\log 10\ p_i)]$, $i = 1\text{-}4$); H=1 indicates a neuron that responds equally to all four tastants.

RESULTS

Figure 1. 129 cells were recorded in 23 rats. In the "within" group, 16 cells were tested both before and after CDP injection and 7 cells were tested both before and after saline. In the "between" groups, 49 cells were sampled prior to a CDP/saline injection. After CDP injection 51 cells were sampled, while 29 were sampled after a saline injection.

The proportion of cells responding best to sucrose in both groups more than doubled after CDP injection. This increase was significant for the "between groups" and was offset by a reduction in acid or QHCl best cells ($X2(3)=10.25$, $p<0.05$). Chi-square could not be analyzed in the repeated measures group due to low sample size. There were no shifts after saline injection in either group.

CDP INCREASED THE PROPORTION OF CELLS BEST-RESPONSIVE TO SUCROSE

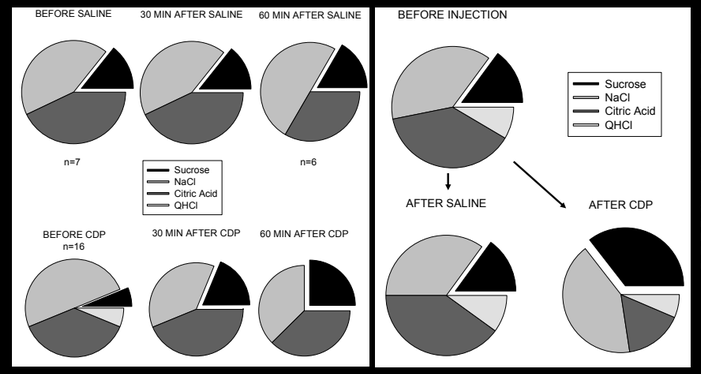


Figure 2. An individual PBN gustatory neuron before and after CDP injection. The same neuron is depicted in all four images. For each image, the bottom trace is the neural signal (V) amplified 10000x and the top traces are stimulus markers. The second upward deflection indicates taste application to the oral cavity (20s), followed by 20s water rinse. **Top:** The unit had a robust citric acid response that was reduced after CDP injection. **Bottom:** The neuron had a small response to 0.1M NaCl that increased after CDP injection.

CDP MODULATED RESPONSES BY TASTE QUALITY IN SOME CELLS

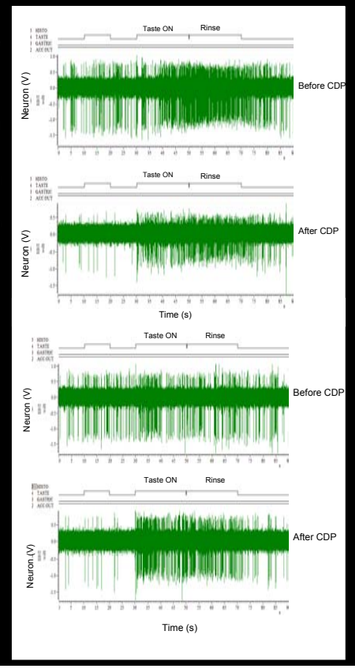


Figure 3. In the repeated measures CDP group, spontaneous activity ($F(2,26)=9.94$, $p<0.01$) and responses to QHCl were significantly suppressed ($F(2,26)=5.82$, $p<0.01$). Population responses to sucrose, NaCl and citric acid were not significantly changed (ps>0.1). Across the "between" groups, there was no effect of CDP on spontaneous rate ($t(96)=-0.77$, $p=0.44$) or water ($F(1,15)=0.41$, $p=0.53$). No significant changes were observed between the "before" and "after" saline injection groups (ps>0.1). After CDP groups the responses to citric acid ($t(57)=-2.43$, $p<0.01$) and QHCl ($t(57)=-2.98$, $p<0.01$) were significantly lower, while sucrose and NaCl responses were not.

Figure 4 (right). In the repeated-measures group, entropy values were reduced after CDP, but the difference was not significant (p=0.10). However, in cells that were broadly tuned initially (n=10; H>0.75), there was a small but significant narrowing of tuning ($t(9)=2.96$, $p<0.01$). All cells that showed a change in best tastant after CDP were in this group. There was no change in entropy after saline injection. No differences were found in the between-subjects groups in terms of their breadth of tuning.

CDP SUPPRESSED RESPONSES TO AVERSIVE TASTE STIMULI

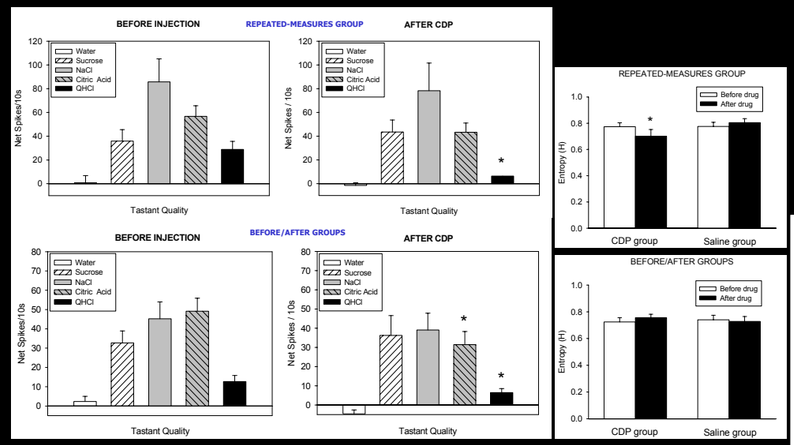
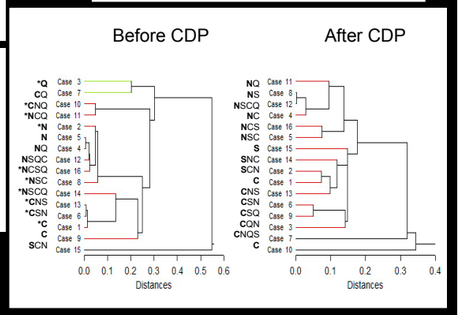


Figure 5 (right). Hierarchical cluster analysis of repeated measures cells before and after CDP. Symbols to the left of each cell (case #) indicates tastants for which a significant response was observed (net response > 2 SD of baseline activity). The first letter in bold indicates the best tastant. S = sucrose, N = NaCl, C = citric acid, Q = QHCl. * = broadly tuned cell (H > 0.75).



DISCUSSION

- Overall, the results provide the best evidence to date that BZ modulation of behavioral gustatory evaluation may be mediated in part via gustatory tuning modulation of PBN neurons.

- CDP reduced both the response magnitude and/or proportion of cells responding best to citric acid and QHCl. These finding suggest that behavioral responses to aversive gustatory stimuli should be affected by BZs. We are working to systematically characterize behavioral responses to aversive tastants through brief access and licking microstructure paradigms.

- CDP increased the proportion of cells responding best to sucrose without altering the number of spikes elicited by sucrose. The results suggest that increased recruitment of S-best cells, perhaps via dynamic change in individual cell responses, may underlie the behavioral responses to sweet stimuli after BZ treatment. The result could be through a selective suppression of responses to aversive stimuli in individual cells, which may also contribute to the finding that the more broadly tuned cells showed a small reduction in entropy after CDP.

- It is not clear whether the effects observed here are mediated by direct action of BZ in the PBN or through actions at other forebrain, hindbrain, or peripheral sites. We are exploring this question through the use of direct nerve recordings, injections targeted directly to the PBN (9, 11), and analysis of the subnuclear distribution of CDP neurons modulated in the PBN.

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