GABA-A in the Parabrachial Nucleus Enhances Taste Palatability during Long-term & Brief-access Testing in Rats.

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The Issue

Obesity continues to be a major health concern affecting increasing numbers of Americans. Advances in the pharmacological treatment of obesity are dependent on a better understanding of the neural control and regulation of food palatability and feeding behavior. Benzodiazepines are a class of anxiolytic drugs that facilitate the effectiveness of the GABA neurotransmitter's inhibitory action in the brain through GABA-A receptors. Over 11% of Americans have been prescribed benzodiazepines for anxiolytic relief. Prior studies in a rat animal model have shown that benzodiazepines potently increase consumption of food, producing a state of hyperphagia and weight gain, which is a common side effect of benzodiazepines.

Taste Palatability, the PBN, & GABA

Benzodiazepine receptors are broadly distributed throughout the CNS including the gustatory nuclei of the hindbrain, the nucleus of the solitary tract (NST) and the parabrachial nucleus (PBN). Recent research has identified neural projections from the hypothalamus to the parabrachial nucleus (PBN) as important regulators of feeding behavior. These fibers co-express GABA and knocking out GABA in these fibers profoundly reduces feeding and body weight. Furthermore, the PBN as been identified as necessary to allow formation of a conditioned taste aversion and may represent a site in the brain responsible for changes in taste associated with cravings and learned taste preferences and aversions. Therefore, the PBN appears to be a good candidate for a central site at which afferent taste signals can be modified based on previous experience and motivational states.

Our research seeks to identify the role of GABA in modifying afferent taste signals in the PBN through application of GABA-A agonists during behavioral and electrophysiological gustatory tests.

Methods

Subjects: Adult male Sprague-Dawley rats (300-400g)

Test Stimuli: Water, sucrose, saccharin, glucose, MSG, NaCl, citric acid, Q-HCl, and capsaicin

Drug Conditions: chlordiazepoxide CDP, 10mg/1ml/kg intraperitoneal (IP). or 33.6µg/4µL/2min intraPBN; buspirone BUS, 1.5mg/1ml/kg IP; saline 150mM NaCl, 1ml/kg IP; and aCSF 4µL/2min intraPBN.

Brief-access behavioral tests were daily sessions in the MS-160 lickometer (DiLog Insturments) in which each lick and the latency until the first lick were recorded. Test sessions contained 3-5 blocks of the taste stimuli plus water in pseudorandom order. There were 10s intervals between trials and per trial, rats had 30s to initiate a lick followed by a 15s trial duration after the first lick.

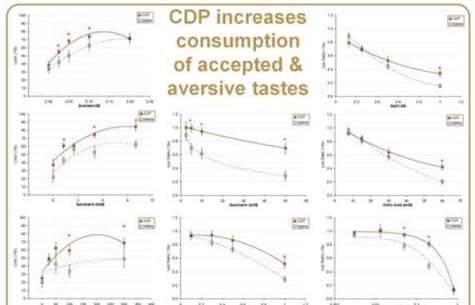
Long-term behavioral tests were daily 60-min sessions in the AC-108 lickometer (DiLog Instruments) in which each lick was recorded. Microanalysis of the data categorized the licking patterns into meals (terminated by 10min pause) and bursts (terminated by 1s pause) of licking.

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. 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.

Data Analysis: All data were statistically analyzed using repeated measures ANOVA and Bonferroni post-hoc paired t-tests ($p<0.05$, SPSS).

Behavioral Evidence: Brief-access Intake Tests

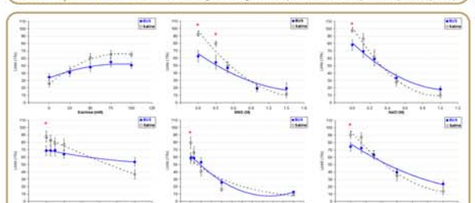
CDP increases consumption of accepted & aversive tastes



ABOVE: The number of licks (15s trials) to normally accepted tastants (sucrose, saccharin, MSG) during water-replete motivation

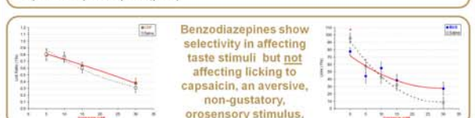
ABOVE: Lick ratios *(Licks to Tastant / Licks to Water; 15s trials)* to normally aversive tastants (saccharin, MSG, NaCl, citric acid, quinine-HCl) during water-restricted motivation

Repeated measures ANOVA revealed significant drug effects compared to saline, stars indicate significant drug effects at specific concentrations (Bonferroni post-hoc, $p<0.05$).



Buspirone(BUS) has no effect on licking to normally accepted tastants (sucrose; water-replete motivation) or normally aversive tastants (saccharin, MSG, NaCl, citric acid, quinine-HCl; water-restricted motivation).

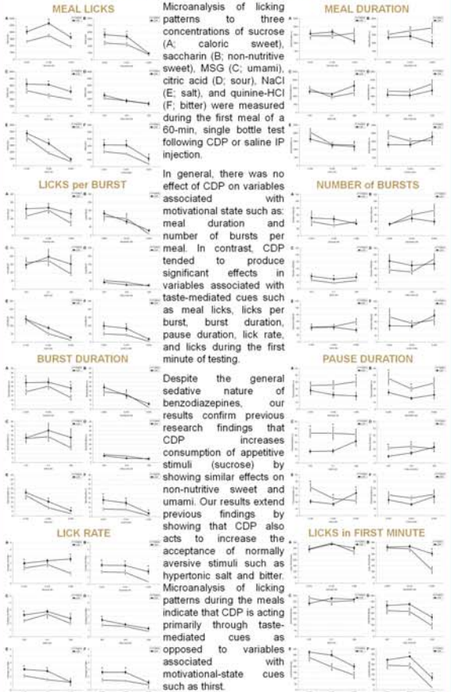
Repeated measures ANOVA revealed no drug effects for tastants, stars indicate significant decreases in licking to water for buspirone compared to saline (Bonferroni post-hoc, $p<0.05$).



Benzodiazepines show selectivity in affecting taste stimuli but not affecting licking to capsaicin, an aversive, non-gustatory, orosensory stimulus.

Behavioral Evidence: Long-term Intake Tests

CDP increases consumption of accepted & aversive stimuli through licking patterns associated with taste-mediated cues



Microanalysis of licking patterns to three concentrations of sucrose (A; caloric sweet), saccharin (B; non-nutritive sweet), MSG (C; umami), citric acid (D; sour), NaCl (E; salt), and quinine-HCl (F; bitter) were measured during the first meal of a 60-min, single bottle test following CDP or saline IP injection.

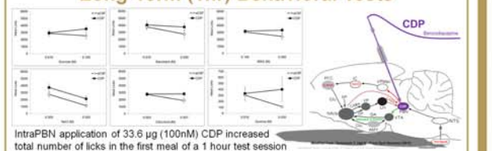
In general, there was no effect of CDP on variables associated with motivational state such as: meal duration and number of bursts per meal. In contrast, CDP tended to produce significant effects in variables associated with taste-mediated cues such as meal licks, licks per burst, burst duration, pause duration, lick rate, and licks during the first minute of testing.

Despite the general sedative nature of benzodiazepines, our results confirm previous research findings that CDP increases consumption of appetitive stimuli (sucrose) by showing similar effects on non-nutritive sweet and umami. Our results extend previous findings by showing that CDP also acts to increase the acceptance of normally aversive stimuli such as hypertonic salt and bitter. Microanalysis of licking patterns during the meals indicate that CDP is acting primarily through taste-mediated cues as opposed to variables associated with motivational-state cues such as thirst.

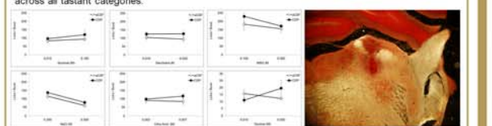
Repeated measures ANOVA revealed significant drug effects compared to saline, stars indicate significant drug effects at specific concentrations (Bonferroni post-hoc, $p<0.05$).

Behavioral Evidence: IntraPBN Application

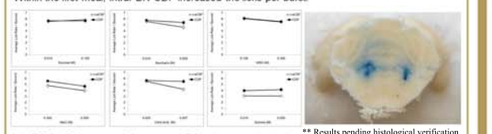
Long-Term (1hr) Behavioral Tests



IntraPBN application of 33.6 µg (100nM) CDP increased total number of licks in the first meal of a 1 hour test session across all tastant categories.



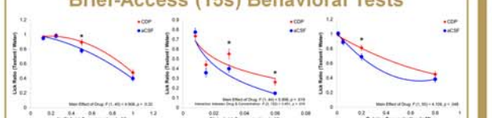
Within the first meal, intraPBN CDP increased the licks per burst.



IntraPBN CDP increased the average lick rate across tastants.

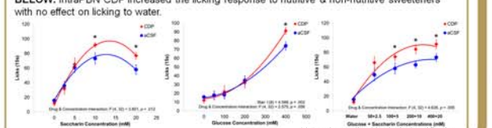
** Results pending histological verification

Brief-Access (15s) Behavioral Tests



ABOVE: IntraPBN CDP increased the licking response to NaCl, citric acid, & quinine in 15s trials.

BELOW: IntraPBN CDP increased the licking response to nutritive & non-nutritive sweeteners with no effect on licking to water.



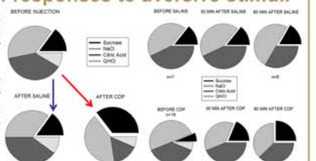
** Results pending histological verification

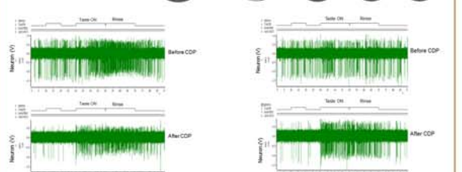
Electrophysiological Evidence: PBN Signaling

CDP increased the proportion of PBN cells best-responsive to sucrose & NaCl with suppression of responses to aversive stimuli

The proportion of cells responding best to sucrose more than doubled after **CDP** IP injection.

This increase was offset by a reduction in acid or bitter best cells ($X2(3)=10.25$, $p<0.05$). There were no shifts after **saline** injection in either group.





An individual PBN gustatory neuron before and after CDP injection. The top stimulus marker indicates taste application to the oral cavity (20s), followed by 20s water rinse. *Left:* The unit had a robust citric acid response that was reduced after CDP injection. *Right:* The neuron had a small response to 0.1M NaCl that increased after CDP injection.

Significance & Importance

Our research indicates that benzodiazepines selectively enhance the hedonic acceptance of gustatory orosensory stimuli, independent of motivational states such as thirst, appetite, or general anxiolytic effects. Furthermore, this effect on palatability appears to be localized to circuits in the PBN and likely involves GABA modulation of afferent taste signals.

Development of pharmacological treatments to control obesity is dependent on understanding the neural systems that regulate the palatability of foods we consume. Collectively, our research is beginning to define a role of GABA in the PBN as influencing taste signals in a manner that makes food more appetitive and thus encourages over-consumption.

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Our latest research on the effect of benzodiazepines on taste can be found at:
http://BenzoTaste.com