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Multiple Processes Underlie Benzodiazepine-Mediated Increases in the Consumption of Accepted and Avoided Stimuli

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Abstract

Hyperphagia is a reported side effect of anxiolytic benzodiazepines such as chlordiazepoxide (CDP). Prior research has focused primarily on the ingestive responses to sweet or solid foods. We examined CDP effects on licking for normally accepted and avoided taste solutions across a range of concentrations. The effect of CDP (10 mg/kg) versus saline on the licking patterns of water-restricted rats for water and 3 concentrations of sucrose, saccharin, NaCl, monosodium glutamate (MSG), citric acid, and quinine (Q-HCl) solutions was evaluated during 1 h tests. CDP increased meal size for all tastants except citric acid. Analysis of licking microstructure revealed 3 dissociable effects of CDP. CDP affected oromotor coordination as indicated by a uniform increase in the modal interlick interval for all stimuli. CDP increased meal size as indicated by shorter pauses during consumption of water, MSG, and weaker saccharin concentrations, and by fewer long interlick intervals (250–2000 ms) for normally avoided tastants. CDP also increased meal size by increasing burst size, burst duration, and the initial rate of licking for most solutions, suggesting increased hedonic taste evaluation. CDP did not affect variables associated with postingestive feedback such as meal duration or number of bursts, and the results also suggest that CDP did not enhance the perceived taste intensity. We hypothesize that the reduction of pause duration is consistent with an increased motivation to sample the stimulus that synergizes with changes in taste-mediated responsiveness to some but not all stimuli to yield increases in the consumption of both normally accepted and avoided taste stimuli.

Key words: CDP, chlordiazepoxide, ingestion, licking, microstructure

Introduction

One of the most studied neurochemical effects on consummatory behavior associated with changes in taste evaluation is the hyperphagic effect of benzodiazepines. Benzodiazepines enhance endogenous $GABA_A$ chloride current hyperpolarization in cells (Costa and Guidotti 1979a, 1979b; Guidotti et al. 1979) that express a subclass of $GABA_A$ receptors that have an α_1, α_2, α_3, or α_5 subunit apposed to a γ_2 subunit (Cooper 2005). Studies have confirmed that benzodiazepine receptor agonists increase behavioral responses to tastants across a variety of experimental paradigms including long-term (Roache and Zabik 1986; Flaherty et al. 1990; Parker 1991; Cooper and Greenwood 1992; Cooper and Barber 1993), brief-access (Higgs and Cooper 1997, 1998; Cooper and Higgs 2005; Cooper and Ridley 2005), operant conditioning (Petry and Heyman 1997; O'Hare et al. 2006), and taste reactivity tests (Berridge and Treit 1986; Treit et al. 1987; Berridge 1988; Treit and Berridge 1990; Gray and Cooper 1995; Soderpalm and Berridge 2000b). A benzodiazepine effect specific for food consumption was confirmed using benzodiazepine antagonists to block (Treit et al. 1987; Higgs and Cooper 1996a, 1996b, 1997; O'Hare et al. 2006) and inverse agonists to reverse these hyperphagic effects (Higgs and Cooper 1996a, 1998; Petry and Heyman 1997). Initially, it was believed that benzodiazepine-induced hyperphagia resulted from effects on perceived hunger or satiety (Margules and Stein 1967; Wise and Dawson 1974). Since these early reports, studies indicate that benzodiazepines modulate affective taste responses to food stimuli rather than perceived physiological states of repletion or depletion. This conclusion is supported by several studies reporting that benzodiazepine receptor agonists enhance ingestive orofacial (taste reactivity) responses or lick rates for primarily sweet ligands in brief-access paradigms, which minimize gastrointestinal stimulation by the taste stimuli

(Grill and Norgren 1978; Berridge and Treit 1986; Treit et al. 1987; Berridge 1988; Treit and Berridge 1990; Smith et al. 1992; Gray and Cooper 1995; Higgs and Cooper 1996a, 1997, 1998; Soderpalm and Berridge 2000b; Cooper and Higgs 2005; Cooper and Ridley 2005).

To date, however, there has been no systematic examination of the effects of benzodiazepines on the ingestive behavior of rats across all primary taste qualities; sweet, sour, salty, bitter, and umami. The majority of studies have focused on sweet ligands, with a few evaluating increases in salt ingestion (Cooper and Greenwood 1992; Cooper and Barber 1993; Cooper and Higgs 2005), and these studies support the interpretation that benzodiazepines increase positive hedonic responses to normally accepted taste stimuli. However, studies investigating benzodiazepine effects on responses to normally avoided stimuli have produced conflicting results. Several studies have reported that benzodiazepines increase the consumption of bitter quinine stimuli (Margules and Stein 1967; Cooper and Green 1993; Gray and Cooper 1995), however, taste reactivity studies have reported little or no benzodiazepine effect on aversive oromotor rejection responses to oral infusions of sour or bitter stimuli (Berridge and Treit 1986; Treit et al. 1987; Berridge 1988; Treit and Berridge 1990). The failure of benzodiazepines to reduce oromotor rejection responses to normally avoided taste stimuli suggests that ingestive processes other than negative affective taste reactivity are influenced by benzodiazepines.

In the current study, we provide the first systematic analysis of the influence of the benzodiazepine chlordiazepoxide (CDP) on licking across 3 concentrations of solutions representing the 5 "basic" taste qualities: sweet (sucrose and saccharin), sour (citric acid), salty (sodium chloride), bitter (quinine hydrochloride), and umami (monosodium glutamate). CDP is the prototypical and most studied benzodiazepine agonist for which taste-mediated hyperphagia has been established, providing the largest database for comparisons, and we chose a dose of 10 mg/kg as it exceeds the threshold to induce hyperphagia, yet it does not sedate the animal (Hodges et al. 1981; Cooper and Webb 1984; Berridge and Treit 1986; Cooper 1987; Treit et al. 1987; Berridge 1988; Flaherty et al. 1990; Parker 1991; Petry and Heyman 1997). We evaluated behavioral responses during a 1-h test session using a licking microstructure analysis. The licking microstructure analysis paradigm has been developed to allow identification of behavioral processes associated with oromotor coordination, affective taste reactivity, sensitivity to postingestive signals, and taste aversion processes (Davis and Levine 1977; Davis and Perez 1993; Davis et al. 1997; Baird et al. 1999, 2005). Solutions that vary in taste quality, intensity, and caloric density produce distinct temporal patterns of licking behavior even though these differences can sometimes result in the same volume consumed (Davis and Levine 1977; Smith 1998; Spector et al. 1998). Since there is little postingestive accumulation of ingesta at the beginning of a session, the initial rate of licking is influenced more prominently by orosensory (gustatory) stimulus properties. For example, rats exhibit a high initial rate of licking and larger mean lick burst sizes (reflecting taste evaluation) for highly preferred solutions such as sucrose. However, weaker sucrose concentrations that elicit smaller gustatory afferent responses yield slower initial lick rates and smaller licking bursts (Davis and Levine 1977; Spector et al. 1998). Normally avoided tastants, such as sour and bitter stimuli, tend to further reduce the initial rate of licking and the size of bursts of licking. When rats lick normally accepted stimuli, 95% of interlick intervals (ILIs) are between 50 and 250 ms (Spector et al. 1998; Baird et al. 2005). Taste stimuli that are normally avoided or conditioned to be avoided introduce a higher proportion of ILIs with durations in the 250–2000 ms range (Baird et al. 2005) reflecting brief "hesitations" in licking at the spout. Microstructure analysis therefore provides an ideal tool to distinguish the behavioral processes underlying CDP hyperphagia. For example, if CDP also modifies the perceived intensity of taste stimuli, responses to normally avoided tastants such as reduced initial licking and reduced burst size would be further suppressed, while comparable responses to the normally accepted stimuli would be increased. Conversely, if CDP modifies the hedonic evaluation of taste stimuli such that they are regarded to be more acceptable, the initial rate of licking and size of licking bursts to both normally accepted and avoided oral stimuli may be expected to increase. Alternatively, CDP may selectively influence responses to normally accepted stimuli with little effect on normally avoided tastants.

Materials and methods

Subjects

Forty-eight adult male Sprague-Dawley rats (Charles River Laboratories) were individually housed in transparent plastic cages in a temperature-controlled colony room on a 12:12 h light:dark cycle with lights on at 0700 h. Animals had free access to Harlan Teklad 8604 rodent chow and were placed on a 23-h water restriction schedule 3 days prior to testing, in order to promote sampling of the taste stimuli. Supplemental water access (15 min) following each test session was sufficient to maintain normal body weight during maintenance of the water restriction during testing with a mean body weight of 401.9 ± 7.7 g for the first test day and 386.7 ± 9.2 g on the last test day. All procedures were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of Wofford College.

Chemical stimuli

CDP was administered via i.p. injection at a dosage of 10 mg/1 mL/kg body weight. Tastants were mixed daily from reagent grade chemicals (VWR) dissolved in deionized water

(dH_2O) and presented at room temperature (22 °C). Taste stimuli consisted of 3 concentrations across the range of prototypical taste categories: sucrose (0.01, 0.1, 1.0 M), saccharin (0.005, 0.01, 0.05 M), monosodium glutamate (MSG, 0.075, 0.1, 0.3 M), citric acid (0.007, 0.15, 0.03 M), sodium chloride (NaCl, 0.1, 0.3, 0.5 M), and quinine hydrochloride (Q-HCl, 0.003, 0.01, 0.05 mM). Deionized water was also included as a test stimulus for all subjects.

Behavioral tests

Twenty minutes prior to testing, rats were given i.p. injections of either CDP or isotonic saline in a counterbalanced order allowing each rat to access a specific tastant for 60 min over 2 consecutive days, alternating one day under each injection condition for each tastant. Single concentrations of 6 tastants and water were presented in random order of these 2-day blocks for a total of 14 consecutive test days. Six groups of rats (n = 8 per group) were cycled through the 1-h test sessions in the AC-108 contact lickometer (DiLog Instruments) from 0900 to 1600 each day. The order of daily testing for each group was rotated across days such that each group was tested 1 h later on the subsequent test day with the group in the last daily test session being first on the following day, thus counterbalancing the time of day that each rat received their 1 h test during the 7 h period. Additionally, groups 1 and 2 received all low concentrations of the tastants, groups 3 and 4 received all middle concentrations, and groups 5 and 6 received the high concentrations of all tastants with water included as a stimulus for all groups. Consumption during test sessions was measured by the change in bottle weight recorded before and after each session. The contact lickometer recorded the time and duration of each lick during the 1-h test session at a resolution of 1 ms. After the test session, rats were returned to their home cages and given 15 min access to deionized water.

Data analysis

Licks for each rat during the 1-h test session were grouped into meals initiated by 5 licks within 1 s and terminated by a pause of 600 s (Spector et al. 1998). The licks within the first meal of the test session were subjected to a microstructure analysis in order to examine whole-meal measures (meal lick count and meal duration), intrameal licking patterns (number of bursts, size of bursts, mean burst duration, mean pause duration, and average lick rate [licks/s]) to provide analysis of taste and postingestive feedback sensitive measures of licking. Oromotor coordination was assessed by analysis of ILIs and duration of tongue contact with the fluid spout. Licking bursts were defined by a 1 s pause in licking. Mean burst size is the total number of licks in the meal divided by the number of bursts in the meal. Mean burst duration is the average length of time for each licking burst within the meal. Mean pause duration is the average length of time from the termination of a burst to the initiation of the next licking burst. Average lick rate was calculated by dividing the meal licks by the meal duration to determine the average number of licks per second. Contact duration was the duration in which the tongue made contact with the spout sufficient to provide electrical bridging. ILIs were analyzed first by calculating the average duration of ILIs in the range of 50–250 ms for each meal, and by calculating the number of ILIs in the meal ranging from 50 to 250 ms and from 250 to 2000 ms. The number of ILIs in the 250–2000 ms range was then divided by the total number of ILIs from 50 to 2000 ms, to yield an ILI ratio (%) reflecting the number of ILIs in the 250–2000 ms range relative to the majority of ILIs in the meal. A mixed factorial analysis of variance was conducted, in order to examine the main effects of drug and concentration as well as any interaction between the two variables. Post hoc Bonferroni t-tests were used to determine significant effects of CDP at each concentration. Significance was defined at $P < 0.05$.

Results

As shown in Figure 1, the total number of licks in the first meal increased under the influence of CDP, demonstrating a hyperphagic effect on all of the taste stimuli except citric acid (Figure 1F). There was a main effect of CDP to increase meal licks for sucrose ($F_{1,45} = 75.056$, $P < 0.01$) and saccharin ($F_{1,45} = 18.858$, $P < 0.01$). Post hoc t-tests show that CDP increased the meal size across all 3 concentrations of sucrose (Figure 1A) but only for the normally accepted weak (0.005 M) and moderate (0.010 M) concentrations of saccharin (Figure 1B). CDP increased meal licks for all concentrations of the umami stimulus MSG ($F_{1,45} = 40.919$, $P < 0.01$; Figure 1C) and all concentrations of the bitter stimulus quinine ($F_{1,45} = 25.434$, $P < 0.01$; Figure 1E). As shown in Figure 1D, CDP increased meal licks for NaCl ($F_{1,45} = 11.434$, $P < 0.01$), but the increase was only significant at the moderate (0.3 M) and strong (0.5 M) concentrations. The lack of a significant increase for the weakest concentration of NaCl may reflect a ceiling effect as rats in both conditions licked the 0.1 M NaCl to a near-maximal level. Meal licks for also water increased ($t_{47} = 7.504$, $P < 0.01$) from 2401 ± 111 under the saline condition to 3281 ± 130 under the influence of CDP. There was no significant increase in meal duration for any tastant or water indicating that the increased meal licks were the result of changes in the patterns or rate of licking within meals rather than prolonged meal length. Analysis of patterns of licking within the meals revealed 3 major behavioral effects of CDP: slowing of the "primary" lick rate through longer ILIs, increased motivation to initiate licking, and increased affective responses to certain tastants/concentrations.

Measures of oromotor coordination

When drinking, rats produce bursts of licks with stereotypical ILIs of consistent duration (Baird et al. 2005). Figure 2

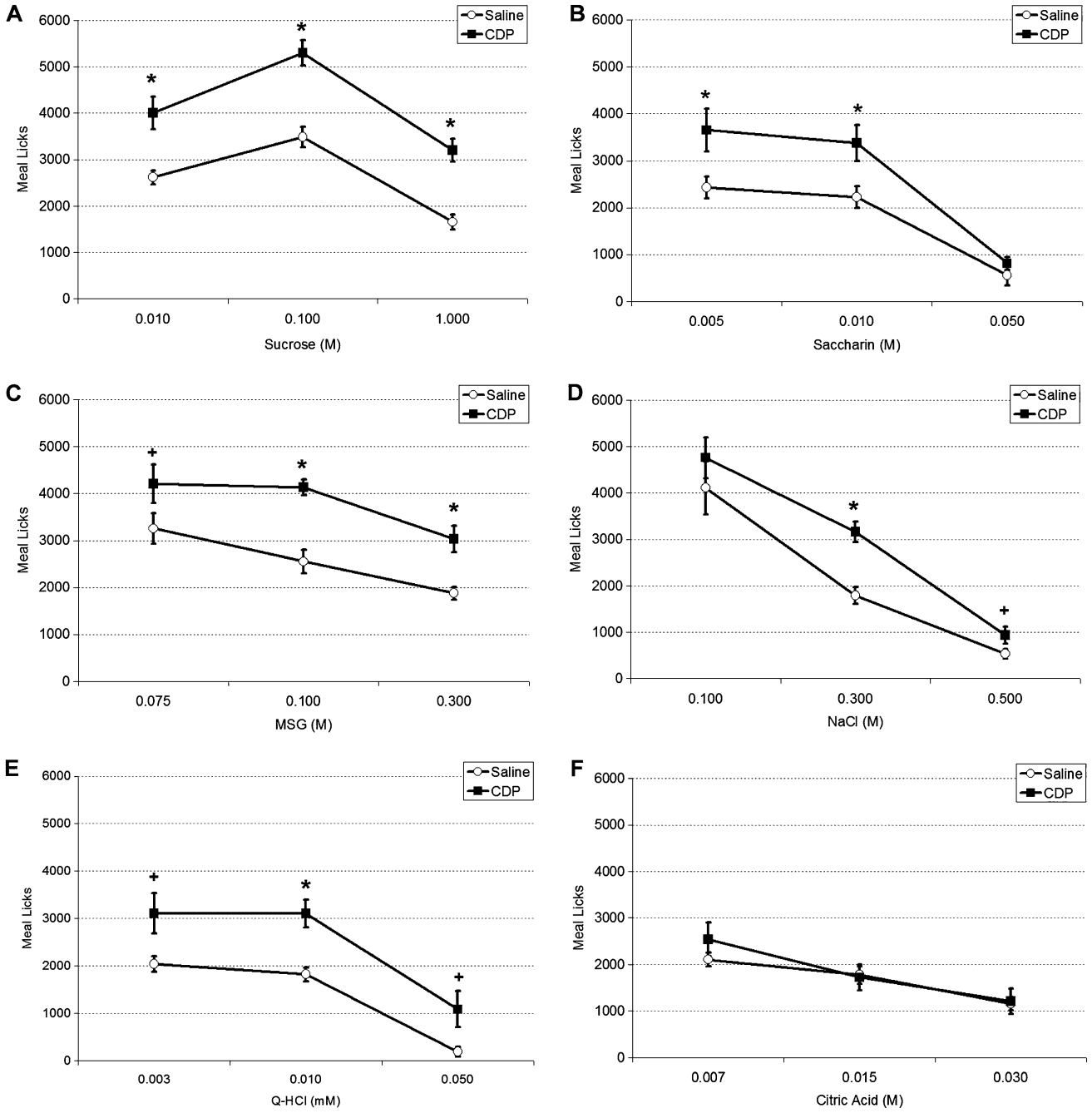

Figure 1 The number of meal licks under CDP and control conditions for (**A**) sucrose, (**B**) saccharin, (**C**) MSG, (**D**) NaCl, (**E**) Q-HCl, and (**F**) citric acid. CDP showed a general increase in meal licks across all tastants. Significant drug effects for specific concentrations are indicated by $^{*}P < 0.01$ or $^{+}P < 0.05$.

shows that CDP increased the average duration of ILIs in the 50–250 ms range, which characterizes the majority of ILIs expressed during normal consummatory behavior. The effect was nearly uniform across all tastants at all concentrations tested, such that the average ILI for CDP was 173.0 ± 0.6 ms compared with 158.8 ± 0.6 ms for saline as supported by a significant main effect of drug for all taste stimuli (sucrose [$F_{1,45} = 110.450$, $P < 0.01$]; saccharin [$F_{1,45} = 51.122$, $P < 0.01$]; MSG [$F_{1,45} = 157.082$, $P < 0.01$]; NaCl [$F_{1,45} = 30.491$, $P < 0.01$]; Q-HCl [$F_{1,45} = 61.662$, $P < 0.01$]; citric acid [$F_{1,45} = 73.788$, $P < 0.01$]). There were no significant drug by tastant interactions except for Q-HCl ($F_{2,45} = 5.055$, $P < 0.01$) indicating that CDP did not increase the mean ILIs for the strongest bitter concentration, for which very few licks were expressed under the saline condition. Significantly longer ILIs for water consumption ($F_{1,45} = 100.951$, $P < 0.01$) after CDP (176.2 ± 1.6 ms) compared with saline conditions (161.6 ± 1.7 ms) indicate that the decrease in the primary lick rate produced by CDP was a generalized oromotor effect not specifically related to taste stimuli.

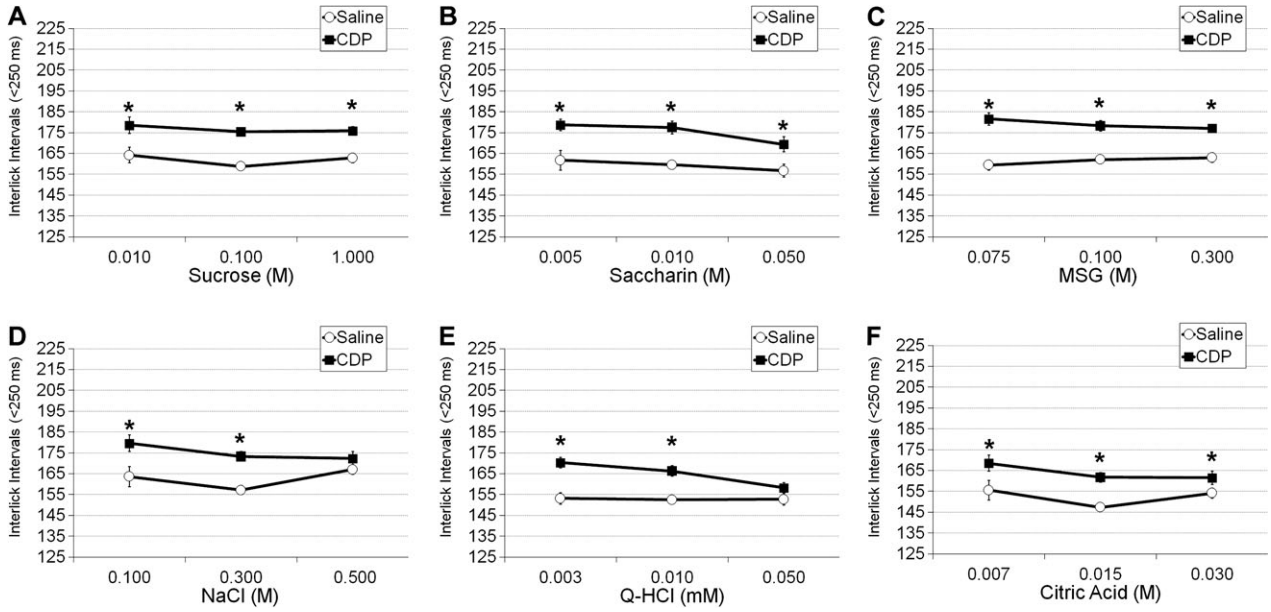

Figure 2 The mean duration (ms) of interlick intervals less than 250 ms during licking under CDP and control conditions for (**A**) sucrose, (**B**) saccharin, (**C**) MSG, (**D**) NaCl, (**E**) Q-HCl, and (**F**) citric acid under CDP and saline. Significant drug effects are indicated by *$P < 0.01$.

As shown in Figure 3H, there was also a general effect of CDP to significantly increase the tongue contact duration for all stimuli (sucrose [$F_{1,45} = 47.555$, $P < 0.01$]; saccharin [$F_{1,45} = 44.282$, $P < 0.01$]; MSG [$F_{1,45} = 44.115$, $P < 0.01$]; citric acid [$F_{1,45} = 47.555$, $P < 0.01$]; NaCl [$F_{1,45} = 47.473$, $P < 0.01$]; Q-HCl [$F_{1,45} = 59.024$, $P < 0.01$]; water [$F_{1,47} = 91.945$, $P < 0.01$]). The overall mean increase in contact duration under CDP was 7.8 ms, which accounted for approximately half of the 14.2-ms CDP-induced increase in the mean duration of ILIs between 50 and 250 ms. Even though the tongue contact duration was prolonged under the influence of CDP, there was no change in the mean volume per lick for any of the tastants or for water (Figure 3I).

As shown in Figure 3, under saline-injected control conditions, the ratio of ILIs in the 250–2000 ms range was increased for concentrations of taste stimuli that were most avoided (open circles). CDP significantly reduced the proportion of ILIs with durations between 250 and 2000 ms for the strongest concentrations of sucrose (interaction: $F_{2,45} = 15.584$, $P < 0.01$; Figure 3A) and saccharin (interaction: $F_{2,45} = 9.665$, $P < 0.01$; Figure 3D). For NaCl (Figure 3E), CDP reduced the proportion of these longer ILIs for the middle and strongest salt concentrations (interaction: $F_{2,45} = 9.966$, $P < 0.01$). For the bitter stimulus, Q-HCl, there was a main effect of CDP ($F_{1,45} = 20.875$, $P < 0.01$) indicating a reduction across all concentrations as shown in Figure 3F. Interestingly, CDP produced no significant reduction in proportion of these ILIs for any concentration of citric acid (Figure 3C), which was the only solution for which CDP did not increase intake. There was generally no effect of CDP where the percent of ILIs in the 250–2000 ms range was already low in the saline condition for the normally accepted stimuli (water, weaker sucrose, saccharin, and NaCl concentrations, and all concentrations of MSG).

Motivation to sample solutions

As shown in Figure 4, CDP significantly reduced the time between bursts of licking within a meal. Since meal duration was similar for CDP and saline conditions, this meant that the average lick rate (licks/s) within meals increased across the tastants as shown in Figure 5. There was a main effect of CDP to reduce mean pause duration for sucrose ($F_{1,45} = 17.630$, $P < 0.01$; Figure 4A), which resulted in a significantly increased average lick rate within the meal ($F_{1,45} = 50.111$, $P < 0.01$; Figure 5A) particularly for the strongest (1.0 M) sucrose concentration (interaction: $F_{2,45} = 13.011$, $P < 0.01$). CDP produced a dramatic decrease in pause duration for the 2 weaker concentrations of saccharin ($F_{1,45} = 11.558$, $P < 0.01$; Figure 4B) producing higher average rates of licking (Figure 5B) for both of the normally accepted saccharin concentrations of 0.005 M ($t_{15} = 3.515$, $P < 0.01$) and 0.010 M ($t_{15} = 2.916$, $P < 0.01$). CDP also significantly reduced the mean pause duration ($F_{1,45} = 25.7615$, $P < 0.01$; Figure 4C) for MSG, thus increasing the average rate of licking ($F_{1,45} = 9.826$, $P < 0.01$; Figure 5C). For NaCl (Figure 4D), Q-HCl (Figure 4E), and citric acid (Figure 4F), there were main effects of CDP (NaCl: $F_{1,45} = 9.116$, $P < 0.01$; Q-HCl: $F_{1,45} = 4.250$, $P < 0.05$; citric acid: $F_{1,45} = 36.323$, $P < 0.01$) to reduce the mean pause duration for the low and middle concentrations with no effect on the more aversive high concentrations, resulting in a significantly faster average lick rate for the 2 weakest concentrations of each tastant (NaCl: $F_{1,45} = 29.544$, $P < 0.01$, Figure 5D; Q-HCl: $F_{1,45} = 17.623$, $P < 0.01$, Figure 5E; citric acid: $F_{1,45} = 8.648$, $P < 0.01$, Figure 5F). The reduced latency to return to the spout after a break was also significant for water ($t_{47} = 4.915$, $P < 0.01$) with the

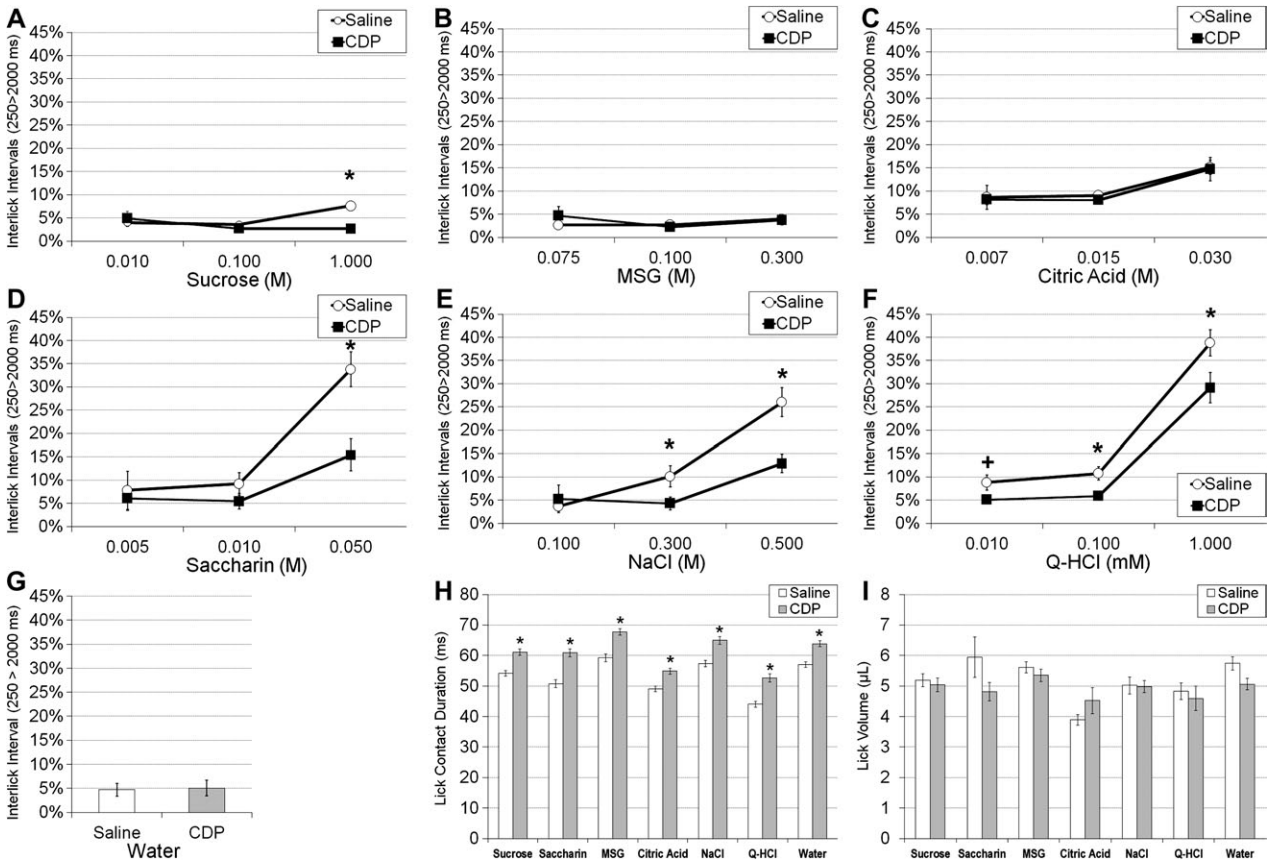

Figure 3 The percent of interlick intervals between 250 and 2000 ms under CDP and saline conditions for (**A**) sucrose, (**B**) MSG, (**C**) citric acid, (**D**) saccharin, (**E**) NaCl, (**F**) Q-HCl, and (**G**) water, the mean duration (ms) of tongue contact per lick (**H**) and the mean volume of solution consumed per lick (**I**). Significant drug effects for specific concentrations are indicated by $^{*}P < 0.01$ or $^{+}P < 0.05$.

mean pause duration of 26.8 ± 3.3 s under the CDP condition compared with 45.4 ± 4.0 s for the saline condition and a subsequent increased average rate of licking within each meal (t_{47} = 4.971, $P < 0.01$; CDP 2.80 ± 0.15 licks/s; saline 1.94 ± 0.17 licks/s).

Affective responses to tastants

Within a meal, the pattern of licking is characterized by bursts separated by pauses defined as breaks in licking longer than 1s. The number of licks within a burst, the burst duration, and the number of licks within the first minute of a meal are influenced by orosensory cues such as taste; whereas the number of bursts and meal duration tend to be influenced by postingestive/postabsorptive feedback signals (Davis and Levine 1977; Smith 1998; Spector et al. 1998; Baird et al. 1999). For water consumption, there were no significant effects of CDP compared with saline for licks per burst (122.0 ± 11.4; 117.2 ± 13.8), burst duration (22.7 ± 1.9; 19.2 ± 2.2), number of bursts (31.6 ± 3.2; 28.6 ± 4.2), or licks in the first minute (306.0 ± 9.8; 295.7 ± 15.2). There was also no effect of CDP on the number of bursts within a meal for any of the tastants with the exception of a subtle increase in the number of bursts for the middle and high concentrations of MSG ($F_{1,45}$ = 12.997, $P < 0.01$) for CDP (31.5 ± 2.9 bursts) compared with saline (21.2 ± 1.6 bursts). Rather, CDP increased burst size, burst duration, and the initial lick rate for sucrose, saccharin, NaCl, Q-HCl, and citric acid solutions.

As shown in Figure 6, burst duration was lengthened under the influence of CDP compared with saline for the low and high concentrations of sucrose ($F_{1,45}$ = 12.939, $P < 0.01$), the highest concentration of saccharin (t_{47} = 2.709, $P < 0.05$), the middle and high concentrations of NaCl ($F_{1,45}$ = 5.188, $P < 0.05$), and all concentrations of Q-HCl ($F_{1,45}$ = 15.860, $P < 0.01$). The longer burst duration can be explained by a significantly increased number of licks within a burst for each of the above mentioned concentrations of tastants mimicking the same patterns shown for burst duration in Figure 6 (data not shown; sucrose: $F_{1,45}$ = 8.900, $P < 0.01$; high concentration of saccharin: t_{47} = 2.870, $P < 0.05$; NaCl: $F_{1,45}$ = 4.211, $P < 0.05$, and Q-HCl: $F_{1,45}$ = 13.766, $P < 0.01$).

In cases where licking during the first minute of a meal was not already maximal, CDP increased the number of licks in the first minute of meals for saccharin, NaCl, Q-HCl, and citric acid. As shown in Figure 7B, rats were maximally licking normally accepted saccharin solutions under both CDP and saline conditions, but CDP did significantly increase licking for the strongest (least preferred) concentration (0.05 M) of saccharin (interaction: $F_{1,45}$ = 12.136, $P < 0.01$). A main effect of CDP to

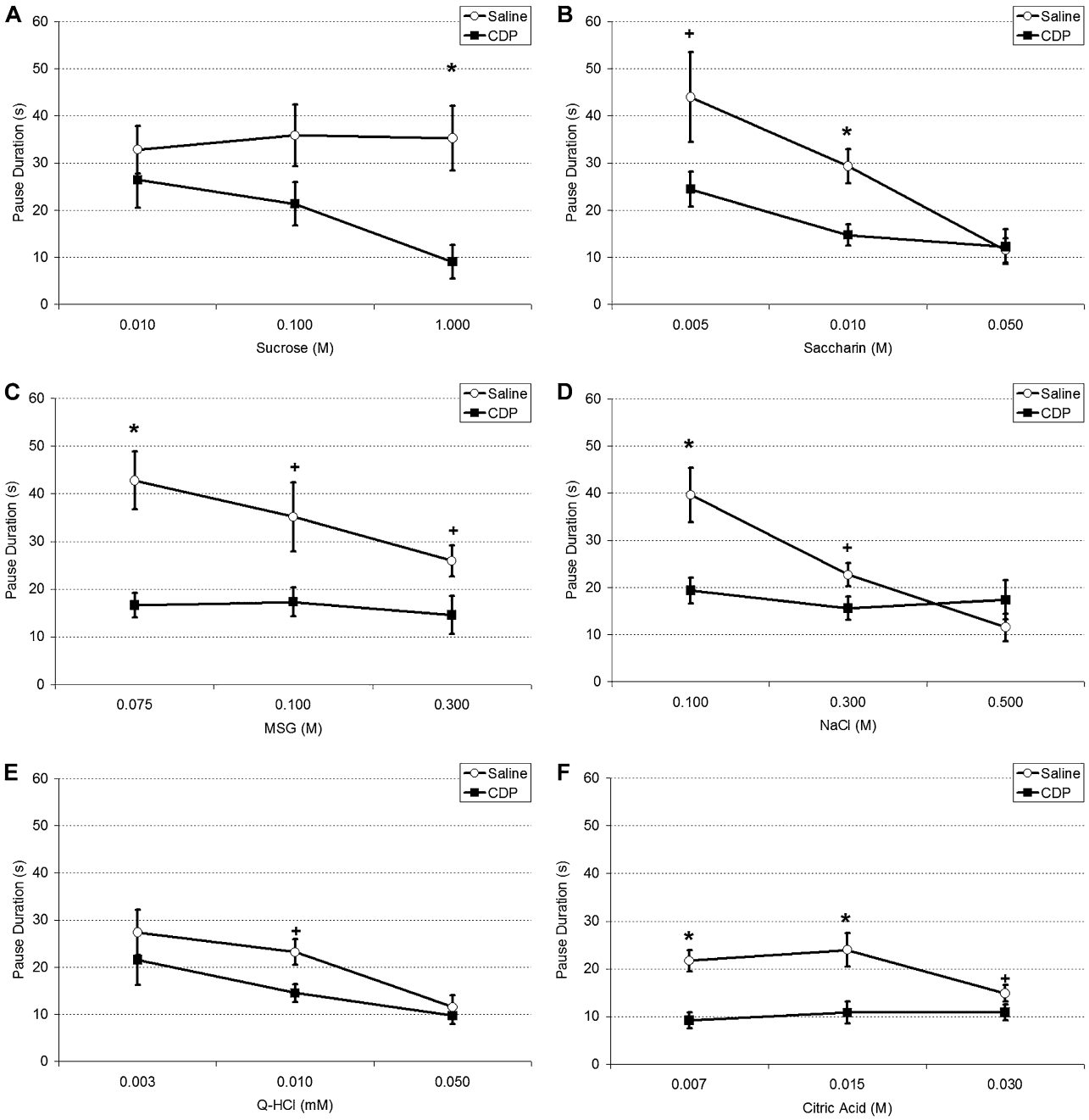

Figure 4 The mean pause duration under CDP and control conditions for (**A**) sucrose, (**B**) saccharin, (**C**) MSG, (**D**) NaCl, (**E**) Q-HCl, and (**F**) citric acid. Significant drug effects for specific concentrations are indicated by $^{*}P < 0.01$ or $^{+}P < 0.05$.

increase first minute licks for NaCl ($F_{1,45} = 13.469$, $P < 0.01$) is evident for both the middle and the strongest concentrations in Figure 7D. CDP also significantly increased first minute licks for Q-HCl ($F_{1,45} = 23.592$, $P < 0.01$; Figure 7E) and citric acid ($F_{1,45} = 7.497$, $P < 0.01$; Figure 7F).

Discussion

To clarify the behavioral processes contributing to benzodiazepine hyperphagia, we conducted a detailed analysis of CDP effects on the pattern of licking for a range of taste stimuli during longer-term consumption, including tastants that are normally avoided. This is the first study to systematically explore benzodiazepine effects on responses to umami and a range of normally avoided taste stimuli. Overall, several novel and corroborating findings in this study support the conclusion that systemic CDP influences brain systems controlling at least 3 distinct feeding processes. First, we determined that the previously reported effect of benzodiazepines to reduce the primary rate of licking within bursts

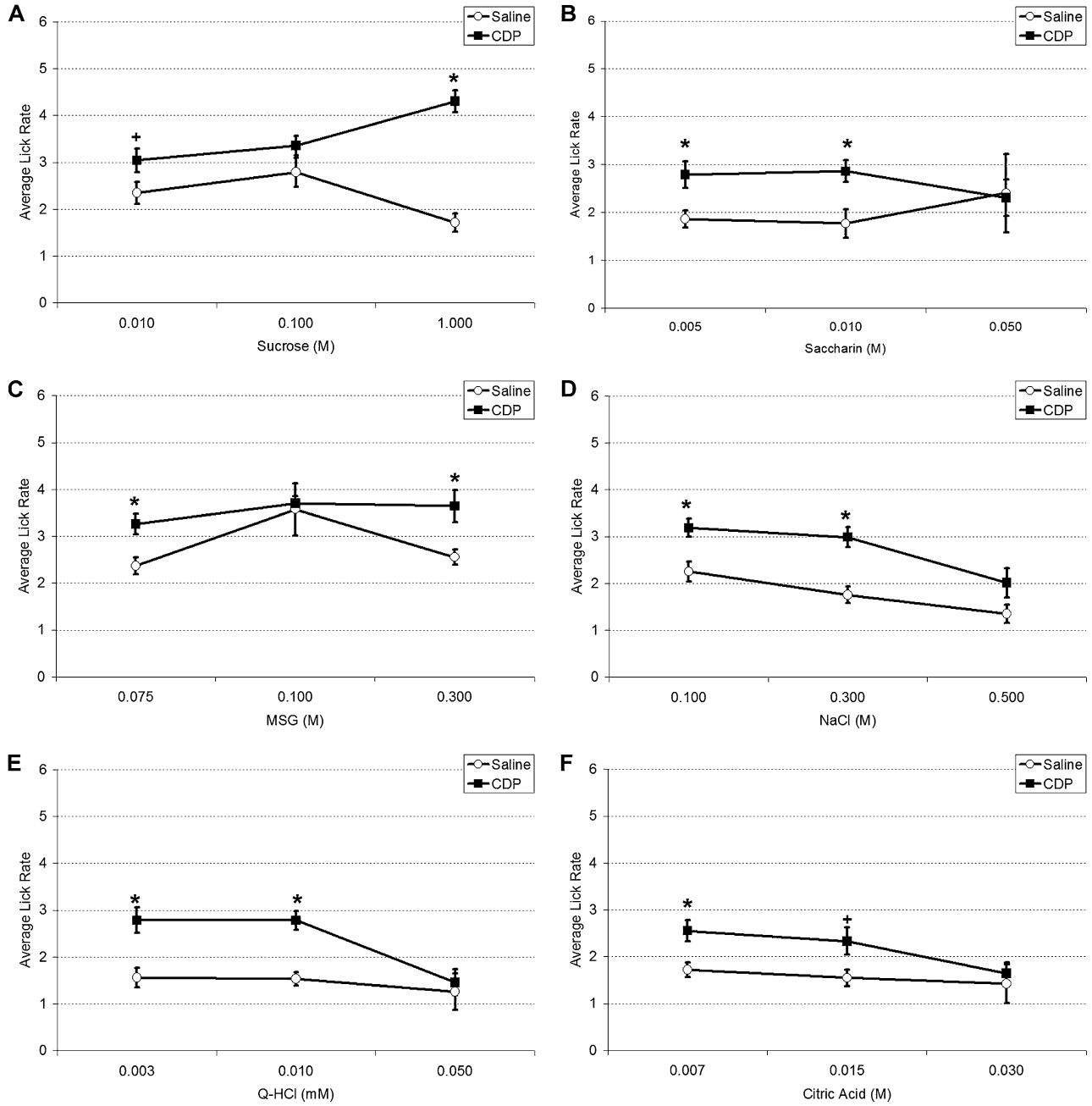

Figure 5 The average lick rate (licks/s) during a meal under CDP and control conditions for (**A**) sucrose, (**B**) saccharin, (**C**) MSG, (**D**) NaCl, (**E**) Q-HCl, and (**F**) citric acid. Significant drug effects for specific concentrations are indicated by $^{*}P < 0.01$ or $^{+}P < 0.05$.

(ILIs 50–250 ms) was dissociated from hyperphagic responses to CDP, and that this effect was in large part due to prolonged contact duration with the spout. Second, CDP-induced hyperphagia (where observed) varied depending on the quality of the taste stimulus; CDP failed to increase consumption of normally avoided concentrations of saccharin and citric acid. Third, the licking patterns underlying CDP hyperphagia also varied according to tastant quality: while CDP enhanced sampling behavior for most stimuli, behaviors associated with hedonic taste evaluation were increased for a subset of these tastants. With the exception of quinine, hedonic taste evaluation increased for tastants (sucrose, saccharin, and NaCl) that were all normally preferred to water. The dissociation of the behavioral effects of CDP across taste stimuli suggests that the brain structures controlling these processes are functionally independent or at least partially nonoverlapping with regard to benzodiazepine sensitivity.

The most common effect of CDP was a prolonging of lick cycles that increased the modal ILI for all taste stimuli tested, including water, and the most strongly avoided taste stimuli. This result was exhibited for stimuli for which intake was not

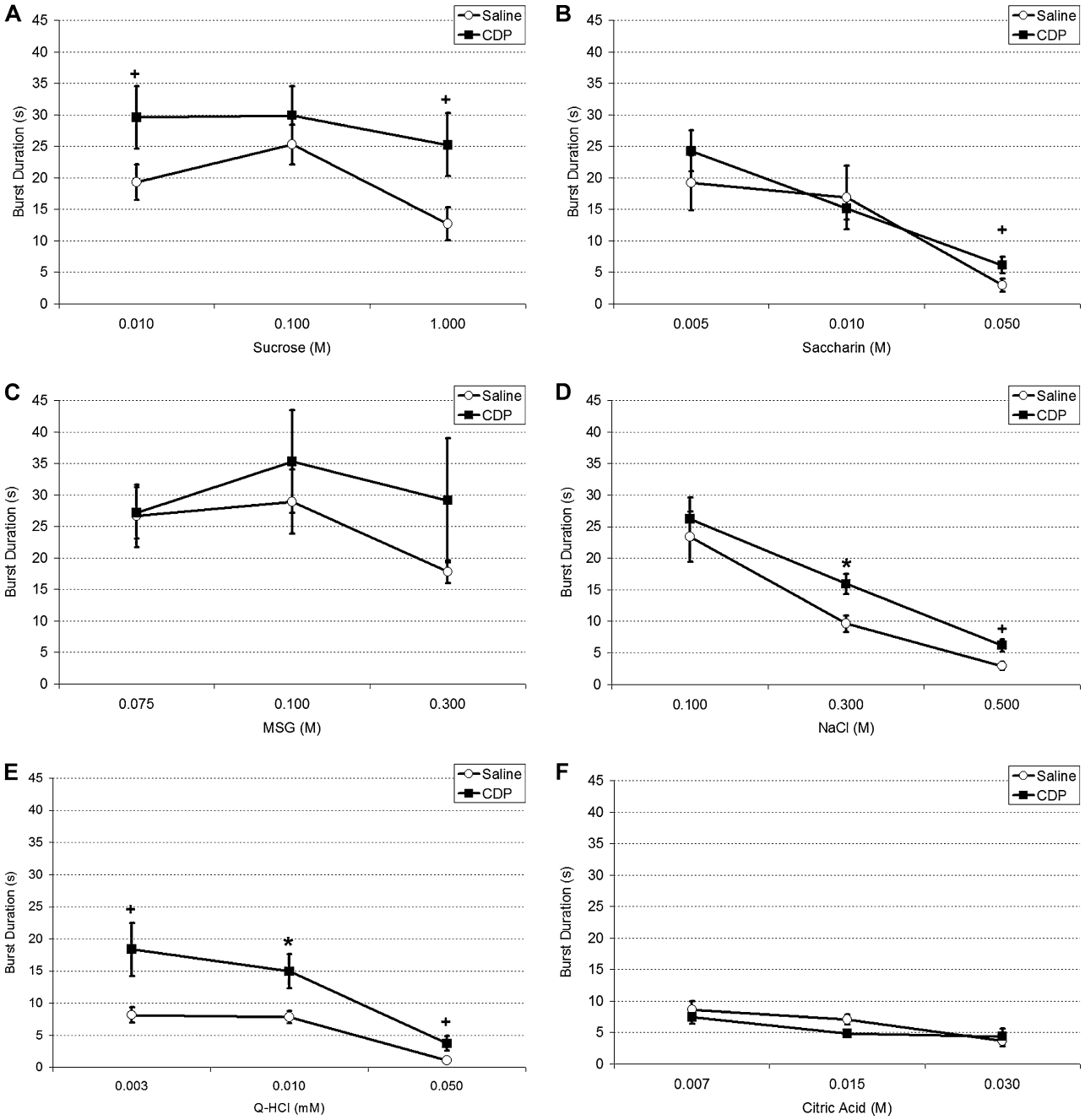

Figure 6 The mean burst duration under CDP and control conditions for (**A**) sucrose, (**B**) saccharin, (**C**) MSG, (**D**) citric acid, (**E**) NaCl, and (**F**) Q-HCl. Significant drug effects for specific concentrations are indicated by $^{*}P < 0.01$ or $^{+}P < 0.05$.

increased by CDP, and the dissociation from CDP hyperphagia suggests independent sites of CDP action in brain areas controlling hyperphagia and areas controlling oromotor coordination. Our finding confirms the prior report that the opioid antagonist naloxone blocked the effect of the benzodiazepine midazolam to increase burst duration, but it did not block midazolam's effect to slow the intraburst lick rate (Higgs and Cooper 1997). We discovered that half of the reduction of the primary lick rate was due to an increased duration of tongue contact with the spout, which suggests a slower transition from tongue protrusion to tongue retraction. Chen et al. (2001) reported that lateral reticular formation infusions of the $GABA_A$ agonist muscimol reduced the electromyographic amplitude of obligate licking muscles (anterior digastric and geniohyoid) and the rate of intraoral licking. We hypothesize that if CDP had reduced tongue-protruder muscle amplitude, it would have resulted in weaker licking, perhaps increasing licks that miss the spout, which would be expected to increase ILIs in the 250–2000 ms range. However, CDP did not increase the ratio of these ILIs;

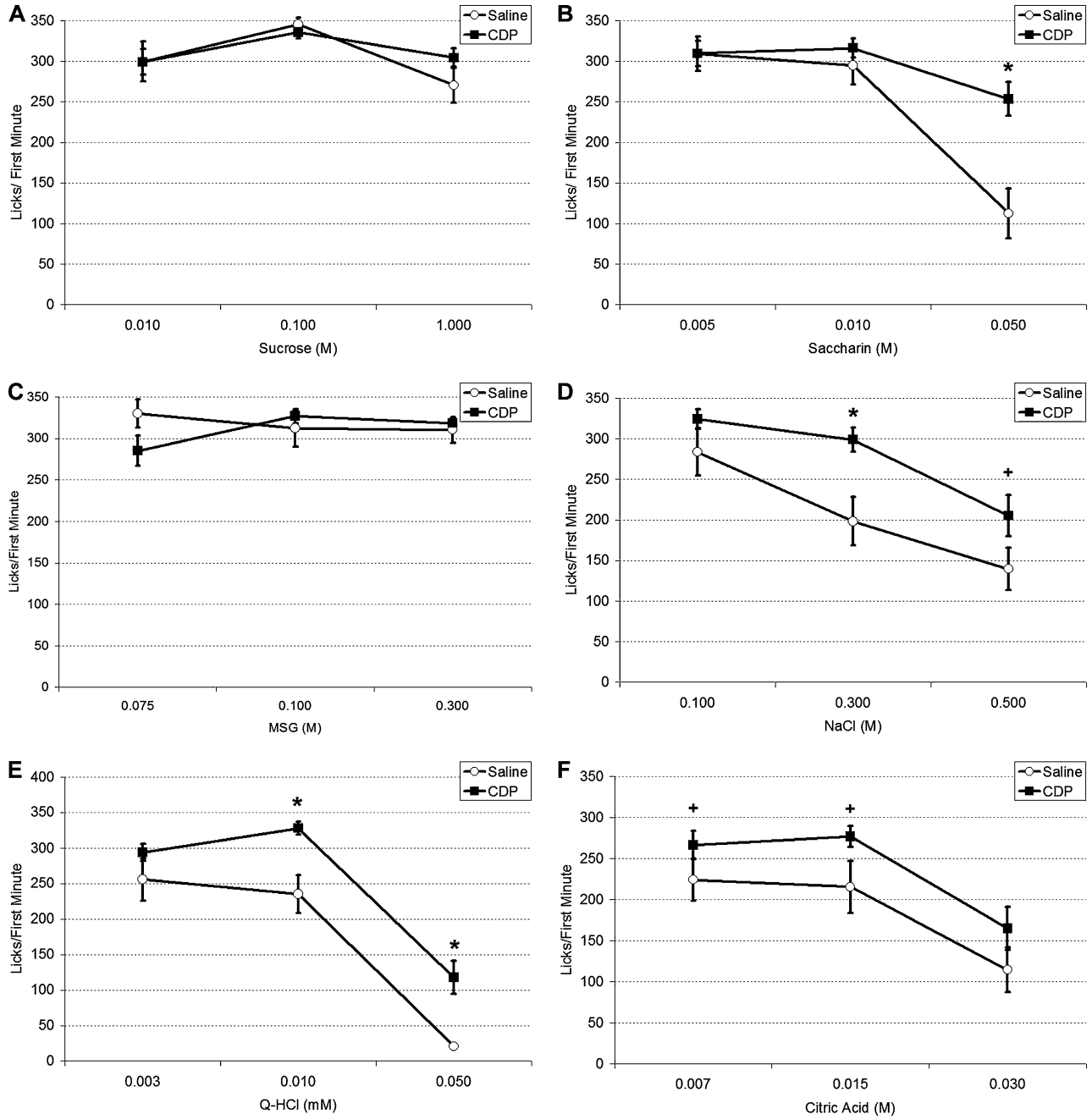

Figure 7 The number of licks in the first minute under CDP and control conditions for (**A**) sucrose, (**B**) saccharin, (**C**) MSG, (**D**) citric acid, (**E**) NaCl, and (**F**) Q-HCl. Significant drug effects for specific concentrations are indicated by $^{*}P < 0.01$ or $^{+}P < 0.05$.

rather, it was often reduced by CDP. Additionally, we would also expect reduced jaw-opener and tongue-protruder muscle amplitude to affect the efficiency of licking, but we were surprised to find that CDP had no effect on lick volume despite prolonged contact with the spout. It is possible that the rats increased the tongue contact duration in order to maximize or maintain the lick volume due to a deficit in fluid capture, possibly related to muscle amplitude reduction, but this is speculative. Alternatively, it is possible that CDP treatment delayed engagement of tongue retraction through enhanced GABAergic hyperpolarization of tongue retractor motor or premotor neurons in the reticular formation (Chen et al. 2001; Travers et al. 2005).

A second effect of CDP was a reduced latency to resample the tastant after termination of a burst of licking, even for solutions that were normally avoided. This was indicated by a reduction of the mean pause duration, which resulted in a faster average rate of licking since meal duration was not prolonged by CDP. In all but 2 cases (the strongest concentrations of saccharin and citric acid), CDP significantly

reduced the mean pause duration, and for both of these exceptions CDP failed to increase the meal size. Conversely, for all tastants where meal size was increased, there was a concomitant reduction of the mean pause duration, which suggests that enhanced sampling motivation was a common and possibly requisite condition for CDP hyperphagia. Consistent with this interpretation, we also observed that the proportion of longer duration ILIs 250–2000 ms in duration was reduced for normally avoided taste stimuli where CDP did increase meal size, but not so for citric acid where no meal size increases were observed. A reduction of these longer ILIs is consistent with a reduced latency to reengage the spout and a reduction in the avoidance of stimuli since conditioned or naturally avoided stimuli typically show increases in this measure (Baird et al. 2005). The results are also consistent with reports that benzodiazepines increase responding on progressive- and cyclic-ratio reinforcement schedules (Thompson 1972; O'Hare et al. 2006), suggesting an increase in motivation to sample or acquire a food stimulus. This appetent effect of CDP may have also contributed to CDP-induced increases in the initial rate of licking during the first minute of a meal.

As established in many prior studies, a third effect of CDP was an increase of the affective response to several taste stimuli as indicated by increases in the initial rates of licking and mean burst size and duration for many of the tastants examined. It is worth noting, however, that CDP-induced increases in the initial lick rate did not translate to an increase in meal size for any citric acid solution or for the strongest saccharin concentration, all of which were normally avoided relative to water. In these cases, the initial lick rate was increased by CDP but the mean burst duration was not, suggesting that for the other taste stimuli an increased motivation to sample the stimulus synergized with changes in taste-mediated responsiveness in order to increase meal size. It is worth noting that the mean pause duration was also not reduced for the strongest concentrations of citric acid and saccharin. If we consider that changes in the burst size and duration are more reflective of changes in affective taste evaluation, we would conclude that CDP influenced the hedonic taste evaluation of NaCl, sucrose, and Q-HCl, with no effect on taste evaluation measures for water, saccharin, MSG, or citric acid. Indeed, in every case where the initial lick rate was less than behavioral maximum under the control condition, CDP increased the initial lick rate. This suggests that increases in initial lick rate reflected 2 independent effects of CDP. First, initial lick rate was increased due to an increase in affective responsiveness to the orosensory properties of several taste stimuli. Second, initial lick rate increases may have also reflected increased motivation to begin sampling the tastant, as also indicated by the reduction of pause duration for most stimuli tested, as discussed above. The latter hypothesis could be supported by observing a parallel reduction in meal-start latency; however, due to training, all rats exhibited minimal meal-start latencies under control conditions. Consistent with our interpretation, however, it was recently reported that a benzodiazepine inverse agonist produced a dissociated effect on licking burst size and the motivation to approach the spout (Martire et al. 2010).

Although CDP affected several behavioral processes, the results discount potential effects of CDP on 2 other factors that could contribute to hyperphagia. We confirmed that intake increases were not related to measures associated with hunger or satiety. Where CDP increased consumption, in most cases, it was not due to a prolonging of the meal duration or due to an increase in the number of licking bursts within the meal. These 2 measures are commonly affected by postingestive/postabsorptive stimuli such as gastric preloads or food deprivation (Davis and Levine 1977; Davis and Perez 1993; Davis et al. 1997; Baird et al. 1999). Although effects on the average rate of licking usually correspond with sensitivity to postingestive feedback, in such cases, the change in lick rate is a function of the treatment effects on the number of bursts and/or meal duration, which were not affected by CDP. Rather, here, the increases in average lick rate were due to rats returning to the spout more quickly after terminating a burst of licking. This CDP-reduced pause duration occurred for both caloric (sucrose) and noncaloric (water, MSG, saccharin, Q-HCl, and NaCl) taste stimuli, further suggesting a lack of CDP influence on postingestive cues. Although sham feeding for sucrose solutions was reported to be respectively increased or suppressed by a benzodiazepine agonist (Cooper et al. 1988) or inverse agonist (Kirkham and Cooper 1987), those results do not fully dismiss a potential benzodiazepine contribution to postingestive sensitivity. A more direct evaluation of benzodiazepine effects on sensitivity to postingestive stimuli (e.g., responses to gastric preloads) is warranted.

Second, the results confirm that CDP is unlikely to enhance the perceived intensity of taste stimuli. Prior studies reported that CDP increased consumption of quinine-adulterated solutions, although it is possible that CDP-induced increases of the intensity of the preferred solution in the mixture exceeded a possibly weaker increase in the perceived aversiveness of quinine (Margules and Stein 1967; Hunt et al. 1988; Petry and Heyman 1997). However, 2 studies noted that benzodiazepines increased intake of unadulterated Q-HCl solutions, in a brief 30-s taste trial and in 29-min 2-bottle preference tests (Cooper and Green 1993; Gray and Cooper 1995). In our study, if CDP enhanced the perceived intensity of normally avoided taste stimuli, then it should have suppressed rather than increased the consumption of 0.3 M NaCl, 0.01 M saccharin, and all concentrations of Q-HCl. Furthermore, CDP was never observed to reduce the initial rate of licking, burst size, or burst duration, nor increase the proportion of ILIs 250–2000 ms, for any of the normally avoided taste stimuli.

Previous studies have evaluated benzodiazepine effects on responses to normally avoided stimuli in order to identify the

motivational underpinnings of benzodiazepine hyperphagia. Margules and Stein (1967) first noted that oxazepam increased consumption of a quinine/milk solution, which led them to suggest that benzodiazepines disinhibited the suppression of punished responses. Cooper and Green (1993) then observed that the benzodiazepine receptor partial agonist bretazenil increased consumption of a Q-HCl solution, but not water, in a 29-min 2-bottle choice test, which led them to also conclude that the benzodiazepine treatment disinhibited the suppressant effect of the bitter tasting quinine on fluid intake. A majority of the studies of unconditioned orofacial taste reactivity to HCl or Q-HCl solutions or Q-HCl/sucrose mixtures, however, have reported little or no effect of benzodiazepine treatment on orofacial rejection responses (Berridge and Treit 1986; Treit et al. 1987; Berridge 1988; Treit and Berridge 1990; Soderpalm and Berridge 2000b, for an exception, note Richardson et al. 2005). Furthermore, CDP increased ingestive reactions, but it did not reduce aversive orofacial reactions to a saccharin solution conditioned to be avoided after prior pairing with a lithium chloride injection to induce gastric distress (Parker 1995). These findings suggest that benzodiazepines do not reduce the suppressant effects of bitter taste stimuli on hedonic taste evaluation. Consistent with this interpretation, Gray and Cooper (1995) observed in rats briefly sampling Q-HCl (30 s trials) that the benzodiazepine midazolam not only increased intake of Q-HCl due to more licks from the spout but that it also increased the number of aversive oromotor rejection responses. They concluded that systemic midazolam promoted sampling behavior but that it did not change the inherent aversive quality of the quinine. Our results are consistent with this interpretation, as we found that CDP broadly enhanced sampling behavior, increasing initial licking for all stimuli and reducing the return-to-spout latency for all but the most strongly avoided stimuli tested, but increases in burst duration and size, with the exception of Q-HCl, were not observed for the normally avoided stimuli, suggesting that their aversive taste properties were also not altered by CDP treatment. The differential results observed for Q-HCl highlight the need to assess a range of aversive taste stimuli in future work. We speculate that benzodiazepines may selectively influence neurons that encode for particular taste qualities, which could be confirmed through direct electrophysiological evidence.

It will be important to evaluate whether the 3 dissociable effects of CDP identified here can be isolated when benzodiazepines are targeted to specific structures of the central nervous system implicated in feeding behavior. We hypothesize that the effects of CDP on oromotor coordination result from benzodiazepine effects in the lateral reticular formation, as discussed above. The taste-affective and motivational effects of CDP are also likely to be centrally mediated, as recent identification of the subclass of $GABA_A$ receptors in peripheral taste receptor cells and taste buds indicated that they do not express benzodiazepine binding subunits (Dvoryanchikov et al. 2011). Within the central nervous system, $GABA_A$ receptors in the nucleus of the solitary tract, the first gustatory relay in the brain, also exhibit little responsiveness to benzodiazepines (Kasparov et al. 2001), whereas benzodiazepine-responsive $GABA_A$ receptors have been found throughout gustatory and viscerosensitive portions of the parabrachial nucleus, a second-order gustatory relay in the brainstem (Guthmann et al. 1998; Wu et al. 2009). Therefore, injections to the hindbrain parabrachial nucleus appear to be a propitious location to evaluate benzodiazepine effects on behavioral measures associated with appetitive and affective taste response behaviors. Consistent with this interpretation, prior studies have reported that midazolam injections to the parabrachial nucleus but not to the nucleus of the solitary tract or the peduncolopontine nucleus increased consumption of, and ingestive but not aversive orofacial taste reactivity responses to, normally accepted foodstuffs (Higgs and Cooper 1996b; Soderpalm and Berridge 2000b). Furthermore, chronically decerebrate rats, which do not express appetitive behaviors, exhibited increased ingestive orofacial responses to taste stimuli after CDP treatment. In contrast, benzodiazepine injections to forebrain nuclei or ventricles (Anderson-Baker et al. 1979; Kelly and Grossman 1979; Pecina and Berridge 1996) have been shown to increase food intake, but benzodiazepine injections into the nucleus accumbens did not affect taste reactivity responses (Soderpalm and Berridge 2000a). It will be important for future work to determine whether the appetitive and the affective taste responses to benzodiazepines are distinctly or conjointly mediated by forebrain and hindbrain structures implicated in feeding control.

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