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Spector, Alan C., and Steven J. St. John. Role of taste in the microstructure of quinine ingestion by rats. *Am. J. Physiol.* 274 (*Regulatory Integrative Comp. Physiol.* 43): R1687–R1703, 1998.—The microstructure of the licking behavior of water-deprived rats presented with either water or quinine during 45-min single-bottle tests was analyzed. The chorda tympani (CT) and glossopharyngeal (GL) nerves, which innervate the taste buds of the tongue, were transected in deeply anesthetized rats to discern their contribution to the behavioral pattern of quinine drinking. Rats were presurgically habituated to the testing protocol and postsurgically tested first with water and then novel 0.2 mM quinine-HCl in a subsequent session. The substantial decrease in intake observed in sham-operated controls (n = 16) when quinine was the stimulus was entirely a function of a decrease in lick volume and burst size (a run of licks with interlick intervals <1 s). Contrary to the intake-suppressing effects of quinine, pause duration decreased and burst number increased. Combined transection of the CT and GL (n = 6) strikingly opposed all of these quinine-induced behavioral changes, whereas CT transection (n = 7) was without effect and GL transection (n = 8) had an intermediate influence. These results suggest that taste acts more on neural circuits governing burst termination as opposed to burst initiation, which, in turn, appears to be more sensitive to signals related to physiological state. These findings are discussed in terms of other known nerve transection effects on quinine responsiveness, and the implications of the microstructural results are considered with respect to probabilistic as opposed to deterministic control of licking behavior.

chorda tympani nerve; glossopharyngeal nerve; gustatory; ingestive behavior; drinking; taste aversion; nerve transection

IT IS WELL ESTABLISHED that taste, postingestive feedback, physiological state, and prior experience interact to influence the amount consumed during a meal or a draft (see Refs. 8 and 45). The effect of these factors on the underlying behavior leading to total intake remains to be fully elucidated but has become an active area of research (e.g., 2, 6, 8, 9, 11, 12, 23). A common experimental model used in the study of energy and fluid regulation involves the measurement of intake in rats drinking liquid stimuli, varying in composition, from a drinking spout during short-term (e.g., 30–60 min) tests. Under these conditions, the rat ingests in bursts of licking behavior. The quantitative nature of these bursts, their number, size, duration, and temporal distribution, make up what is referred to as the microstructure of ingestion (see Ref. 6). Any treatment that affects total intake during a drinking episode must operate at the level of burst generation. In other words, total intake is entirely a function of the size and number of licking bursts. Accordingly, any comprehensive understanding of the neural basis of feeding and drinking under these test conditions will ultimately require students to link the influence of controlling variables to behavioral action, not just to outcome measures (e.g., amount ingested).

The present report focuses on the influence of oral input on the control of ingestive behavior within a temporally circumscribed drinking episode. More specifically, the contribution of the lingual gustatory nerves to the microstructure of quinine drinking is examined in water-deprived rats. The sensory receptors of the oral cavity, most notably the taste buds, provide the brain with an initial chemical analysis of the ingested substance. Clearly, such input is instrumental in guiding ingestive responses. Although this general notion remains uncontested, the influence of orosensory factors on the microstructure of drinking behavior has only begun to be explored. With few exceptions (23), relatively little work has focused on taste compounds that are avoided, such as quinine. Most studies on microstructure have involved taste solutions that are preferred by the rat. That is, stimuli that are hedonically positive or rewarding have been examined. One crucial adaptive function of the gustatory system is to prevent the animal from ingesting potentially toxic substances, which humans often report as "bitter" (see Ref. 15). Our experiment was designed to create a conflict between the rat's "thirst" and the motivation to avoid the ingestion of quinine. It is likely that rats and other animals are occasionally confronted with similar conflicts in their natural environments. In fact, it has been suggested that the study of such homeostatic conflicts provides a more relevant analysis of the regulation of drinking behavior from an evolutionary standpoint (35).

The generality of any functional principles derived from microstructural analyses of ingestive behavior will ultimately depend on the number of different experimental contexts in which the primary licking data were collected. For example, burst size (the number of consecutive licks before a criterion pause) has been suggested to reflect stimulus palatability (9, 11). In support, burst size monotonically increases as the concentration of sucrose is raised (9, 11, 23, 39), a finding that corresponds with the reinforcement efficacy of sucrose (21). This appears to be generally true, because aversive taste compounds such as quinine decrease burst size. Hsiao and Fan (23) found that water-deprived rats decreased burst size as a function of quinine concentration during a 15-min single-bottle test. In the latter study, however, the explicit contribution of the oral sensory receptor fields to the various microstructural components of ingestion, including burst size, was not examined. Moreover, differences in critical analytic parameters across studies, such as

the criterion chosen to define a pause, can potentially lead to different microstructural outcomes of treatments (39).

When a chemical stimulus is ingested, it contacts various receptor systems positioned at various oral, gastric, intestinal, and postabsorptive sites. Therefore, one cannot assume that any difference between the ingestion of the chemical stimulus and the vehicle in which it is dissolved (i.e., water) is based necessarily on input from one or another of these receptor systems without applying a specific experimental design that dissociates the possibilities (see Ref. 45). In any attempt to examine what role gustatory input plays in guiding ingestive behavior, there are several strategies that can be adopted. First, various concentrations of a taste stimulus (or stimuli) can be presented in brief-access tests, and the animal's licking response can be quantified (e.g., Refs. 5, 38, 40–42, 53). This strategy would include procedures in which the stimulus is delivered through a drinking spout as well as those that involve infusing the stimulus directly into the oral cavity through a chronically implanted cannula (see Ref. 19). In the latter procedure, referred to as taste reactivity, oromotor and somatic responses are quantified. The fact that small stimulus volumes are used and immediate responses to the chemical compound are measured increases the confidence that the behavior is under the control of orosensory events. Rats display avoidance and aversion responses to quinine in a concentration-dependent manner in these brief-access tests. Such tests, however, do not yield direct information on how taste input is influencing ongoing behavior during an ingestive episode such as a meal or a draft.

A second strategy that has been successfully used is the sham drinking paradigm, in which ingested solutions drain from an open gastric cannula (or esophageal fistula) that is surgically implanted (e.g., Refs. 11, 29, and 50). Accordingly, the stimulus is prevented from both accumulating in the stomach and reaching postgastric compartments [although see caveat raised by Sclafani and Nissenbaum (37)]. Thus this technique assesses the influence of orosensory factors in isolation of postingestive events.

A third strategy, complementing the second, is the study of postoral influences on ingestive behavior dissociated from oral events. This can be accomplished in two ways. Chemical solutions can be infused directly into the stomach as an animal drinks some "neutral" chemical stimulus (see Ref. 36). Alternatively, the lines of transmission linking oral receptors to the brain can be surgically interrupted. It is the latter strategy that was used in the present study. This, of course, is a derivative of the time-honored ablation behavior techniques that have made significant contributions toward our current concepts of brain function.

We chose to focus on the lingual gustatory nerves, which collectively innervate ~80% of the total taste buds in the rat oral cavity (28). Specifically, the chorda tympani (CT) branch of the facial nerve, which innervates taste buds on the front of the tongue, and the glossopharyngeal nerve (GL), which innervates taste buds in the back of the tongue, were transected alone or in combination. The lingual branch of the trigeminal nerve, which carries tactile, thermal, and pain input from the anterior tongue, was left intact. Both the CT and GL are responsive to quinine as assessed in electrophysiological experiments (4, 13, 14). In fact, the GL is the most responsive to quinine compared with the other gustatory nerves and contains fibers (Q units) that are rather narrowly tuned to this alkaloid (13). Combined transection of these two nerves raises the quinine detection threshold by ~1.5 log units (43), substantially attenuates unconditioned taste-guided lick avoidance to suprathreshold concentrations of quinine during brief-access trials in water-deprived rats (42, 51), and essentially eliminates aversive taste reactivity to intraorally infused quinine solutions (17). Accordingly, any feature of the ingestive microstructure that differed between animals with and without gustatory nerve transection could be considered directly or indirectly under the control of taste input, provided that general neurotomy-induced interruptions in licking behavior could be dismissed analytically.

METHOD

Subjects

Forty-two naive adult male Sprague-Dawley rats (Charles River Breeders, Wilmington, MA) served as subjects. They were housed individually in hanging stainless steel cages in a room in which temperature, humidity, and lighting were automatically controlled. A 12:12-h light-dark cycle was used. Rats had free access to laboratory chow (5001 Purina, St. Louis, MO) and water except where noted below.

Apparatus

The lickometer apparatus consisted of six stainless steel hanging cages, an electronic interface that signaled the occurrence of licks, and a microcomputer. The rats had access to a single drinking spout through a 25 × 7-mm slot in the stainless steel face of the holder. The slot was located 7.0 cm above the floor of the cage. The orifice of the drinking spout was retracted 8 mm away from the front of the cage to prevent incidental contact. The curved drinking spout was attached to a 100-ml graduated cylinder suspended above the drinking spout holding unit. Upon tongue contact, a circuit, passing no more than 50 nA through the animal, was completed. For each rat, the onset time of licks, within 1 ms accuracy, was saved for later analysis. All six cages were used simultaneously in a room separate from the animal housing area.

Procedure

The restricted fluid access schedule was designed as a series of 3-day cycles. On the first day of the cycle, water bottles were removed from the home cage. On the second day, 24 h later, the rats were placed in the test cages and given 45 min access to fluid. Food was removed from the home cage 2 h before this fluid presentation and was not replaced until after the test. This was done in an attempt to minimize the influence of recently ingested meals on fluid intake. Water was withheld from the animals until 24 h after the start of the intake test. This latter feature of the procedure was designed to prevent rats from learning that fluid would be forthcoming shortly after the test. The rats participated in squads that were staggered in time throughout the day. A given rat was always tested in the same cage at the same time of day. On the third day of the cycle, the animals had food and water

available ad libitum in the home cage. Thus animals had 1 day of ad libitum food and water before the start of each 3-day cycle. This was done to help circumvent any cumulative effects of water deprivation across test sessions.

The first three fluid test cycles involved the presentation of distilled water and served to acclimate the rats to the test schedule. The last of these cycles was used as a presurgical assessment of water-elicited ingestive behavior. The rats then received their prescribed surgical treatments (see *Surgery*). After the recovery period, the rats received a test cycle with distilled water as the stimulus. This served as a postsurgical assessment of water-elicited ingestive behavior. The next test cycle involved the presentation of 0.2 mM quinine hydrochloride (reagent grade; Fisher Scientific, Orlando, FL) as the test stimulus. This concentration of quinine was determined to be midrange on the basis of behaviorally derived concentration-response curves in intact rats tested in a brief-access taste procedure (42). There was a technical failure in one cage during the baseline water testing for one animal in the control group; this animal's data were discarded from the entire analysis, reducing the sample size.

Surgery

The rats were deeply anesthetized intraperitoneally with a combination of ketamine hydrochloride (86 mg/kg) and xylazine (13 mg/kg). The surgical procedures were based on those described by St. John et al. (42). Four rats died during or shortly after surgery presumably due to complications with the anesthesia; the group sizes listed in parentheses represent the animals used in the data analysis. The CT nerve was bilaterally transected (CTX, $n = 7$) in the middle ear, and the ossicles were removed. The GL nerve was bilaterally transected (GLX, $n = 8$) near the outside wall of the tympanic bulla underneath the posterior belly of the digastric muscle. An additional group had both nerves transected (DBLX, $n = 6$). The control group (Con, $n = 16$) received sham surgery in which the GL was exposed bilaterally but left intact and the tympanic membrane was bilaterally punctured. All rats were given 7–12 days before testing resumed.

Data Analysis

Interlick intervals (ILI; time interval between the onset of consecutive licks) that were >1 s were considered pauses that terminated bursts of licking. This pause criterion was based on prior observations in our laboratory suggesting that pauses less than this may actually represent a small series of consecutive missed spout contacts and that 1 s serves as a reasonable filter, a finding confirmed in the present work. Longer pause criteria decrease the number of bursts entering into the analysis but do not appear to represent distinct units of drinking until they reach values close to 30 s or more (see Ref. 39). Total intake (in ml), total licks, initial lick rate (1 min from second lick), burst and pause number, burst size (licks), drinking duration (of session from start to end of last burst), and ILI were all measured and averaged for the session. In the analysis of ILIs, only pauses <250 ms were included, because these generally represent >95% of the total ILI durations and we did not wish to contaminate the analysis with 5% of extreme values falling well outside of the primary distribution of scores. The behavior during the postsurgical water session was compared with that during the postsurgical quinine session for each surgical group separately with the use of *t*-tests. Consequently, effects within the groups could not be attributed to a surgically induced nonspecific disturbance of licking.

A percentage change across the water and quinine session was also calculated for each rat and each measure. The change score was computed to determine if the various surgical manipulations differentially affected the dependent variables. These values were compared in a one-way ANOVA, and differences between each nerve-transected group and the control group were tested for statistical significance with Dunnett's procedure. In some cases, there was a discrepancy between the percentage change calculated on the basis of the mean water and quinine values and the mean of the percentage change values calculated for each rat. The latter provides a measure of variability.

In addition to the above analyses, bursts in the water and quinine sessions were serially ordered for each rat. These were then broken into one-third portions. In cases in which the total number of bursts was not divisible by three, the first and last one-third always had the same number of bursts in the distribution. For example, if an animal had 31 bursts in a session, the first 10 bursts would contribute to the mean for the first one-third, the last 10 bursts would contribute to the mean for the last one-third, and the middle 11 bursts would contribute to the second one-third. Consequently, the mean burst size of the first one-third of the bursts produced in a given session could be compared with those appearing in later one-third portions. We refer to this arranged sequence of bursts as the serially ordered distribution. A similar analysis was conducted for pauses. The objective of these latter analyses was to examine how the experimental manipulations affected the burst size and pause duration as the behavior progressed toward satiation. For example, consider an animal that initiates 30 bursts during one fixed time period (e.g., water session) and 90 during another (e.g., quinine session). Is the mean size of the first third of the serially ordered distribution of bursts for both sessions similar or not, and do the respective means change in the same fashion as the session progresses? In addition, we calculated the drinking rate (licks/min) associated with the time period for each one-third segment of bursts and pauses. Consequently, it was possible to examine how changes in burst size and pause duration affected drinking rate.

Survival functions were determined for both burst size and pause duration for each control rat for both the postsurgical water and quinine tests. These were expressed as the probability that a given burst size or pause duration is greater than a given value (see Ref. 7). As proposed by Davis (7), a Weibull equation

$$y = e^{-(x/\alpha)^{\beta}} \quad (1)$$

was used to fit (least squares) the burst size survival function to the data, where y is the proportion of total bursts with sizes greater than x licks and α is the rate parameter, which estimates the arithmetic average of the distribution provided that a simple exponential fit accounts for a high percentage of the variance (i.e., $\beta = 1$). When β, a shape parameter, is equal to 1, the Weibull function reduces to a simple exponential. When $\beta > 1$, the initial portion of the function has a shoulder. When $\beta < 1$, the function consists of a longer tail and the initial decay is steeper. In cases in which β significantly departs from 1.0, the mean can be estimated by scaling α accordingly (32).

The double exponential function

$$y = \rho e^{(-\gamma t)} + (1 - \rho)e^{(-\delta t)} \quad (2)$$

was used to fit a survival function to the pause duration data, where y represents the proportion of total pauses with durations greater than t seconds, γ represents the rate parameter for the first exponential (i.e., short pauses), δ represents the rate parameter for the second exponential (i.e., long pauses), and ρ represents the proportion of pauses associated with the first exponential (see Ref. 7 for more discussion about these curve fits). The reciprocal of the rate

Table 1. *Histological results*

	% of Fungiform Papillae with Intact Taste Pores	*P* Value
Con	92.6 ± 0.85	
CTX	12.5 ± 3.55	$P < 0.001$ vs. Con
DBLX	12.7 ± 3.98	$P < 0.001$ vs. Con
	Number of Taste Pores in Circumvallate Papilla	***P* Value**
Con	347 ± 14.5	
GLX	46 ± 12.3	$P < 0.001$ vs. Con
DBLX	32 ± 16.3	$P < 0.001$ vs. Con

Percentages and no. of taste pores values are means ± SE. Con, control; CTX, bilateral chorda tympani nerve transection; DBLX, combined transection of the chorda tympani and glossopharyngeal nerves; GLX, bilateral glossopharyngeal nerve transection.

parameters provides the durations for the respective categories of pauses.

Histology

The rats were transcardially perfused with isotonic saline followed by 4% buffered formaldehyde. The tongues were removed and stored in the fixative. The anterior tongue was treated with 0.5% methylene blue using the procedure previously described (43), and the number of fungiform papillae with an identifiable taste pore was determined for each rat. The circumvallate papilla was embedded in paraffin, cut into 10-μm sections, mounted on glass slides, and stained with hematoxylin and eosin. The number of taste pores was then counted. These procedures were done to confirm that the nerves were transected, in which case taste buds degenerate. A random subsample of eight control rats was used for histological comparisons.

RESULTS

Histology

All of the nerve-transected animals showed markedly reduced numbers of taste buds in the appropriate receptor fields, confirming the success of the surgeries (Table 1). Some of the animals, however, showed signs of regeneration. This is most likely because these animals were not perfused until at least 28 days after surgery due to the fact that they took part in a second phase of this experiment (not reported here). On the basis of published (43) and unpublished data from our laboratory, regeneration of taste buds should not yet have occurred at the time of the present studies (at most 17 days after surgery). Moreover, every nerve-transected rat had at least a 5.8 SD reduction in the number (or percentage) of taste pores in the denervated receptor field compared with the control mean.

Ingestive Behavior

As the analysis of the individual measures below reveal, the Con and CTX groups showed similar disruptions in their total intake and ingestive microstructure when water was adulterated with quinine. The transection of the GL countered these changes, and for some measures the additional transection of the CT further mitigated the effects of quinine on drinking behavior.

Total intake and total licks. Fluid intake was significantly lower in all groups when quinine was the stimulus compared with water (Fig. 1, all $P \leq 0.014$). The extent of the decrease was noticeably less in the GLX and DBLX groups. The percentage change in intake between postsurgical water and quinine tests differed significantly among the groups [$F(3,33) = 13.7$, $P < 0.0001$]. A Dunnett's test revealed that the GLX and DBLX group were significantly less affected by quinine adulteration of the fluid compared with the control group (both $P < 0.012$).

Similar statistical tests conducted on the total licks measure resulted in similar, but not identical, outcomes (Fig. 2). Total licks decreased significantly during the quinine presentation for all groups (all $P \leq 0.035$). The percentage change in total licks between the water and quinine sessions also differed significantly between the groups [$F(3,33) = 3.30$, $P = 0.032$], but these changes did not appear to be as great as they were for total intake in the control, CTX, and GLX groups. In contrast to the Dunnett's comparisons involving total intake, only the DBLX group differed significantly from the control group ($P = 0.041$); the GLX did not.

Volume per lick. The apparent difference in the degree of change for total intake compared with total licks suggested that the volume ingested per lick decreased when water was replaced by quinine. In fact, this was the case for the control, CTX, and GLX groups (Fig. 3, all $P \leq 0.008$). In contrast, the volume per lick did not change significantly in the DBLX group. A

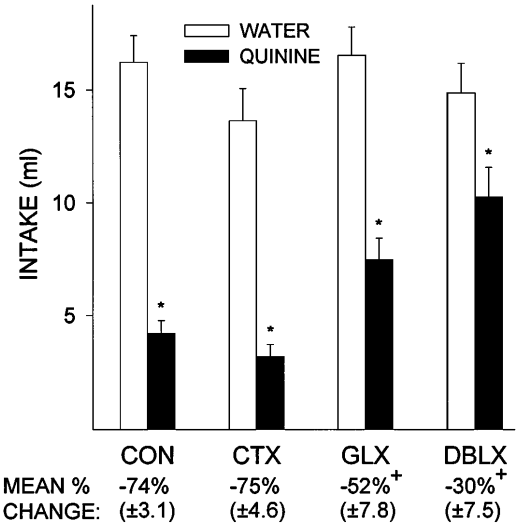

Fig. 1. Mean + SE intake (ml) of water (open bars) and quinine (solid bars) after sham surgery (Con), bilateral chorda tympani nerve transection (CTX), bilateral glossopharyngeal nerve transection (GLX), or combined transection of the chorda tympani and glossopharyngeal nerves (DBLX). Values under bars indicate mean ± SE percentage change in intake between water and quinine sessions. In some groups, there were minor discrepancies between percentage change calculated on the basis of mean water and quinine values and mean of percentage change values calculated for each rat. The latter provides a measure of variability. * Statistically significant ($P < 0.05$) intake difference between quinine and water sessions, + statistically significant ($P < 0.05$) difference from percentage change in Con group.

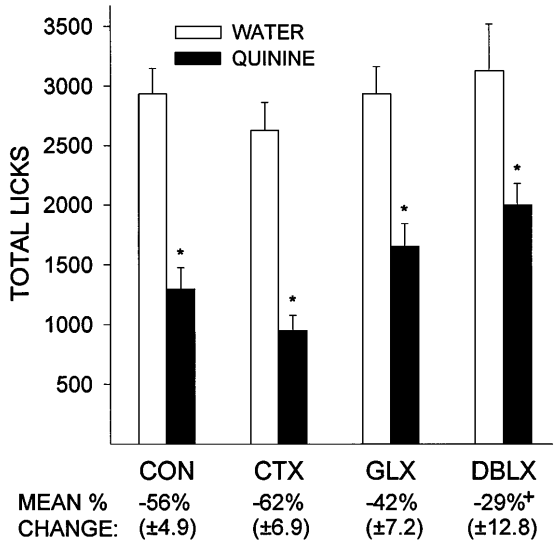

Fig. 2. Mean + SE of total licks of water (open bars) and quinine (solid bars) in Con, CTX, GLX, or DBLX groups. Values under bars indicate mean ± SE percentage change in total licks between water and quinine sessions. In some groups, there were minor discrepancies between percentage change calculated on the basis of mean water and quinine values and mean of percentage change values calculated for each rat. The latter provides a measure of variability. *Statistically significant ($P < 0.05$) intake difference between quinine and water sessions, + statistically significant ($P < 0.05$) difference from percentage change in Con group.

one-way ANOVA of the percentage change in volume per lick across the two sessions revealed a significant effect of surgical treatment [$F(3,33) = 8.41$, $P = 0.0003$]; a Dunnett's test indicated that only the DBLX group differed significantly from controls ($P = 0.0001$).

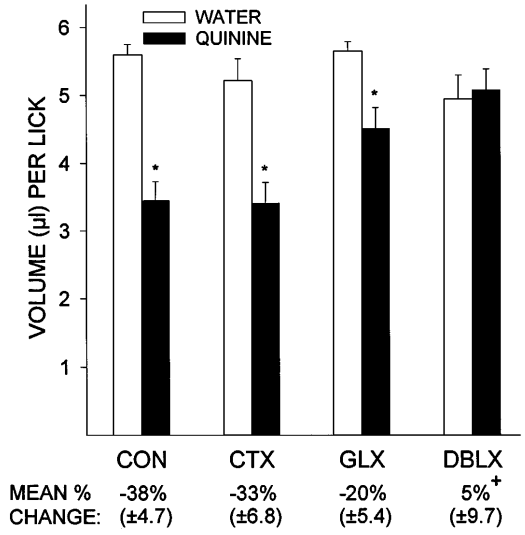

Fig. 3. Mean + SE volume (µl) per lick of water (open bars) and quinine (solid bars) in Con, CTX, GLX, or DBLX groups. Values under bars indicate the mean ± SE percentage change in volume per lick between water and quinine sessions. In some groups, there were minor discrepancies between percentage change calculated on the basis of mean water and quinine values and mean of percentage change values calculated for each rat. The latter provides a measure of variability. *Statistically significant ($P < 0.05$) intake difference between quinine and water sessions, + statistically significant ($P < 0.05$) difference from percentage change in Con group.

Initial drinking rate. The number of licks produced during the first minute after the second lick, a measure thought to be strongly influenced by the orosensory properties of the stimulus relatively uncontaminated by postingestive events (9, 10), dropped by more than one-half in the Con and CTX groups when quinine was presented in comparison with water (Fig. 4; both $P < 0.0001$). The initial drinking rate also dropped significantly in the GLX group when quinine was the stimulus ($P < 0.015$), but not by as large a margin as in the previous groups. There was essentially no change in the initial drinking rate across stimulus conditions in the DBLX group ($P > 0.57$). The percentage change in initial drinking rate across the two test sessions seen in control rats did not significantly differ from that in the CTX group ($P > 0.79$) but did differ from that in both the GLX ($P < 0.005$) and DBLX ($P < 0.0001$) groups [overall $F(3,33) = 17.3$, $P < 0.0001$].

Burst size. Burst size (Fig. 5) significantly decreased in the Con, CTX, and GLX groups when quinine was presented in place of water (all $P \leq 0.029$), and the decrease approached the statistical rejection criterion in the DBLX group ($P = 0.07$). The effect, however, was clearly greatest in the Con and CTX groups. The percentage change in burst size across the two test sessions seen in the control rats did not significantly differ from that in the CTX group ($P > 0.99$) but did differ from that in both the GLX ($P = 0.0006$) and DBLX ($P < 0.0001$) groups [overall $F(3,33) = 17.4$, $P < 0.0001$].

Burst number. When quinine was presented, the number of bursts (and pauses) increased significantly relative to water in both the control and CTX groups

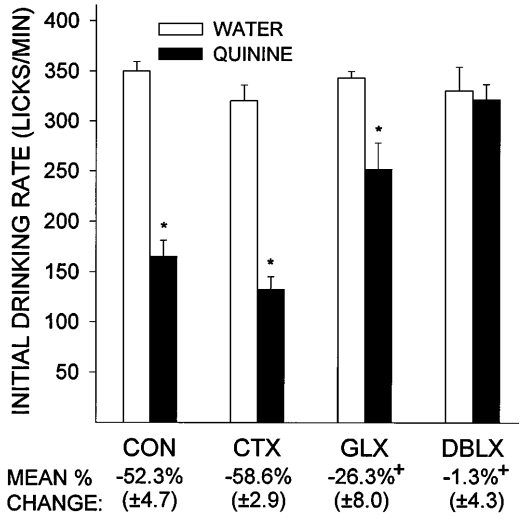

Fig. 4. Mean + SE of initial lick rate (licks/min) during first minute after second lick of water (open bars) and quinine (solid bars) in Con, CTX, GLX, or DBLX groups. Values under bars indicate mean ± SE percentage change in total licks between water and quinine sessions. In some groups, there were minor discrepancies between percentage change calculated on the basis of mean water and quinine values and mean of percentage change values calculated for each rat. The latter provides a measure of variability. *Statistically significant ($P < 0.05$) intake difference between quinine and water sessions, + statistically significant ($P < 0.05$) difference from percentage change in Con group.

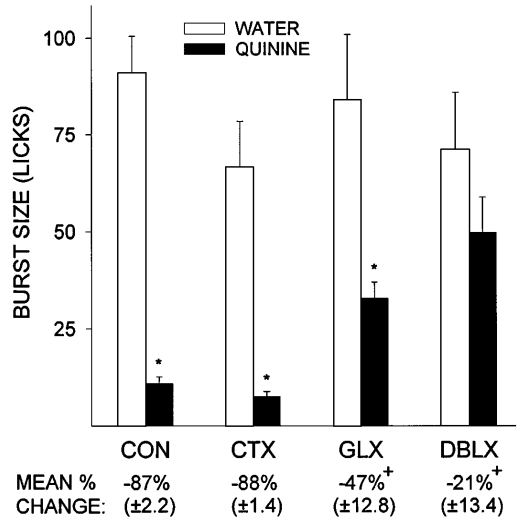

Fig. 5. Mean + SE burst size (licks) of water (open bars) and quinine (solid bars) in Con, CTX, GLX, or DBLX groups. Values under bars indicate mean ± SE percentage change in burst size between water and quinine sessions. In some groups, there were minor discrepancies between percentage change calculated on the basis of mean water and quinine values and mean of the percentage change values calculated for each rat. The latter provides a measure of variability. *Statistically significant ($P < 0.05$) intake difference between quinine and water sessions, + statistically significant ($P < 0.05$) difference from percentage change in Con group.

(both $P \leq 0.0027$, Fig. 6). In contrast, no significant change was observed in either the GLX or DBLX groups (both $P > 0.25$). The percentage change across the two test sessions seen in the control rats did not significantly differ from that in the CTX ($P = 0.64$) and GLX ($P = 0.077$) groups but did differ from the DBLX ($P = 0.05$) group [overall $F(3,33) = 2.95$, $P = 0.047$]. Judging from the SE values for each group, one should regard the statistical analysis of the percentage change in burst number with extreme caution. Nevertheless, the primary analysis of the number of bursts produced between the two sessions clearly indicates that quinine adulteration only affected the control and CTX groups, and the magnitude of this effect was quite substantial.

Pause duration. Pause duration (Fig. 7) was reduced by the addition of quinine to water in the control ($P = 0.0001$) and CTX ($P = 0.0038$) groups but was left relatively unchanged in the GLX ($P = 0.49$) and DBLX ($P = 0.77$) groups. The percentage change across the two test sessions in the control group did not significantly differ from that in the CTX group ($P > 0.99$) but did from that in both the GLX ($P = 0.0125$) and DBLX ($P = 0.027$) groups [overall $F(3,33) = 5.24$, $P = 0.0045$].

ILI. Quinine adulteration of water caused the ILI to decrease in the control and the GLX groups ($P < 0.04$) but not in the CTX or DBLX groups (Fig. 8). The extent of these changes was small and therefore of questionable functional significance. An ANOVA of the percentage change in ILI across the two test sessions did not reveal any significant differences among the groups [overall $F(3,33) = 0.76$, $P = 0.52$].

Drinking duration. Drinking duration (Fig. 9) significantly increased in the control group in response to quinine adulteration of water, but only by 13% ($P < 0.03$). The other groups did not exhibit significant changes in this measure ($P > 0.10$). An ANOVA of the percentage change in drinking duration did not reveal any significant differences among the groups [overall $F(3,33) = 1.32$, $P = 0.28$].

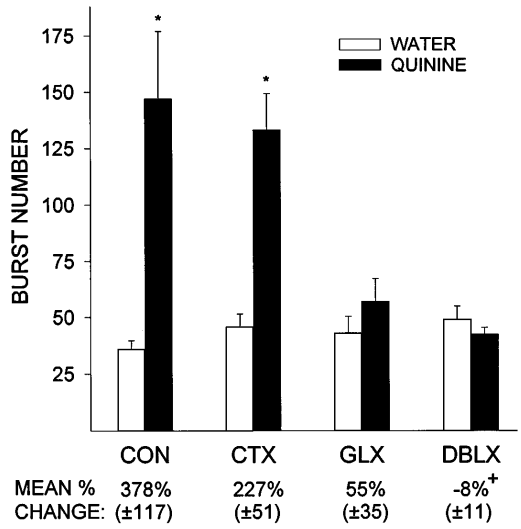

Fig. 6. Mean + SE burst number of water (open bars) and quinine (solid bars) in Con, CTX, GLX, or DBLX groups. Values under bars indicate mean ± SE percentage change in burst number between water and quinine sessions. In some groups, there were minor discrepancies between percentage change calculated on the basis of mean water and quinine values and mean of percentage change values calculated for each rat. The latter provides a measure of variability. *Statistically significant ($P < 0.05$) intake difference between quinine and water sessions, + statistically significant ($P < 0.05$) difference from percentage change in Con group.

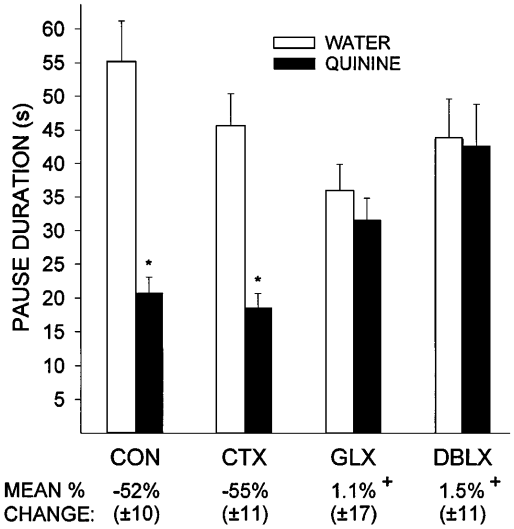

Fig. 7. Mean + SE of pause duration (s) between bursts of water (open bars) and quinine (solid bars) in Con, CTX, GLX, or DBLX groups. Values under bars indicate mean ± SE percentage change in pause duration between water and quinine sessions. In some groups, there were minor discrepancies between percentage change calculated on the basis of mean water and quinine values and mean of percentage change values calculated for each rat. The latter provides a measure of variability. *Statistically significant ($P < 0.05$) intake difference between quinine and water sessions; + statistically significant ($P < 0.05$) difference from percentage change in Con group.

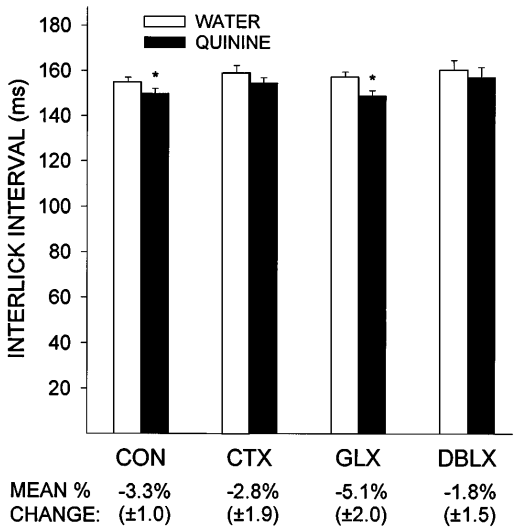

Fig. 8. Mean + SE interlick interval (ms) associated with water (open bars) and quinine (solid bars) in Con, CTX, GLX, or DBLX groups. Values under bars indicate mean ± SE percentage change in interlick interval between water and quinine sessions. In some groups, there were minor discrepancies between percentage change calculated on the basis of mean water and quinine values and mean of percentage change values calculated for each rat. The latter provides a measure of variability. *Statistically significant ($P < 0.05$) intake difference between quinine and water sessions.

Survivorship analysis: burst size. A Weibull function accounted for the survival curves representing burst size (Table 2) for both water (average $r^2 = 0.96 \pm 0.007$) and quinine (average $r^2 = 0.99 \pm 0.001$) drinking by control rats after surgery. The mean α for the quinine-related Weibull was quite close to the mean burst size. This is due to the fact that the Weibull accounted for

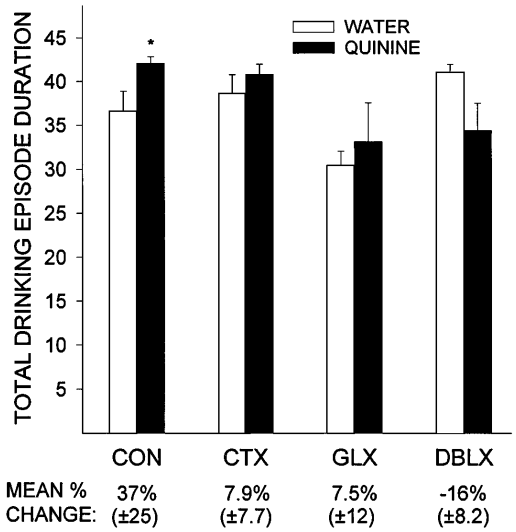

Fig. 9. Mean + SE drinking episode duration (min) for water (open bars) and quinine (solid bars) in Con, CTX, GLX, or DBLX groups. Values under bars indicate mean ± SE percentage change in duration between water and quinine sessions. In some groups, there were minor discrepancies between percentage change calculated on the basis of mean water and quinine values and mean of percentage change values calculated for each rat. The latter provides a measure of variability. *Statistically significant ($P < 0.05$) intake difference between quinine and water sessions.

Table 2. *Weibull fits for burst size survival functions*

	Water Burst Size			Quinine Burst Size		
Rat	α	β	r^2	α	β	r^2
5	29.3	0.490	0.985	3.62	1.36	0.996
6	84.4	0.626	0.974	15.9	0.725	0.992
10	47.8	0.345	0.900	4.00	0.958	0.999
11	56.1	0.878	0.988	6.40	1.04	0.993
12	65.4	0.497	0.963	4.63	1.36	0.994
15	106.9	0.566	0.895	4.11	1.04	0.997
18	139.5	0.586	0.937	8.32	1.22	0.995
20	185.6	1.30	0.935	9.30	0.989	0.983
24	51.2	0.714	0.983	17.7	0.868	0.992
25	134.1	1.41	0.970	15.2	0.708	0.994
30	38.1	0.916	0.982	8.55	1.13	0.994
35	46.7	0.673	0.984	7.87	1.45	0.995
36	37.3	0.493	0.953	5.37	0.971	0.997
39	64.6	1.50	0.974	5.64	1.34	0.994
41	70.1	0.585	0.978	23.1	0.749	0.993
42	51.9	0.989	0.974	11.3	1.33	0.978
Mean	75.6	0.785	0.961	9.44	1.08	0.992
SE	11.0	0.088	0.007	1.43	0.061	0.001

>99% of the variance and the shape parameter (β) approximated 1.0. In contrast, the mean α for the water-related Weibull departed somewhat from the mean burst size, because the shape parameter (β) substantially departed from 1.0. Davis (7) has shown that the burst size (i.e., cluster size) survival functions from rats drinking sucrose solutions during 30-min tests are accounted for by a Weibull function with a shape parameter close to 1.0, as was seen here with quinine drinking in water-deprived rats. The fact that the shape parameter was <1.0 for water-deprived rats drinking water suggests that physiological state interacts with the stimulus to produce survival functions that can depart from a simple exponential. Both Weibull parameters (α and β) significantly changed (both $P < 0.02$) across stimulus conditions (quinine vs. water; Table 2).

Survivorship analysis: pause duration. The double exponential function accounted for the survival curves representing pause duration (Table 3) for both water (average $r^2 = 0.96 \pm 0.008$) and quinine (average $r^2 = 0.95 \pm 0.011$) drinking by control rats.[1] When water was the stimulus, 61% (i.e., $\rho \times 100$) of the pauses were short, with an average duration of 3.4 s (i.e., $1/\gamma$) and 39% [i.e., $(1 - \rho) \times 100$] of the pauses were long, with an average duration of 84 s (i.e., $1/\delta$). This profile changed significantly across stimulus conditions. When quinine was the stimulus, the percentage of short pauses (72%) significantly increased ($P = 0.014$), the duration of the short (2.3 s) pauses significantly decreased ($P < 0.0001$), and the decrease in the duration of long (58.5 s) pauses approached the statistical rejection criterion ($P =$

[1] The double exponential fit to the survival function for quinine pause duration for one rat from the control group (*rat 10*) had a ρ that was essentially 1. Thus δ is technically undefined, and the double exponential reduces to a single exponential. Accordingly, the δ value provided by the fit is highly suspect, and this animal was not included in the statistical analyses comparing the changes in the exponential parameters between the quinine and water sessions ($n = 15$).

Table 3. *Double exponential fits for pause duration survival functions*

	Water Pause Duration				Quinine Pause Duration			
Rat	ρ	γ	δ	r^2	ρ	γ	δ	r^2
5	0.895	0.342	0.0021	0.899	0.944	0.456	0.0039	0.839
6	0.627	0.325	0.0107	0.974	0.623	0.607	0.0128	0.935
10	0.504	0.392	0.0122	0.944	1.00	0.392	0.8897	0.912
11	0.554	0.205	0.0217	0.986	0.468	0.335	0.0381	0.993
12	0.570	0.263	0.0128	0.974	0.879	0.422	0.0139	0.920
15	0.613	0.307	0.0081	0.956	0.900	0.333	0.0071	0.949
18	0.547	0.313	0.0082	0.989	0.696	0.540	0.0278	0.959
20	0.464	0.181	0.0097	0.982	0.836	0.396	0.0063	0.946
24	0.568	0.310	0.0187	0.982	0.566	0.322	0.0325	0.979
25	0.406	0.267	0.0096	0.986	0.412	0.524	0.0286	0.992
30	0.586	0.290	0.0124	0.975	0.727	0.375	0.0157	0.990
35	0.669	0.379	0.0130	0.965	0.623	0.607	0.0128	0.956
36	0.758	0.346	0.0106	0.958	0.754	0.428	0.0239	0.971
39	0.707	0.402	0.0232	0.886	0.878	0.511	0.0050	0.885
41	0.518	0.183	0.0097	0.992	0.730	0.381	0.0184	0.982
42	0.703	0.152	0.0076	0.983	0.705	0.253	0.0091	0.986
Mean	0.608	0.291	0.0119	0.964	0.716	0.433	0.0171	0.952
SE	0.030	0.019	0.0013	0.008	0.041	0.027	0.0028	0.011

For *rat 10* quinine pause duration is unreliable because ρ was essentially 1.0. Means and SE for quinine pause duration are based on $n = 15$; *rat 10* was not included.

0.066). These results demonstrate how the mean pause duration for the session significantly decreased when water was replaced by quinine.

Change across session: burst size. The survivorship analyses confirmed the momentary probabilistic nature of burst size as suggested by Davis (7). We ordered the bursts serially and divided the total distribution into thirds to examine whether there was a tendency for subsequent bursts to be smaller as the session progressed. On average, burst size decreased as drinking behavior progressed when water was the stimulus for all groups (see Table 4 and Fig. 10). A test of simple effects for the control, CTX, and GLX groups revealed burst size significantly decreased across one-third segments of the serially ordered distribution when water was the stimulus (all $P \leq 0.0002$). In striking contrast, adulterating water with quinine not only lowered the overall burst size but essentially eliminated the change in burst size observed across one-third portions of the burst sequence in the control and GLX groups (simple effect of thirds for quinine; both $P > 0.97$) and severely attenuated it in the CTX group ($P = 0.039$). Interestingly, average burst size for the DBLX group decreased as drinking behavior progressed during the quinine session in much the same fashion as it did for the water session, as supported by a significant effect of thirds in the absence of both a significant main effect of stimulus and interaction (Table 4 and Fig. 10). A test of simple effects indicated that burst size was smaller for each third of the serially ordered distribution of bursts when quinine was the stimulus for the control and CTX groups (all $P < 0.03$); this was also true for the first one-third portion of bursts in the GLX group ($P < 0.009$).

Change across session: pause duration. In the control group, pause duration was affected by both stimulus type and behavioral progress, and there was a significant interaction between these two factors (Table 5 and Fig. 11). A test of simple effects indicated that pause duration increased as drinking behavior progressed for both water ($P < 0.0008$) and quinine ($P = 0.001$). The pause duration increased much more steeply across one-third portions of the pause sequence when water was the stimulus, as supported by the fact that pause duration was significantly longer for both of the latter two thirds of the serially ordered distribution when water was the stimulus (both $P < 0.02$). As was the case for many of the other dependent measures, the statistical results for the CTX group were similar to that for the control rats, except that pause duration did not increase significantly with distribution thirds when quinine was the stimulus ($P = 0.12$), and the difference between the water and quinine pauses did not reach significance until the last one-third ($P < 0.008$).[2] Interestingly, both the GLX and DBLX rats increased their pause duration progressively across the quinine session in a manner that was similar to water. In the GLX group, this increase was not statistically distinguishable from water (all $P > 0.219$), and in the DBLX group, there was no evidence that pauses during any of the one-thirds of the serially ordered distribution were significantly shorter when quinine was the stimulus compared with water. Clearly, the magnitude of this water vs. quinine difference in pause duration in the DBLX group was small and in the opposite direction compared with the size of the difference observed in the control and CTX groups during the latter two-thirds of the serially ordered distribution.

Change across session: drinking rate. The changes seen across one-third portions of the serially ordered distributions of bursts and pauses resulted in changes in the drinking rate associated with those time periods (Table 6 and Fig. 12). When water was the stimulus, the control group decreased burst size and increased pause duration, leading to a decrease in drinking rate as the session progressed ($P < 0.0001$). The drinking rate associated with the one-third portions of bursts and pauses when quinine was the stimulus also decreased as the session progressed ($P < 0.0001$) but took a much more shallow descent. In fact, the drinking rate for quinine during the period of the first one-third of bursts and pauses was similar to that produced during the period of the last one-third for water. The water drinking rate was significantly higher than the quinine drinking rate for each one-third segment in the control group (all $P < 0.05$). Thus, despite the short pause durations and the increase in burst number when quinine was the stimulus relative to water, the rate of intake was consistently low due to the small burst size.

[2] The difference between quinine and water pause durations during the second one-third portion of the serially ordered distribution approached statistical significance for the CTX group ($P = 0.053$).

Table 4. *ANOVA results of burst size as a function of distributional thirds and stimulus*

	Stimulus		Thirds		Interaction	
Group	*F*	*P*	F	*P*	*F*	*P*
Con	$F(1,15) = 70.3$	$P < 0.0001$	$F(2,30) = 36.8$	$P < 0.0001$	$F(2,30) = 27.3$	$P < 0.0001$
CTX	$F(1,6) = 27.9$	$P = 0.0019$	$F(2,12) = 50.8$	$P < 0.0001$	$F(2,12) = 40.3$	$P < 0.0001$
GLX	$F(1,7) = 7.45$	$P = 0.0293$	$F(2,14) = 14.6$	$P = 0.0004$	$F(2,14) = 11.5$	$P = 0.0011$
DBLX	$F(1,5) = 5.22$	$P = 0.0710$	$F(2,10) = 12.8$	$P = 0.0018$	$F(2,10) = 3.29$	$P = 0.0797$

When water was the stimulus, the changes in drinking rate for the nerve-transected groups were similar to that observed in the control animals. When quinine was the stimulus, the drinking rate profile in the GLX and DBLX groups was more waterlike but was nevertheless statistically distinguishable from that seen when water was the stimulus (Table 6).

DISCUSSION

The adulteration of water with quinine had striking effects on the pattern of drinking in rats. This outcome was expected, because it has been clearly established that total intake of quinine solution during both short- and long-term drinking tests is markedly attenuated relative to water (e.g., 1, 3, 18, 33, 34, 42, 48). In our study, total intake, total licks, volume per lick, pause duration, and burst size decreased notably, whereas burst (and pause) number increased in intact control rats when quinine replaced water in the drinking test. Transection of the GL, especially in combination with the CT, opposed these quinine-induced changes in ingestive microstructure, suggesting that these behavioral alterations were related to taste. The fact that both quinine and water lick patterns were assessed and

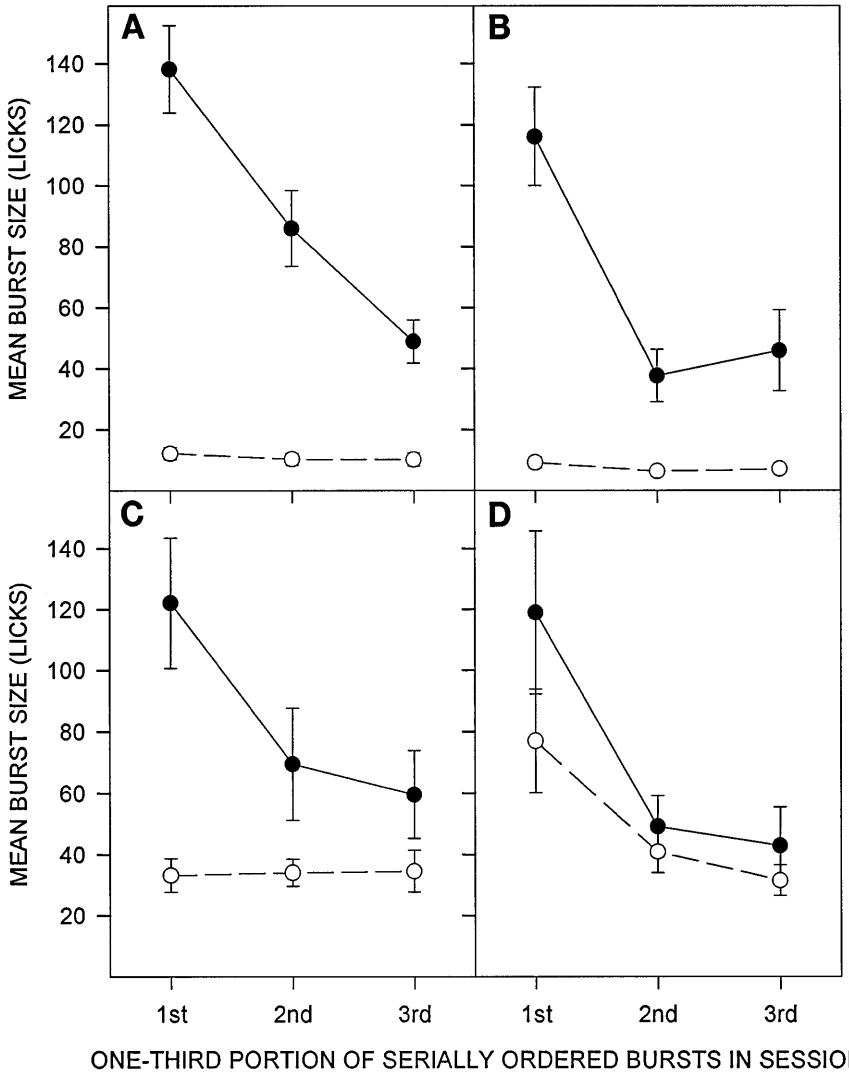

Fig. 10. Bursts for each rat were serially ordered for water (●) and quinine (○) sessions and broken down into roughly equal one-third segments. Mean ± SE for each segment is presented for Con (*A*), CTX (*B*), GLX (*C*), or DBLX (*D*) groups.

Table 5. *ANOVA results of pause duration as a function of distributional thirds and stimulus*

Group	Stimulus		Thirds		Interaction	
	F	*P*	*F*	*P*	*F*	*P*
Con	$F(1,15) = 26.4$	$P = 0.0001$	$F(2,30) = 14.3$	$P < 0.0001$	$F(2,30) = 5.55$	$P < 0.0089$
CTX	$F(1,6) = 20.9$	$P = 0.0038$	$F(2,12) = 27.8$	$P < 0.0001$	$F(2,12) = 11.9$	$P = 0.0014$
GLX	$F(1,7) = 0.41$	$P = 0.5417$	$F(2,14) = 9.16$	$P = 0.0029$	$F(2,14) = 0.47$	$P = 0.6364$
DBLX	$F(1,5) = 0.09$	$P = 0.7783$	$F(2,10) = 19.1$	$P = 0.0004$	$F(2,10) = 7.35$	$P = 0.0109$

compared after surgery rules out any simple explanation of these stimulus-based differences between the groups arising from a neurotomy-induced impairment in general licking competence. Furthermore, it is unlikely that nongustatory orosensory input played a large role in these stimulus-related behavioral effects. Quinine is not noted as an especially effective excitatory stimulus for trigeminal sensory receptors even at rather high concentrations that stimulate gustatory afferents in the rat (25, 26). Moreover, the lingual branch of the trigeminal nerve, a major conduit for transmitting tactile, thermal, and pain signals from the anterior tongue, was left intact in all of the groups. Given that these nerves contain autonomic efferents primarily targeting salivary glands, we cannot completely rule out some change in the salivary environment as a contributor to the effects seen here, but it seems unlikely. Transection of the CT only partially denervates the sublingual and submaxillary salivary glands (22, 52), and extirpation of these glands does not appear to alter the rat's responsiveness to quinine in brief-access taste tests (42). The taste buds of the circumvallate and foliate papillae are bathed by secretions from the von Ebners glands (see Ref. 20), which

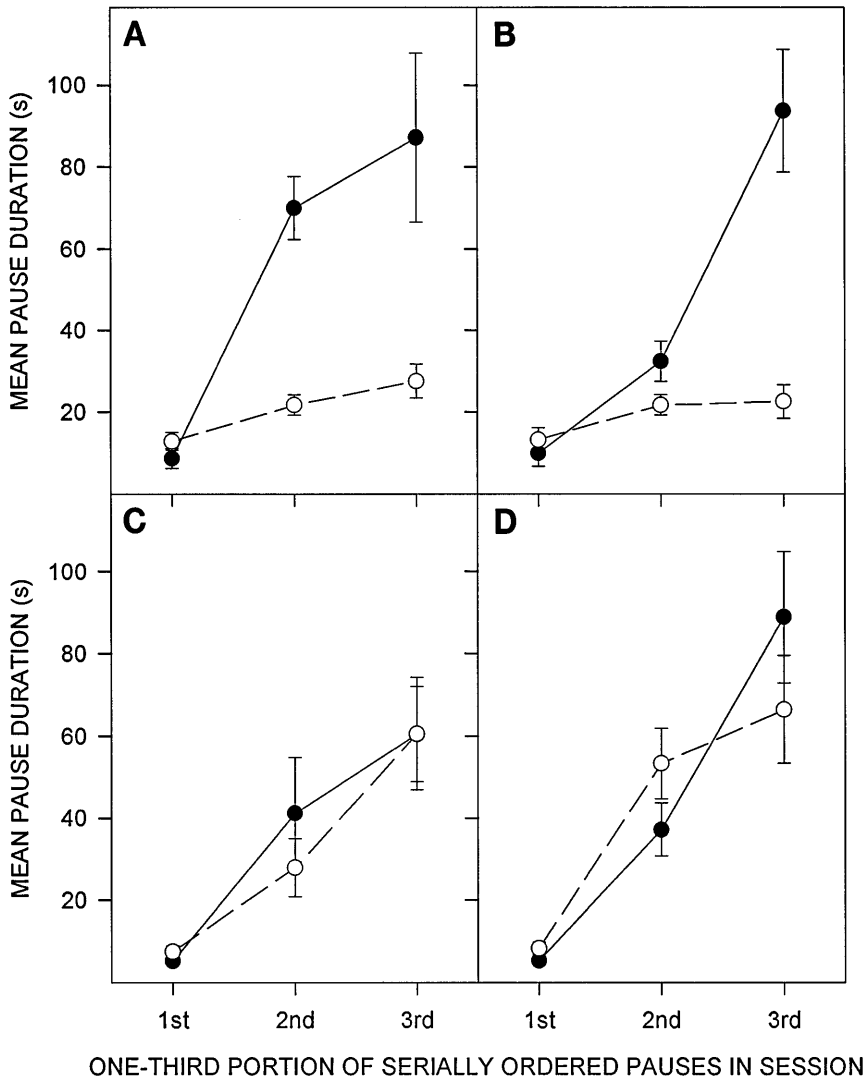

Fig. 11. Pauses for each rat were serially ordered for water (●) and quinine (○) sessions and broken down into roughly equal one-third segments. Mean ± SE for each segment is presented for Con (*A*), CTX (*B*), GLX (*C*), or DBLX (*D*) groups.

Table 6. *ANOVA results of drinking rate as a function of stimulus and distributional thirds of pauses and bursts*

	Stimulus		Thirds		Interaction	
Group	*F*	*P*	*F*	*P*	*F*	*P*
Con	$F(1,15) = 87.8$	$P < 0.0001$	$F(2,30) = 266$	$P < 0.0001$	$F(2,30) = 94.9$	$P < 0.0001$
CTX	$F(1,6) = 43.6$	$P < 0.0001$	$F(2,12) = 59.5$	$P < 0.0001$	$F(2,12) = 35.6$	$P < 0.0001$
GLX	$F(1,7) = 7.12$	$P < 0.0001$	$F(2,14) = 53.1$	$P < 0.0001$	$F(2,14) = 3.61$	$P = 0.054$
DBLX	$F(1,5) = 17.4$	$P < 0.0088$	$F(2,10) = 163$	$P < 0.0001$	$F(2,10) = 8.10$	$P < 0.0081$

are denervated by transection of the GL, but this same surgery results in the degeneration of those taste buds. All things considered, the effects of the nerve transections likely have a gustatory origin, and the microstructural components that are affected by these surgeries can therefore be considered directly or indirectly taste mediated.

Interpretation of Taste Effects on Ingestive Microstructure

Given that the oral sensory properties of quinine obviously exert a suppressive effect on total intake, the increase in burst number and shortening of average pause duration when quinine was the stimulus does not appear to be a direct effect of taste. Rather, the available evidence suggests the following sequence of events. *1*) The water-deprived rat approaches and begins to sample the fluid stimulus, *2*) the "aversive" taste (i.e., quinine) acts upon neural circuits that promote burst termination, *3*) these shortened bursts reduce the rate at which the rat can rehydrate, thus maintaining a lower level of postingestive inhibition longer into the test session (relative to rats drinking a more acceptable stimulus such as water), *4*) this low-

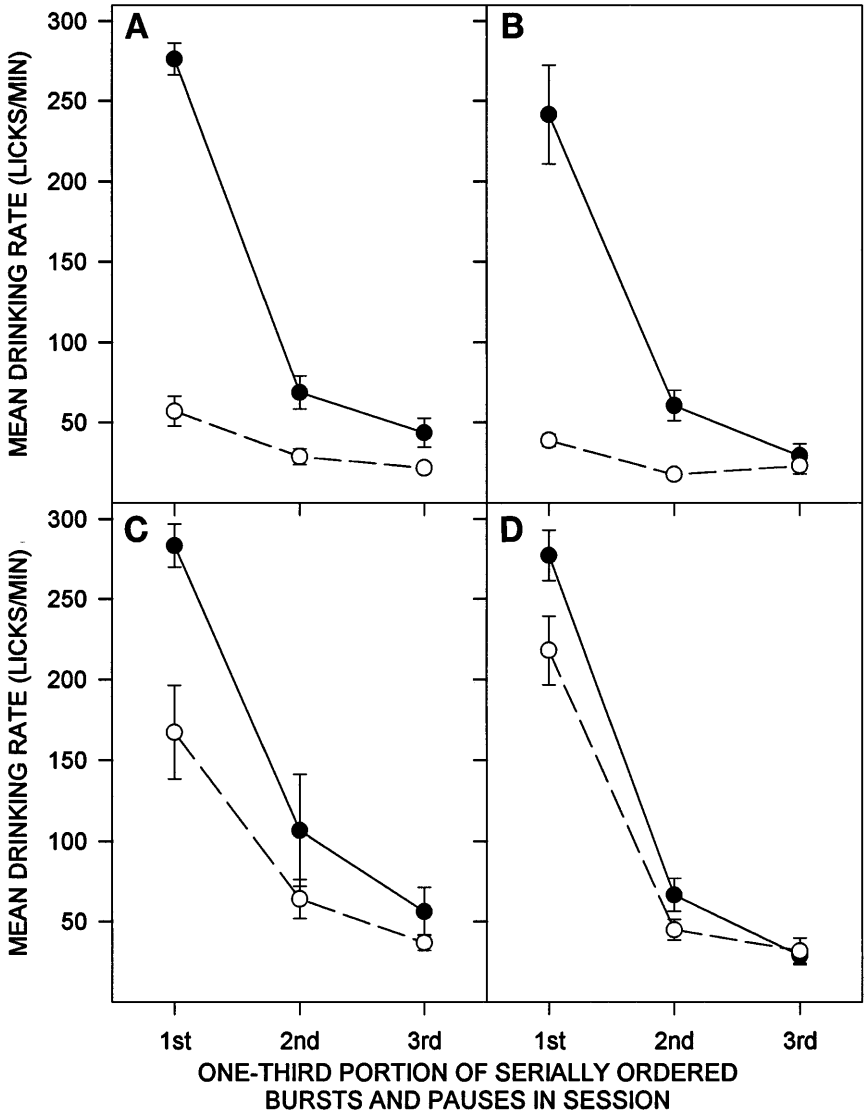

Fig. 12. Bursts and pauses for each rat were serially ordered for water (●) and quinine (○) sessions and broken down into roughly equal one-third segments. Mean ± SE drinking rate (licks/min) associated with time segments for each segment is presented for Con (*A*), CTX (*B*), GLX (*C*), or DBLX (*D*) groups.

ered postingestive inhibition, or perhaps an undiminished drive to rehydrate, acts upon neural circuits that promote burst initiation,[3] and *5*) this results in the behavioral outcome observed: short but frequent bursts of licking. There are two lines of evidence supporting this inferred sequence of events. The first is based on the change in burst size, pause duration, and the associated intake rate as the drinking behavior progressed during respective sessions. The second is based on the effect of varying gustatory input (i.e., the comparison of surgical groups).

At the beginning of the session (i.e., when the first one-third of serially ordered bursts and pauses were compared), there was no difference in the mean pause duration for control rats drinking either quinine or water (Fig. 11*A*). This might be expected because postingestive inhibition should be low at the beginning of the session before much fluid is consumed. Note, however, that the average size of those bursts in the first one-third is much greater in control rats drinking water compared with quinine (Fig. 10*A*), and this concomitantly leads to a relatively greater rate of water intake early in the drinking episode (Fig. 12*A*). Thus, by the time the middle (and final) one-third of bursts is initiated, control rats have ingested far more fluid during the water session than during the quinine session. Therefore, postingestive inhibition will be greater in the middle and final one-thirds during the water session relative to the quinine session. In contrast, the influence of the aversive taste should remain relatively constant over the session, because the same quinine stimulus is present for the duration of the test.[4] In other words, behavioral changes seen over the course of the sessions can be attributed to physiological state, because taste is held relatively constant. On the other hand, during the first one-third of the session, physiological state is relatively equal across treatment conditions, whereas taste varies *1*) as a function of stimulus (quinine or water) and *2*) as a function of surgical group (e.g., taste input is substantially reduced in the DBLX group relative to controls). In the extreme, it is clear that in the first minute of the drinking episode, when postingestive effects should be negligible, the rate of licking is halved when quinine is the stimulus in comparison to that elicited by water, and this reduction is entirely eliminated by lingual gustatory denervation. This lends support to the view that initial drinking rate is heavily influenced by taste (9, 10).

From this perspective, an aversive taste exerts its influence on intake by promoting burst termination, not by inhibiting burst initiation. This is not to say, however, that burst termination is only influenced by taste. The fact that burst size decreased across the one-third segments of the burst sequence when water was the stimulus suggests that postingestive load can also affect burst termination processes. It is also noteworthy that average volume per lick decreased when quinine was added to water, and this, like all of the other microstructural changes, was opposed by lingual gustatory denervation. This finding implies that average lick topography can be adjusted when an aversive stimulus contacts lingual receptors.

On the basis of these collective findings, we hypothesize that when "thirsty" rats (i.e., water deprived) are presented with an "unpalatable" taste solution (i.e., one that is normally avoided and unconditionally elicits oromotor rejection behaviors), the taste input acts primarily on neural circuits governing lick topography and burst termination, whereas signals related to hydrational state primarily influence neural circuits involved in burst initiation. The putative neural circuits underlying burst and/or pause generation can be considered as "switches" for the central pattern generator (CPG) thought to control licking in the rat (see Ref. 46). One process turns the CPG on, and the other turns it off. It would appear that postingestive load affects both burst termination (off) and initiation (on) processes, because burst size decreased and pause duration increased, on average, as the session progressed when water was the stimulus. It is possible that postingestive load is exerting its effect by altering the animal's hydrational state during the drinking episode. The extent to which this occurs depends partly on the rate of water absorption from the gut and cannot be addressed by the present experiment. Furthermore, our hypothesis is based on the assumption that processes that promote the start of a drinking episode can be functionally discriminated from those that encourage burst initiation once the episode has begun.

Taste, postingestive load, and physiological state need not interact to influence the microstructure of ingestion exclusively as hypothesized. It is more likely a matter of degree, in which one factor (e.g., taste) affects one process (e.g., burst termination) more than another (e.g., burst initiation). After all, these conclusions are based on an admittedly limited set of conditions. For example, it is possible that an increase in quinine concentration or a decrease in water deprivation would attenuate both the increase in the number of bursts and the shortening of pause duration. Under those conditions, aversive taste input might more successfully compete with the physiological state-related drive to initiate bursts of ingestive behavior. Of course, if total intake were completely suppressed, then there would be no drinking behavior past the first burst (of a few licks). In the present experiment, the quinine concentration used resulted in a substantial decrease in total intake (~75% drop) in intact rats; despite this marked suppression of intake, average pause duration did not increase, but actually decreased, and burst number increased in contrast to a striking decrease in burst size.

[3] Burst initiation processes could just as easily be conceived as pause termination processes. Likewise, burst termination processes could be considered pause initiation processes. It is not our intent to distinguish between the burst and pause perspectives.

[4] It is possible that some degree of gustatory adaptation or habituation may occur, but this would presumably reduce the aversiveness of the stimulus and predict changes in the opposite direction as the session progresses (i.e, increases in drinking rate and burst size over time).

In the discussion here, we have been dealing with the unconditioned motivational properties of the taste stimulus. In our experiment, there was no opportunity for conditioning to influence quinine responsiveness, because quinine was presented only once.[5] It is important to recognize, however, that the hedonic evaluation of taste signals from the periphery is known to be malleable. Clearly, the affective character of a gustatory stimulus can be modulated by experience, as evidenced by the relative ease with which conditioned taste aversions and preferences, as well as taste contrast effects, can be demonstrated in rats. It is important to stress therefore that in our conceptual framework, it is the perceived hedonic value of the taste, conditioned or unconditioned, that exerts its influence on microstructure.

Comparison with Hsiao and Fan Study

Hsiao and Fan (23) examined the ingestive microstucture of intact water-deprived rats licking various weak concentrations of sucrose and quinine alone and mixed during 15-min single-bottle tests. Their results for the highest concentration of quinine used, 0.01% (which is close to the concentration used here), compare favorably with ours with some notable differences. Hsaio and Fan (23) found only about a 30% reduction in intake when quinine was added to water compared with our 74% suppression. Accordingly, the decrease in burst size in the Hsiao and Fan (23) study was not as substantial compared with that observed in our experiment. In both studies, average volume per lick decreased when quinine was added to water, but Hsiao and Fan (23) found no difference in the number of bursts initiated between these two stimuli (water vs. 0.01% quinine); the latter finding is a significant departure from the remarkable increase in burst number observed in our study when water was adulterated with quinine. There are several procedural differences between the two experiments that could serve as the basis for these discrepancies. The duration of our test was three times longer than theirs. Also, our rats had no prior experience with the taste solution, whereas the rats in the Hsiao and Fan (23) study had significant experience with sucrose and quinine at various concentrations under test conditions. As discussed above, prior experience may alter the hedonic value of taste stimuli. Perhaps more importantly, Hsiao and Fan (23) used a liberal pause criterion of 230 ms. It is quite possible that some ILIs that were categorized as pauses could have been 1 or 2 missed tongue contacts in a continuous stream of licking. Symmetrical distributions of pauses are commonly observed around integer multiples (especially 2×) of the fundamental ILI, a finding strongly implicating the normal occurrence of missed spout contacts (11, 49). Thus the use of too liberal a pause criterion might lead, at times, to the conclusion that the CPG is not engaged when in fact it is. The importance of pause criterion selection in microstructural analyses was recently addressed by Spector et al. (39), who reported significant differences in the way that food deprivation and sucrose concentration were reflected in the underlying ingestive microstructure during a 60-min single-bottle test when a 300-ms pause criterion was applied in comparison with a 1-s criterion. Nevertheless, in both the study of Hsiao and Fan (23) and in our study, quinine adulteration appeared to affect processes controlling burst termination and lick topography. Our study demonstrated that these quinine-induced effects are dependent on input from lingual taste receptors, notwithstanding the caveats detailed at the outset of this discussion.

Deterministic Versus Probabilistic Control of Burst and Pause Generation

Recently, Davis (7) elegantly argued that the control of burst size and pause duration is probabilistic rather than deterministic. In other words, the factors known to affect ingestive microstructure do not operate in any systematic way on a single burst or pause. Rather, these factors affect the probability that a burst of a given size or a pause of a given duration will be generated. When Davis (7) examined the survivorship functions for bursts of licking and the pauses between them in a single session, the functions emulated a Poisson process, at least in rats ingesting sucrose solutions. Thus factors influencing burst size and pause duration appear to operate on the "rate" parameter defining the Poisson process. We have now extended this finding to include water-deprived rats licking either water or quinine. The survivorship functions associated with burst size and pause duration had a distinctly exponential character. One difference between the work presented here and that done by Davis (7) is that in the latter study a single exponential function described the survivorship functions for sucrose drinking quite well. That is, when Davis fit the Weibull function (see *Eq. 1*) to his burst size data,[6] the shape parameter did not substantially depart from 1.0 (except for some minor changes). Although this is consistent with the survivorship functions from water-deprived control rats drinking quinine in our study, when water was the stimulus, the shape parameter was, on average, substantially <1.0, demonstrating that there were more smaller, relative to larger, bursts than would be predicted from a simple exponential. The latter finding suggests that the shape parameter can be influenced by test conditions (e.g., physiological state,

[5] Although it is true that rats had no opportunity to form conditioned responses to quinine, they nevertheless had the opportunity to form expectations about the test stimulus. That is, it is possible that as a result of the repeated testing with water in the 45-min task, the rats "expected" to find water in the bottle on the quinine test day.

[6] Davis and his colleagues make a distinction between "bursts" and "clusters." Bursts are defined as runs of licks separated by pauses >250 ms. Clusters are runs of licks separated by pauses >500 ms. Interburst intervals are pauses that are >250 ms and ≤500 ms, whereas intercluster intervals are pauses that are >500 ms. In our terminology, a burst is more analogous to the cluster definition of Davis and co-workers, except that our pause criterion was more conservative (≥1 s).

taste, etc.) as well as the rate parameter. The pause survivorship functions in the present experiment were well represented by a double exponential distribution. Quinine adulteration caused both an increase in the proportion of pauses in the "short" category and a decrease in their average duration. The duration of long pauses also decreased, but this change just missed the statistical rejection criterion. Overall, our survivorship analyses confirm, in principle, the probabilistic nature of the burst and pause structure of licking during an ingestive episode by the rat as suggested by Davis (7). Thus the Poisson-like generation of bursts and pauses appears to be a fundamental principle of microstructure that generalizes across taste stimuli and physiological states.

The fact that the mean burst size decreased and the mean pause duration increased across one-third portions of the sequence of bursts and pauses when water was the stimulus suggests that the rate parameter of the survivorship function (as well as the cumulative probability distribution) changed as a function of postingestive load. The arithmetic average of values in a distribution characterized by an exponential survivorship function represents the reciprocal of the rate parameter defining that function (if the rate parameter is already expressed in the equation in reciprocal form, as in *Eq. 1*, then the mean is equal to the rate parameter itself). Because the survivorship functions do not necessarily represent a simple single exponential (although in the case of burst size for quinine they do), we would not expect the means to correspond perfectly with rate parameters. Although we did not attempt to fit survivorship functions to the one-third portions of the bursts and pauses in a session, it is noteworthy that the standard deviations of the burst sizes and pause durations for each one-third portion of the respective sequence in individual rats often approximated the mean values, a trend consistent with the sampling expectations from a Poisson-like process (32). It appears that quinine opposed the changes in both pause duration and burst size as the behavior progressed in the control (and CTX) group because the aversive taste stimulus kept intake rate, and thus postingestive load, below some threshold for such changes to be expressed.

Relative Contribution of the GL and CT to Taste-Related Behavior

The fact that bilateral transection of the GL had an appreciable effect on spout licking of quinine in the rat is at odds with other studies demonstrating that such neurotomy does not affect quinine intake in either brief-access or long-term intake tests. In two-bottle preference tests (quinine vs. water), rats with combined transection of the CT and GL have both higher quinine avoidance thresholds and quinine concentration-aversion functions shifted to the right, but no shifts are generally observed in rats with transections of the CT or GL alone (1, 17, 18, 24, 33, 48).[7] When tested for licking responses during brief-access trials (e.g., 10 s), rats shift their quinine concentration-response functions rightward after combined transection of the CT and GL but not after CT or GL section alone (42, 51). Although combined transection of the CT and GL raises the quinine detection threshold by ~1.5 $\log_{10}$ units, transection of either the CT or GL alone has no effect (43). On the other hand, transection of the GL alone substantially attenuates the gape response to a range of quinine concentrations as measured in taste reactivity tests in which the stimulus is intraorally infused (18, 47). Combined transection of the CT and GL reduces other aversive taste reactivity responses even further (17). All of these studies, including the present one, share the finding that CT transection alone has little or no effect on taste-guided behavior toward quinine, whereas combined transection of the CT and GL causes sizeable impairments in quinine responsiveness. These studies differ, however, in the extent to which GL transection alone causes disruptions in such behavior. Why?

One possibility relates to the extent of pre- and postsurgical experience the rats have with quinine stimulation. In the present experiment, the rats were tested in a single session in which they experienced quinine presumably for the first time in their lives. In cases for which the rat has some presurgical exposure to quinine, especially in the test setting, expectation may help guide postsurgical performance. For example, we recently tested GL-transected and control rats for their licking responses to quinine in brief-access trials but used a between-subjects design as opposed to the within-subjects design of our previously published work (42) in which we failed to find any effects of GL transection. These GL-transected rats, which had no presurgical experience with quinine, shifted their concentration-response curve ~0.5 $\log_{10}$ unit to the right (27). The extent of the shift was not as great as that seen with combined CT and GL transection in the within-subjects design but was nonetheless significant. The rats in the taste reactivity experiments, which showed marked reductions in quinine-induced gapes, also had no presurgical experience with quinine infusion (18). The detection threshold experiment, which revealed no effect of GL transection, was a within-subjects design (43). On the other hand, the two-bottle intake tests described above were all based on between-subjects design, and none revealed significant effects of GL transection. Long-term intake tests, however, are not considered optimal measures of taste responsiveness. The procedural differences between long-term

[7] Akaike et al. (1) reported that, although GL transection was without consequence, transection of the CT significantly reduced the aversion to quinine in a two-bottle preference test in rats. This finding is in contrast to similar experiments conducted in other laboratories in which CT transection was without effect. The Akaike et al. (1) study was published as a very brief report, so it is difficult to compare the methods across these studies. Also, in the Vance (48) and Jacquin (24) studies, the pharyngeal branch of the vagus nerve was transected in addition to the CT and GL.

preference tests and the method used in our study may underlie the disparity in the effects of GL transection on behavior. In long-term preference tests, the animals have access to two solutions for a prolonged period of time and testing occurs over days. In our experiment, rats were water deprived and had access to a single novel solution for only 40 min. Under test conditions involving two alternative choices during an extended period, a modest effect on stimulus intensity may not be expressed in preference behavior.

St. John et al. (42) hypothesized that the difference between the effects of GL transection on quinine responsiveness in taste reactivity experiments compared with spout licking experiments is based on the distinction between consummatory and appetitive behavior. In taste reactivity procedures, the stimulus is infused directly into the oral cavity under experimenter, not animal, control. Thus there is no appetitive component involved. In contrast, spout licking involves both appetitive and consummatory components. Perhaps the GL differentially contributes to such processes with respect to the quinine stimulus. Although this hypothesis may have some validity, it cannot solely explain the disparity of results across the various studies, because, as noted above, GL transection has been found to compromise responsiveness to quinine in some spout licking contexts but not in others.

The fact that CT transection alone was without consequence but markedly exacerbated the attenuating effect of GL transection on quinine avoidance behavior suggests that CT and GL input converge centrally such that removal of input from one nerve is compensated by the presence of input from the intact nerve on the relevant neuronal population (42, 51). The observation that impairments in taste responsiveness after combined nerve transection is greater than the sum of the effects from single nerve cuts is common (17, 18, 40, 41, 43) and has been consistently observed for quinine (33, 42, 48, 51). An alternative to the convergence hypothesis is that there may simply be a nonlinear relationship between quinine taste receptors and avoidance behavior. Transection of the CT may remove a subthreshold number of receptors for behavioral effects to be expressed, but when combined with the removal of a greater number of receptors as a result of GL transection, CT transection adds insult to an already compromised system. It should also be emphasized that this discussion is limited to procedures that measure avoidance or aversion responses to quinine and do not demand high-resolution taste quality processing. This qualification is important because St. John and Spector (44) have recently found that transection of the CT results in deficits in a presurgically conditioned operant taste discrimination task involving quinine and KCl; GL transection alone is without effect and does not enhance the CT-induced impairment. The varying profile of effects of peripheral gustatory neurotomy on taste-related behaviors underscores the likelihood that the various taste nerves are not functionally homogeneous (44).

Perspectives

The interpretive framework hypothesized can be applied to the microstructure of ingestive behavior observed under other experimental contexts. For example, Spector et al. (39) have recently found that rats presented with sucrose in a 1-h test will systematically increase their burst size (1-s pause criterion) as a function of concentration and the actual size of the burst is not influenced by the food deprivation state. This supports the idea that burst size, and thus burst termination circuitry, is heavily dependent on oral sensory cues and is not strongly modulated by deprivation state. Food deprivation enhanced total sucrose intake by lengthening the duration of the meal and concomitantly increasing the number of bursts initiated, apparently delaying satiation. Grill et al. (16) reported that although the size of the first burst (1 s pause criterion) of oral motor behavior after a 15-s intraoral glucose infusion varied directly with concentration, deprivation had no significant effect. It should be noted, however, that the failure of deprivation state to affect sucrose burst size has not been universally observed (9).[6] Nevertheless, the consistent finding that burst size monotonically increases with sucrose concentration coupled with concentration sharing monotonic relationships with *1*) responsiveness in gustatory neurons (e.g., Refs. 30 and 31), *2*) licking in brief-access taste tests (e.g., Refs. 5, 38, 40, 41, and 53), *3*) intake in sham-drinking preparations (e.g., Ref. 50), and *4*) operant bar pressing (e.g., Ref. 21) supports the claim that burst size reflects stimulus palatability (9, 11). The fact that quinine adulteration decreases burst size as shown here and by others (23) and that this effect is completely reversed by lingual gustatory denervation is in agreement with this view. Moreover, from this perspective, postingestive load would appear to alter stimulus palatability, because burst size declines on average as the ingestive episode progresses. As a cautionary note, however, in any use of this measure to infer treatment effects (e.g., drug injections, lesions) on stimulus palatability, it is important for the investigator to rule out potential motor explanations (e.g., general licking impairments).

We believe, as do others (e.g., Refs. 2, 6, 7, 9, 11, 23, and 39), that microstructural analysis of ingestive behavior provides a functional framework from which to understand the neural controls of feeding and drinking. Having described the effects of natural variations in stimulus and test conditions on ingestive microstructure, an experimenter can use the paradigm as a tool to compare and contrast exactly how neural manipulations (drugs, lesions, etc.) exert their influence on the behavior underlying the intake outcome.

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