Learning and Meal-Associated Drinking: Meal-Related Deficits Produce Adjustments in Postprandial Drinking

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LUCAS, G A., W TIMBERLAKE AND D. J. GAWLEY Learning and meal-associated drinking. Meal-related deficits produce adjustments in postprandial drinking PHYSIOI BEHAV 46(3) 361-367, 1989.—Two experiments examined the effect of meal-related water deficits on the distribution of meal-associated drinking. In the first procedure free-feeding rats received 10-, 20-, or 30-min delays between the end of a meal and the subsequent availability of postprandial water. Each delay condition remained in effect for 10 consecutive days. The primary effect of the delay was to postpone the intake of postprandial drinking. None of the delay conditions produced an increase in preprandial drinking. However, when the rats were returned to baseline following the delay conditions a pronounced rebound effect was obtained in the proportion of postprandial drinking. The second experiment followed the same general procedure except that 5-min access to water was always presented after each meal and before the postprandial water restrictions. Under this procedure the rats increased their water intake during the 5-min period when the postprandial restrictions were imposed. These findings show that meal-related water deficits can affect the timing and proportion of postprandial drinking, but provide no evidence that meal-related deficits increase preprandial drinking. The results suggest that the anticipation of meal-related water deficits may play a role in shaping the rat's postprandial drinking pattern.

Anticipation Drinking Preprandial drinking Postprandial drinking Rats

IT is well recognized that there is a close association between feeding and drinking in the rat such that a large proportion of the rat's daily water intake occurs around meals (12). However, the basis of this temporal association is complex. In the case of postprandial drinking, there is evidence to suggest that much of the drinking may be regulatory. The passage of food into the stomach postprandially results in cellular dehydration, evidenced by a 1% to 4% increase in blood osmolality, is correlated with the initiation of postprandial drinking in the rat after large meals (1,10). Postingestional fluid deficits may also result in hypovolemia, a deficit in extracellular fluid volume. The activation of the renin-angiotensin system in response to extracellular fluid deficits can also contribute to postprandial drinking (2), although the elevation of plasma angiotensin II in the rat following feeding does not appear to be sufficiently large to initiate postprandial drinking by itself (22).

In addition to the regulatory components of drinking, there is evidence that nonhomeostatic mechanisms contribute to the expression of spontaneous drinking. For example, infusions of water to preclude homeostatic deficits do not eliminate diurnal drinking rhythms nor the tendency for drinking to occur around mealtimes in the rat (6, 13, 27). As much as 30% of a free-feeding rat's daily water intake occurs shortly before ingesting a meal (7), a timing that appears to anticipate postprandial fluid deficits (8). Some preabsorptive mechanisms associated with feeding also appear to elicit drinking (15). For example, pregastric stimulation during sham feeding is sufficient to elicit drinking in rats (17,31). This effect has been attributed to the release of gastric mucosal histamine in direct response to oropharyngeal and esophageal stimulation and secondarily in response to insulin (15,18). In fact, Krahl (14,20) suggests that over half of the postprandial drinking in the free-feeding rat may be attributed to the activation of peripheral histamine receptors. Taken together, these studies suggest that part of the rat's spontaneous meal-associated drinking occurs in anticipation of, and thus serves to attenuate, meal-related fluid deficits (12, 13, 16, 23).

The suggestion that some nonhomeostatic drinking mechanisms associated with feeding may anticipate fluid deficits suggests the possibility that some meal-associated drinking may depend on learning to anticipate water deficits. For example, associations could be formed between environmental or behavioral cues and a subsequent deficit state, such that the cues would come to predict the deficit condition and thereby motivate drinking before the deficit occurred. In support of this possibility, Weisung (32) reported that previously neutral stimuli (e.g., odors) repeatedly associated with thirst inducing treatments (e.g., subcutaneous formalin injections) come to elicit drinking in the absence of the treatment. Such learning might account for preprandial drinking, and contribute to the initiation of postprandial drinking as well.

However, it is not clear whether learning is necessary to account for the rat's pattern of meal-associated drinking. Toates
(29) has suggested that the timing of meal-associated drinking may depend more on motivational cross-linkages between hunger and thirst rather than on learning. In the case of preprandial drinking this may occur because drinking to relieve thirst would disinhibit hunger [previously inhibited by thirst (24,25)]. Such a linkage would favor a short preprandial time course between drinking and subsequent feeding. The postprandial time course may be influenced by a sequence of events, including anticipatory mechanisms partly mediated by peripheral histamine (16), and homeostatic mechanisms responding to cellular and extracellular fluid deficits. Toates (29) reported that in computer simulations relatively weak motivational-linkages of this sort could effectively couple free-feeding and free-drinking rhythms, producing the familiar meal-associated drinking pattern.

However, even within such a system of coupled feeding and drinking rhythms learning might still contribute to the anticipation of deficits, at least at the level of adjusting the timing or distribution of meal-related drinking. An example consistent with this type of learned adjustment was reported by (8), who found that rats shifted from a low protein diet to a protein rich diet gradually increased the amount of meal-associated drinking to accommodate the greater fluid requirements associated with the high protein diet. However, in that study the distribution of meal-associated drinking was not reported. Further, the rat's overall need for water was effectively increased by the change in diet, so the changes obtained with that procedure may not have been specific to meal-related water deficits.

EXPERIMENT 1

The aim of the present study was to evaluate the extent to which learning about meal-related deficits would affect the rat's pattern of meal-associated drinking. Recent work in our laboratory has shown that the rat is able to anticipate marked restrictions on access to water under some conditions (9). The present study examined the effects of repeated exposure to meal-related deficits on the rat's ability to anticipate postprandial restrictions on water access. Because systemic water is needed for the digestion of dry food, the postprandial restriction of water was expected to increase the probability that the rats would encounter a temporary homeostatic fluid deficit following feeding. Of major interest was whether the rats would learn to change their pattern of meal-associated drinking in anticipation of this deficit.

METHOD

Subjects

Four female Sprague-Dawley albino rats were obtained from a local breeding colony (Indiana University). The rats had been adapted to living in the experimental test chambers for 6 months prior to the start of this procedure. During part of this time they were exposed to a schedule of reduced rate of access to water. They were 270 days old at the start of the present procedures.

Apparatus

The four test chambers each consisted of a 30 × 30 × 30 cm main compartment in which food, water, a sniff hole, and a wood block were available. An Acme running wheel (36 cm in diameter × 14 cm wide) was located adjacent to the left side of the main chamber. A small L-shaped nest enclosure approximately 20 cm deep × 10 cm wide × 13 cm high was accessible through an opening in the wall opposite the feeder wall. A more detailed description of the apparatus is given in (21).

The feeder opening (3.2 cm wide × 5 cm high) was recessed 2.5 cm into the left side of the front wall. Food pellets (94 mg rodent pellets, Bio Serv, Frenchtown, NJ) were delivered into a V-shaped trough at the bottom of the opening. An eat was recorded whenever a pellet was removed from the feeding trough for a least 0.5 sec. This criterion prevented occasional nosing or pawing movements that temporarily displaced the pellet from being mistaken for eats.

The water opening (3.2 cm wide × 5 cm high) was recessed 2.5 cm into the right side of the front wall. A 1.0 cm diameter brass bowl was situated at the bottom of this opening. Water was delivered into the bowl in 0.05-ml units through a 2-mm opening in the bottom of the bowl via a solenoid operated valve. Drinking was detected by the interruption of a continuity circuit between the bowl and the water source. Continuity was broken when the water was depleted to less than 10% of the 0.05 ml volume. Because the water could be repleted within 0.1 sec, this procedure provided a continuous source of water as the animal drank while measuring intake to a 0.05-ml resolution.

Each test chamber was enclosed in a sound attenuating shell. The four chambers were further isolated within a dedicated temperature controlled room maintained at 21°C (± 1°C) and 60% (± 10%) relative humidity. Exhaust fans vented to the outside to isolate the odor cues from each box.

A 12:12 hr light:dim cycle was in effect at all times. An F8T5-CW fluorescent bulb located in the ceiling of the main chamber provided direct lighting of the chamber and wheel area. Illuminance measured at the center floor level of the main chamber was 30 lux using an Electro-Optics (Salem, MA) model 550 radiometer/photometer. When the houselight was off an indirect light source located in ceiling of the experimental room provided dim ambient light (0.02 lux) through the observation window.

Control of all chambers was arranged by an IBM-personal computer and locally constructed solid state interface located in a separate room. All contingencies and timing were managed by Spyder Systems Conman software (Bloomington, IN) operating at 0.1 sec resolution.

Procedure

Equipment maintenance. Routine cleaning, replenishing of pellets, maintenance and adjustments of equipment was performed every two to three days. These maintenance periods were distributed irregularly early in the phase period (when the rat typically remained in the nest). During these periods the rat was restricted to the nest by manually closing the nest door. To minimize the effects of handling, body weight was assessed by removing the nest and weighing the rat and nest together (after removing the waste pan). Maintenance procedures typically took less than 5 min per subject. The nest doors were programmed to reopen 15 min after all maintenance was completed.

Postprandial restrictions. During the first 30 days each subject was given free access to food and water. Baseline measures of drinking were based on the last 6 days of this condition (Predelay baseline). Following the predelay baseline access to water was restricted for some minimum delay following eating. This procedure was implemented by resetting the delay each time a pellet was removed. Three delay conditions, 10, 20, or 30 min following a meal, were imposed in ascending order. Each delay condition was in effect for 10 consecutive days. Following the three delay conditions the subjects were returned to baseline for 14 days (Postdelay baseline).

Data Analysis

In order to establish the distribution of drinking relative to
feeding, episodes of feeding and drinking were digitally recorded on a 32 channel event log (synthesized in software) stepped at 4-sec intervals. These records were subsequently used to determine the number of 4-sec periods of feeding and drinking that occurred during consecutive 1-min bins across days. Each 1-min bin in which drinking occurred was scored to determine the time to the nearest preceding meal and the nearest following meal in a manner similar to Kissileff (12). If the time between drinking and the following meal was shorter, then those drinks were assigned to the preprandial distribution. If the time between drinking and the preceding meal was shorter or equal, then those drinks were assigned to the postprandial distribution. If the shortest time between feeding and drinking exceeded 30 min then those drinks were assigned to the nonprandial distribution. A small number of cases (less than 2%) in which drinking and feeding occurred during the same minute were categorized as prandial drinking and were excluded from subsequent analyses.

In order to provide stable estimates of intake for each condition subject means were averaged across the last 6 days of the condition. The group means and standard errors reported for each condition were based on the average of the 4 subject means for that condition. Statistical comparisons across conditions were based on within-subjects comparisons using repeated-measures ANOVAs and subsequent contrast tests. The analyses of the recovery of drinking patterns in the postdelay baseline were based on means across successive 2-day blocks. The changes in intake across these blocks were analyzed using a repeated measures ANOVA and subsequent contrast tests comparing the measure in each 2-day block with the mean of the respective measure form the predelay baseline.

RESULTS

Imposing a delay between the end of the meal and the availability of water had no systematic effect on total daily intake. Table 1 shows the mean food and water intake and the water to food ratio (ml/g) averaged across the last 6 days of each condition. An ANOVA for each of these measures indicated no significant difference in daily food or water intake across conditions, all

<table>
<thead>
<tr>
<th>Condition</th>
<th>Measure</th>
<th>Pre-BL</th>
<th>Delay-10</th>
<th>Delay-20</th>
<th>Delay-30</th>
<th>Post-BL</th>
</tr>
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<tr>
<td>Food (g)</td>
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<td>19.8</td>
<td>20.9</td>
<td>19.6</td>
<td>20.6</td>
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<tr>
<td>(SE)</td>
<td>(1.6)</td>
<td>(1.8)</td>
<td>(1.9)</td>
<td>(1.9)</td>
<td>(1.9)</td>
<td></td>
</tr>
<tr>
<td>Water (ml)</td>
<td>25.3</td>
<td>24.2</td>
<td>24.6</td>
<td>22.9</td>
<td>25.3</td>
<td></td>
</tr>
<tr>
<td>(SE)</td>
<td>(2.3)</td>
<td>(2.1)</td>
<td>(3.2)</td>
<td>(1.7)</td>
<td>(3.8)</td>
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<tr>
<td>W/F Ratio</td>
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<td>1.24</td>
<td>1.23</td>
<td>1.31</td>
<td></td>
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<tr>
<td>(SE)</td>
<td>(0.26)</td>
<td>(0.19)</td>
<td>(0.27)</td>
<td>(0.23)</td>
<td>(0.34)</td>
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</tr>
</tbody>
</table>

Pre-BL and Post-BL refer to the pre- and postdelay baselines conditions respectively. Numbers in parentheses are standard errors of the mean.
The primary effect of imposing a delay between feeding and drinking was to postpone drinking until after the delay. At longer delays this procedure had the effect of significantly increasing the proportion of nonprandial drinking, $F(4,12) = 23.1, p<0.01$. However, much of this drinking might still be considered postprandial in origin. Even under the 30-min delay condition, more than half of the nonprandial drinking occurred in the first 10 min following the end of the delay.

The return to baseline following the 30-min delay condition revealed a persistent change in the pattern of meal-associated drinking. In order to illustrate this change the proportion of preprandial, postprandial, and nonprandial drinking during the postdelay baseline was scored in 2-day blocks, and compared with the appropriate measure from the final block of the predelay baseline. These comparisons are summarized in Fig. 2. As shown, the proportion of postprandial drinking varied significantly across blocks, $F(7,21) = 9.80, p<0.01$. Subsequent contrast tests indicated that postprandial intake was elevated across the first 4 blocks (8 days) of the recovery baseline (all $p's<0.05$). The proportion of postprandial drinking also varied significantly across blocks, $F(7,21) = 4.73, p<0.01$. Contrast tests showed that postprandial intake was suppressed below the predelay baseline across the first 3 blocks (6 days) of the recovery period (all $p's<0.05$). However, both preprandial and postprandial measures closely recovered their predelay values by the end of the recovery period. The proportion of nonprandial drinking returned to the predelay baseline level within the first 2-day block and did not vary significantly across blocks, $F(7,21)<1.0, p>0.5$.

**DISCUSSION**

We found no evidence that postprandial restrictions on access to water affected overall intake, or increased the proportion of preprandial drinking. Postprandial restrictions did produce a temporary increase in the motivation to drink, as seen in the increased rate of intake following the end of the delay period. Note, in particular, the rapid recovery of water intake that occurred when access to water was reinstated in the 10- and 20-min delay conditions (Fig. 1). Such compensatory changes in the rate of water intake have previously been reported following shorter periods of restriction on access to water [see (9)]. As an aside these data suggest that the postprandial effects on drinking may well extend over 30 min following feeding [cf. (26)]. But more importantly, this pattern suggests that the postprandial motivation to drink was not a transient effect, but was sustained across the delay.

Two characteristics of the drinking pattern that emerged at the beginning of the postdelay baseline suggested that this increase in postprandial drinking motivation was in part a learned adjustment that extinguished across the postdelay baseline. First, inspection of individual drink bout revealed that for three of the four subjects the longest drinking bout of the recovery period occurred during the first night of postdelay baseline. And second, the initial 8 days of this phase were characterized by a marked rebound effect in which the proportion of postprandial drinking significantly exceeded the proportion found in the predelay baseline. However, the proportion of preprandial and postprandial drinking found in the predelay baseline was recovered after 14 days of postdelay baseline.

**EXPERIMENT 2**

The results of the previous study provided no evidence that the rats learned to change their proportion of preprandial drinking in anticipation of meal-related deficits. But, the data did suggest that the rats learned to adjust their pattern of postprandial drinking following experience with meal-related deficits. However, in the previous study any changes in the postprandial motivation to drink could not be expressed during the restriction conditions. Thus, it was not possible to determine if the apparent changes in postpran-
TABLE 2
DAILY FOOD AND WATER INTAKE ACROSS CONDITIONS IN EXPERIMENT 2

<table>
<thead>
<tr>
<th>Condition</th>
<th>Measure</th>
<th>Pre-BL</th>
<th>Delay-10</th>
<th>Delay-20</th>
<th>Delay-30</th>
<th>Post-BL</th>
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<tr>
<td></td>
<td>Food (g)</td>
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<td>17.5</td>
<td>17.9</td>
<td>16.6</td>
</tr>
<tr>
<td></td>
<td>(SE)</td>
<td>(0.5)</td>
<td>(0.3)</td>
<td>(0.6)</td>
<td>(1.0)</td>
<td>(0.5)</td>
</tr>
<tr>
<td></td>
<td>Water (ml)</td>
<td>23.1</td>
<td>22.6</td>
<td>23.1</td>
<td>23.2</td>
<td>23.2</td>
</tr>
<tr>
<td></td>
<td>(SE)</td>
<td>(1.9)</td>
<td>(2.1)</td>
<td>(2.7)</td>
<td>(2.1)</td>
<td>(1.9)</td>
</tr>
<tr>
<td></td>
<td>W/F Ratio</td>
<td>1.28</td>
<td>1.29</td>
<td>1.31</td>
<td>1.30</td>
<td>1.39</td>
</tr>
<tr>
<td></td>
<td>(SE)</td>
<td>(0.09)</td>
<td>(0.11)</td>
<td>(0.11)</td>
<td>(0.09)</td>
<td>(0.09)</td>
</tr>
</tbody>
</table>

Pre-BL and Post-BL refer to the pre- and postdelay baselines conditions respectively. Numbers in parentheses are standard errors of the mean.

dial motivation would have been expressed in anticipation of the postprandial restriction. The present study addressed this question by providing a 5-min period of free access to water following meals. Postprandial restrictions on access to water were imposed at the end of this 5-min access period. If the results of Experiment 1 represent a learned change in postprandial motivation, then this change should be expressed during the 5-min access period in anticipation of the postprandial restriction.

METHOD

Subjects

Four female Sprague-Dawley albino rats served as subjects. These rats had been adapted to living in the experimental test chambers for 7 months prior to the start of this procedure. The subjects had previous experience with various schedules of food delivery, but no experience with water restriction. They were approximately 300 days old at the start of the present procedure.

Apparatus and Procedure

The same four test chambers described above were used. The general procedures were identical to those used in Experiment 1, except as noted below.

During the first 30 days each subject was given free access to food and water. Baseline measures of drinking were based on the last 4 days of this condition. Following the predelay baseline access to water was restricted beginning 5-min after the end of each meal. That is, for the first 5-min after the end of each meal water was always freely available, but after this period access to water was restricted for some minimum delay period. Once the postprandial water restriction began it remained in effect for its full duration. Three delay conditions, 10, 20, or 30 min were imposed in ascending order. Each delay condition was in effect for 10 consecutive days. Following the three delay conditions the subjects were returned to a postdelay baseline for 10 days.

Data collection and analysis procedures were the same as those used in the previous study, except that the measures of terminal performance were based on means across the last 4 days of each condition. Changes in intake across conditions were analyzed using repeated measures ANOVAs and subsequent contrast tests.

RESULTS AND DISCUSSION

As in the previous study, imposing a restriction on access to water following each meal had no systematic effect on total daily food or water intake. Table 2 presents the mean food and water intake and the water to food ratio (ml/g) averaged across the last 4 days of each condition in Experiment 2. An ANOVA for each of these measures revealed no significant difference in daily intake across conditions, all F's(4,12)<1.0, all p's>0.4.

The percentage of drinking occurring in preprandial and postprandial time periods is shown across conditions in Fig. 3. Restricting access to water after 5-min following the end of each meal produced relatively minor changes in the overall distribution of meal-associated drinking. Neither the percentage of preprandial drinking during the 30 min preceding feeding, nor the percentage of nonprandial drinking (not shown) occurring more than 30 min away from feeding varied significantly across conditions, both F's(4,12)<2.5, p's>0.1. In fact the change in the percentage of total postprandial drinking across conditions was not significantly different despite the postprandial restrictions on access to water, although this measure approached significance, F(4,12)=2.80, p=0.075.

However, the percentage of drinking occurring during the 5-min access period immediately following each meal increased
significantly when the postprandial restrictions on access to water were imposed. F(4,12) = 4.85, p<0.02. Subsequent contrast tests showed that the percentage of water intake in the 5-min access period was significantly elevated above the predelay baseline intake for the 10-min and 20-min delay conditions (all p's<0.05), but not for the 30-min delay. The lack of significance at the 30-min delay occurred because for two of the rats the proportion of drinking during the 5-min access period decreased and became irregular at this delay, increasing on some days but not on others. However, for the other two subjects intake during the 5-min access period in this condition remained elevated and stable.

GENERAL DISCUSSION

Despite increasingly severe restrictions on the availability of postprandial water across a 30-day period, our rats did not learn to increase their preprandial intake of water in anticipation of these restrictions. In this regard, the present results contradict the hypothesis that preprandial drinking develops in learned anticipation of meal-related deficits. One reason that the rats might not have learned to show preprandial anticipation is that the preprandial period may not be a discriminable cue. For example, if the preprandial timing of drinking results form a disinhibition of hunger after drinking (24), then there would be no increase in feeding motivation to distinguish the timing of preprandial drinking as "preprandial." In effect, the rat may not be able to learn about the preprandial period because at the time of its occurrence the rat cannot discriminate a preprandial drinking bout from any other nonprandial drinking bout.

However, there appears to be little evidence that preprandial drinking is enhanced at times when food clearly can be anticipated. For example when food is restricted to one period per day robust anticipatory patterns have been reported for wheel running and barpressing, but not for drinking [e.g., (4,5)]. Thus, the weight of the findings for preprandial drinking, including the present data, are more consistent with Toates' (29) proposal that preprandial drinking depends on motivational linkages between thirst-reduction and eating rather than depending on a learned anticipation of meal-related deficits.

In contrast to preprandial drinking, our results suggest that rats can adjust both the timing and the relative amount of postprandial drinking in anticipation of meal-related water deficits. However, these changes in postprandial drinking were brought about by changing the proportion of drinking occurring in the postprandial period rather than changes in overall daily water intake. Thus it appears that the learned adjustments were limited to changes affecting the timing or initiation of drinking and did not alter the homeostatic regulation of water intake. Such changes would be expected if learning served to fine tune the timing of drinking in anticipation of homeostatic deficits. In this regard, such learned adjustments in drinking should not be considered inconsistent with the homeostatic regulation of drinking, but rather supplementary to it.

Although the present data cannot identify the specific mechanisms involved in the learned modification of drinking patterns, it seems likely that any learned changes in meal-related drinking would be related to the "cephalic" phase (3,30) of physiological responses to food-related cues and be mediated by the vagus. The vagus is known to play a role in spontaneous meal-related drinking (19,28). Anticipatory drinking elicited by sham feeding also depends on an intact abdominal vagus (15). Further, vagal efferents have been implicated in the release of gastric mucosal histamine (11) and pancreatic insulin (3) during feeding, which in turn have been implicated in the control of meal-associated drinking (15,18). And there is evidence that the cephalic phase of both histamine release (30) and insulin release (33,34) are susceptible to conditioning.

In summary, our results suggest that the rat can learn to anticipate meal-related restrictions on access to water, and that this learning can produce a change in the distribution of meal-associated drinking. These findings suggest that the rat's pattern of meal-related drinking is not determined exclusively by negative feedback signals and that the anticipation of restrictions on access to water may play an important role in shaping the rat's spontaneous pattern of drinking. However, the anticipatory changes that we obtained here were limited to postprandial drinking. We found no evidence that learning about meal-related deficits changed the distribution of preprandial drinking. As such the present results favor the conclusion that preprandial drinking probably depends more on reorganized motivational linkages between feeding and drinking than on a learned anticipation of water deficits.

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