Calcium deprivation alters gustatory-evoked activity in the rat nucleus of the solitary tract

STUART A. MCCAUHEY AND MICHAEL G. TORDOFF
Monell Chemical Senses Center, Philadelphia, Pennsylvania 19104

Received 8 February 2001; accepted in final form 22 May 2001

McCaughey, Stuart A., and Michael G. Tordoff. Calcium deprivation alters gustatory-evoked activity in the rat nucleus of the solitary tract. Am J Physiol Regulatory Integrative Comp Physiol 281: R971–R978, 2001.—Calcium-deprived rats develop a compensatory appetite for substances that contain calcium. To investigate the role of gustatory factors in calcium appetite, we recorded the extra-cellular activity of single neurons in the nucleus of the solitary tract of calcium-deprived and replete rats. The activity evoked by a broad array of taste stimuli was examined in 51 neurons from replete rats and 47 neurons from calcium-deprived rats. There were no differences between the groups in the responses of all neurons combined. However, neurons with sugar-oriented response profiles gave significantly larger responses to 3, 10, and 100 mM CaCl₂ in the calcium-deprived group than did corresponding cells in the replete group. This difference in taste-evoked responding may underlie an increase in the palatability of CaCl₂ and, in turn, contribute to the expression of calcium appetite.

palatability; sodium; taste; electrophysiology; nucleus tractus solitarii

THE INCREASED APPETITE for calcium produced by calcium deprivation is a complex behavior mediated by several physiological and neural factors, including changes in taste sensitivity (8, 32). Calcium-deficient rats ingest more CaCl₂ than do replete controls, even when the concentration series of CaCl₂. Responses to 30–300 mM CaCl₂ are significantly higher in deprived than in replete animals, whereas responses to 30–300 mM CaCl₂ are significantly reduced (20).

The effects of calcium status on calcium taste perception may parallel the effects of sodium status on sodium taste perception. Sodium appetite is evident within the first minute of access to NaCl (13, 27) and under sham-drinking conditions (16, 36, 45). Sodium depletion is also accompanied by changes in gustatory neural responding to the taste of NaCl. Recordings from gustatory-sensitive neurons in the CT, nucleus of the solitary tract (NST), and parabrachial nucleus indicate that responsiveness to hypertonic NaCl declines following sodium depletion, and the effect is largest in the subset of neurons that are tuned most narrowly to sodium salts (11, 21, 28, 37). There is also evidence that sodium deprivation increases the response to NaCl of NST neurons that are most sensitive to sugars (21). This does not necessarily mean that NaCl tastes sweet to a sodium-deprived rat, but it may taste unusually palatable, and there is behavioral data to support this view. In replete rats, intraoral infusion of 0.5 M NaCl results in a combination of ingestive and aversive reflexive facial reactions. However, when rats are sodium deprived, infusion elicits only ingestive facial reactions, such as those seen after infusion of highly palatable stimuli like sucrose (3). Sodium-deprived rats will also forego mild brain stimulation reward to consume NaCl, a behavior that replete rats demonstrate for sucrose but not NaCl (10). Sodium appetite may also involve an increase in dopaminergic activity in the nucleus accumbens (35), which is normally associated with rewarding stimuli such as sugars.

Calcium deprivation affects the ingestion of compounds that do not contain calcium (8, 9). In some cases, deprived rats increase their intake (e.g., NaCl, HCl, MgCl₂, SrCl₂), and in others they decrease it (e.g., sucrose, sodium phosphate) relative to controls. Little is known about the mechanisms underlying these behavioral differences, although for some compounds they are observed under sham-drinking conditions and/or in short-term tests (9, 25), and thus changes in gustatory factors may be involved.

We investigated the role of taste in calcium appetite by measuring single-unit NST responses in calcium-deprived and replete rats. In all neurons, gustatory-evoked activity was recorded in response to a broad stimulus array that included representatives of the basic taste qualities, compounds that include calcium, and noncalcium stimuli that are consumed differentially by replete and calcium-deprived rats. In a subset of these neurons, responses were also recorded to a concentration series of CaCl₂.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
METHODS

Subjects and Diets

Subjects were 119 male Sprague-Dawley rats [Charles River, Crl-CD(SD)IGS BR], age 21–23 days old when they arrived in the laboratory. They were kept at ~23°C on a 12:12-h light-dark cycle, and they had ad libitum access to food and deionized H2O. Sixty-five rats (calcium-deprived group) were maintained on a powdered diet containing 25 mmol Ca2+/kg for 6–11 wk to induce calcium deficiency, and 54 rats (replete group) were maintained on a 150-mmol Ca2+/kg diet for the same time. The commercially prepared diets were based on a calcium-free version of the AIN-76A diet (1), to which enough calcium carbonate was added to generate 25- or 150-mmol Ca2+/kg diet (Dyets, Bethlehem, PA; cat. no. 113059 and 113060). The calcium content of the 150-mmol Ca2+/kg diet is effective at stimulating a calcium appetite and reducing plasma calcium concentrations, without greatly compromising growth or survival rates (8, 9, 41, 43). It was chosen instead of a calcium-free diet to facilitate comparison with prior studies (9, 20) and to allow for a range of time over which deprived animals would be in approximately the same state.

Neural Recording

On the day of electrophysiological recording, each rat was weighed, and a 40-µl blood sample was taken from the tip of its tail. Blood samples were centrifuged and the plasma frozen, and the plasma total calcium concentration was determined later using a colorimetric method, in which 7.5 µl of plasma were mixed with 300 µl of working solution containing o-cresolphthalein complexone (kit no. 587-A; Sigma Chemical, St. Louis, MO).

The rat was then anesthetized using ketamine HCl (100 mg/kg im) followed by chloral hydrate (48 mg ip, with further doses as necessary). A tracheotomy was performed to prevent suffocation, and a fistula was inserted into the esophagus to prohibit ingestion of stimuli. The head was secured in a nontraumatic head holder (14) to avoid damaging the CT. A 5 × 5-mm section of skull was removed, and a portion of the cerebellum was aspirated to expose the surface of the medulla. Body temperature was maintained at 35–37°C, and subcutaneous electrodes were used to monitor heart rate.

The activity of a single unit in the rostral NST was isolated using a glass microelectrode filled with 1.6 M potassium citrate and with a tip diameter of ~1 µm (Z = 5–10 MΩ at 1 kHz). Typical recording coordinates were 2.7 mm anterior to obex, 1.7 mm lateral to the midline, and 1 mm ventral to the surface of the medulla. The signal was amplified, filtered, displayed on an oscilloscope, and stored on videotape for off-line analysis.

Presentation of Taste Stimuli

When the activity of a single neuron was isolated, responses were recorded to a broad stimulus array that included the compounds listed in Table 1. If the cell remained well isolated after this array was applied, a concentration series of CaCl2 ranging from 0.01 to 300 mM in half-log molar steps was presented in ascending order. All stimuli were mixed in distilled water, with the exception of glucose and sucrose, to which 10% tap water was added to ensure activation of an automatic stimulus-onset marker.

The stimulus delivery procedure followed that of Chang and Scott (7). Five milliliters of each stimulus were presented at room temperature and at a rate of 1 ml/s. Solutions were delivered as a spray that contacted the entire tongue and oral cavity. Stimulus presentations were separated by at least a minute and were followed by at least 25 ml of deionized water as a rinse. The stimulus array was given in a semirandom order, in which compounds with similar taste qualities were not presented consecutively to avoid adaptation effects. Some stimuli were presented more than once to assess the stability of neural responding.

Three stimuli (30, 100, and 300 mM CaCl2) were applied first as part of the stimulus array and then again during the CaCl2 concentration series. Statistical analyses indicated that there was no difference between the first and second responses to these stimuli in either the replete or calcium-deprived groups. Therefore, for each cell, the average response across all presentations of these stimuli was used in all analyses.

Analysis

Action potentials were counted with a window discrimination program created using the DasyLab software package (Dasytec USA, Amherst, NH). Activity was monitored for 3 s before stimulus onset to determine spontaneous firing rate and for 5 s afterwards to determine evoked firing. Responses were then expressed as net spikes per second (evoked – spontaneous). A detailed analysis of the temporal patterns of responding was also conducted by assigning evoked spike counts to 50 bins of 100 ms each. However, these results did not reveal any differences between the replete and calcium-deprived groups, and so they are not presented.

Comparisons were made between the two groups on the following measures: spontaneous rate, evoked rates in response to each stimulus, and breadth of tuning. T-tests, repeated-measures ANOVAs, and post hoc comparisons were performed using the Statistica package, with $P < 0.05$ considered significant.

Neurons were assigned to subgroups using cluster analysis. Within each group of rats, correlation coefficients were calculated between every pair of cells based on their profiles of responding across the four basic stimuli (100 mM NaCl, 10 mM HCl, 10 mM quinine HCl, and 500 mM sucrose). The resulting matrix of correlations was then subjected to cluster analysis, with the result being a dendrogram indicating the relative similarity among response profiles. Clusters of neu-

Table 1. Taste stimuli in the broad stimulus array and their abbreviations

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration, mM</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>100</td>
<td>100 Na</td>
</tr>
<tr>
<td>Sodium phosphate (dibasic)</td>
<td>100</td>
<td>NP</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>10</td>
<td>H</td>
</tr>
<tr>
<td>Citric acid</td>
<td>10</td>
<td>Ci</td>
</tr>
<tr>
<td>Glucose</td>
<td>1000</td>
<td>G</td>
</tr>
<tr>
<td>Sucrose</td>
<td>500</td>
<td>S</td>
</tr>
<tr>
<td>Quinine hydrochloride</td>
<td>10</td>
<td>Q</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>100</td>
<td>K</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>100</td>
<td>Mg</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>100</td>
<td>NH</td>
</tr>
<tr>
<td>Calcium lactate</td>
<td>100</td>
<td>L</td>
</tr>
<tr>
<td>Strontium chloride</td>
<td>100</td>
<td>Sr</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>30</td>
<td>30 Ca</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>100 Ca</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>300 Ca</td>
</tr>
</tbody>
</table>

Downloaded from http://ajpregu.physiology.org/ by 10.220.33.1 on October 6, 2016
neurons were identified visually, and ANOVAs and post hoc comparisons were performed to confirm that groups differed significantly on responses to their defining (best) stimuli. Each statistically identifiable group was then investigated independently for an effect of calcium deprivation.

Comparisons between stimuli were also made within each group of subjects. Correlation coefficients were calculated between each pair of stimuli, based on the similarity of the profiles they generated across all neurons, and the resulting correlation matrices were used to perform multidimensional scaling.

RESULTS

Effectiveness of Dietary Calcium Deprivation

Consistent with previous work (8, 46), calcium-deprived rats weighed significantly less than did replete rats [429 ± 8 and 507 ± 8 g, respectively; t(117) = 6.6, P < 0.001] and had significantly lower plasma total calcium concentrations [2.50 ± 0.05 and 2.79 ± 0.02 mM, respectively; t(117) = 4.8, P < 0.001]. Maintenance on the low-calcium diet was therefore effective at inducing calcium deficiency.

Electrophysiology

The activity of 98 neurons was recorded successfully following application of the broad stimulus array. Fifty-one of these cells were from replete rats, and 47 cells were from the calcium-deprived group.

Spontaneous firing rate. There was no significant difference in mean (±SE) spontaneous firing rates of neurons in the replete and calcium-deprived groups (14.6 ± 1.6 and 16.9 ± 1.6, respectively). There were also no differences in spontaneous rate when corresponding subgroups of neurons were compared.

Response reliability. For each group of subjects, we identified instances in which a stimulus was presented more than once for a given cell. A Pearson product-moment correlation was then calculated between the responses to the first and second stimulus applications. In the replete group, the value of the coefficient was +0.91, and in the calcium-deprived group, it was +0.90, indicating that there was a high degree of stability in the recording preparations.

Breadth of tuning. For each cell, a breadth of tuning metric was calculated according to the method of Smith and Travers (38). This metric assesses the distribution of responding across the four basic stimuli, with a value of one resulting from the broadest possible tuning (25% of the total response to each stimulus) and a value of zero from the narrowest (100% of the total response to one stimulus). In the replete group, the mean (±SE) breadth of tuning was 0.82 ± 0.02, and in the calcium-deprived group, it was 0.80 ± 0.02. As is typical for the rat NST, the distributions for both groups were negatively skewed, so they were compared with a Mann-Whitney U test. The groups did not differ significantly on breadth of tuning across all cells or in any of the corresponding subgroups of neurons.

Responses of all neurons. Figure 1 shows the responses across all neurons to the broad stimulus array. Although there was a significant group × chemical interaction, F(15,1,440) = 2.6, P < 0.001, post hoc t-tests did not reveal significant differences between the replete and calcium-deprived groups in response to any individual stimulus.

Thirty-two neurons in the replete group and 29 neurons in the calcium-deprived group were tested with the CaCl2 concentration series. Neural responsiveness to CaCl2 across all cells did not differ between the groups (Fig. 2).

Responses of neural subgroups. Neurons were assigned to subgroups using cluster analysis (see METHODS; Fig. 3). As is typically found for rat NST neurons, there were clusters of cells that were generally most responsive to sucrose (S-cells), NaCl (N-cells), and HCl (H-cells). The small number of quinine-best cells (one per group) prevented any meaningful comparison between groups of subjects, and in replete rats, there were four additional neurons that were separate from the other clusters and were considered outliers. In the calcium-deprived group, there was a larger percentage of N-cells and smaller percentages of S- and H-cells than in the replete group. This difference in the distribution of neurons into subgroups was significant (χ² = 15.6; P < 0.001).

There was no significant difference in neural responding to the broad stimulus array between replete and calcium-deprived subjects within any of the subgroups (Fig. 4). However, in the CaCl2 concentration series, significant differences were found in the S-cells (Fig. 5). Neural responding was generally greater in the calcium-deprived rats [main effect of group, F(1,18) = 4.8, P < 0.05], although this effect was larger for some concentrations than others [group × chemical interaction, F(9,162) = 2.5, P < 0.05]. Post hoc comparisons indicated that responses were significantly greater to 3, 10, and 100 mM CaCl2 in the calcium-deprived group.
deprived group \( t(18) = 2.1, P < 0.05 \) in all cases, and the results approached significance \( P < 0.1 \) for 1 and 30 mM CaCl\(_2\). For the N- and H-cells, there was no difference in responding to CaCl\(_2\) between the groups.

**CaCl\(_2\) response thresholds.** For each cell in which the CaCl\(_2\) concentration series was presented, the neural response threshold was defined as the lowest concentration that caused a net response that was at least 2.33 SD greater than that cell’s mean spontaneous rate \( P < 0.01 \), one-tailed). Values were then expressed logarithmically because of the large range that resulted. Response thresholds in S-cells were significantly lower in calcium-deprived than in replete rats, \( t(18) = 3.3, P < 0.005 \), but there were no group differences across all neurons or in N- or H-cells (Table 2).

**Across-neuron profiles of responding.** In each group of subjects, multidimensional scaling was used to compare stimuli based on their profiles of responding across all neurons (see METHODS). The resulting multidimensional spaces (Fig. 6) are typical of those found for rat NST activity. Stimuli with similar taste qualities (as described by humans) are located near each other, indicating a high degree of similarity in their across-neuron profiles of responding. The relative location of the calcium-containing stimuli was similar in the replete and calcium-deprived groups. Additional spaces were also generated that incorporated responses to all concentrations of CaCl\(_2\) and that likewise appeared similar in the two groups of subjects (not shown).

**DISCUSSION**

Richter and Eckert (33) suggested that changes in calcium appetite were due to “chemical changes in the taste mechanisms in the oral cavity, making the calcium more desirable.” Although we cannot pinpoint the underlying mechanisms, the results of our experiment add to a growing body of evidence that is consistent with this hypothesis (see Introduction). NST neurons with sugar-oriented response profiles (S-cells) had lower thresholds and gave significantly larger responses to oral CaCl\(_2\) in rats that had been calcium deprived than in replete controls. This appeared to be a specific effect because calcium deprivation did not influence neural responding to other stimuli or in other subgroups of neurons.

The elevated responding to CaCl\(_2\) observed in S-cells may contribute to an increased palatability for this stimulus when rats are calcium deprived. A similar effect on NST responding has been observed in the S-cells of sodium-deprived rats, which gave significantly larger responses to NaCl than did corresponding cells in sodium-replete controls (21). Although it is not known whether NaCl tastes sweet to sodium-deprived rats, there is other evidence that NaCl may activate neural pathways that normally are activated only by highly palatable stimuli such as sugars (see Introduction). Experiments using these techniques have not been conducted in calcium-deprived rats, but the ra-

![Fig. 2. Mean (±SE) net responses to the CaCl\(_2\) concentration series across all neurons in the replete and calcium-deprived groups. Only neurons that received the entire concentration series are included, so the values for 30–300 mM CaCl\(_2\) vary slightly from those shown in Fig. 1.](image-url)

![Fig. 3. Dendrograms resulting from cluster analysis, in which cells from replete (A) or calcium-deprived (B) rats were compared based on their profiles of responding across the four basic chemicals. Letters along the bottom of each dendrogram indicate which of the basic stimuli evoked the largest response from each neuron (N, 100 mM NaCl; other abbreviations as in Fig. 1). Dark horizontal bars indicate the extent of the three neural subgroups (S, S-cells; N, N-cells; H, H-cells).](image-url)
pidity and avidity with which calcium appetite is expressed suggest that CaCl₂ may be highly palatable to a calcium-deprived rat. If this is the case, one possibility is that the increased responding to CaCl₂ that we observed in S-cells may contribute to increased neural activation of brain areas, such as the lateral hypothalamus and nucleus accumbens, that are thought to be involved in hedonic aspects of feeding and that mediate the ingestion of palatable stimuli.

Although calcium-deprived rats had significantly reduced plasma ionized calcium levels, there was no impairment in the ability of NST neurons to give robust responses or in their spontaneous firing rates. Only responses to certain concentrations of CaCl₂ were affected and only in a particular subgroup of neurons (S-cells). Moreover, neural responding was increased relative to replete controls.

There were also no differences in the across-neuron profiles of responding between the groups. Thus there was no evidence that CaCl₂ or other stimuli possess different taste qualities when a rat is calcium deprived.

These results contrast with those found in the NST and parabrachial nucleus of sodium-deprived rats, where the across-neuron profile evoked by NaCl becomes more similar to those evoked by sugars (21, 37).

The range of CaCl₂ concentrations that evoked different responses in S-cells included those that are most preferred by calcium-deprived rats (8). At the lowest concentrations tested, responding was subthreshold for both groups of subjects. It should be pointed out, though, that there were concentrations where no significant differences were observed in neural responding (1, 30, and 300 mM), despite the fact that there are differences in intake following calcium deprivation (8, 9). There were also similar neural responses in the two groups of rats to other stimuli that are consumed differentially based on calcium status. In the case of strontium chloride, this result is not surprising, because behavioral differences are found during long-term but not short-term tests and thus may depend on postdigestive rather than gustatory factors (8, 9). However, in other cases (calcium lactate, NaCl, HCl), there is a discrepancy between the behavioral results and those observed in the present study. In part, this may be due to the fact that it was not practical for us to

Fig. 4. Mean (±SE) net responses to the broad stimulus array in S-cells (A; replete, n = 19; calcium deprived, n = 11), N-cells (B; replete, n = 7; calcium deprived, n = 25), and H-cells (C; replete, n = 20; calcium deprived, n = 10) of the replete and calcium-deprived groups.

Fig. 5. Mean (±SE) net responses to the CaCl₂ concentration series in S-cells (A; replete, n = 13; calcium deprived, n = 7), N-cells (B; replete, n = 5; calcium deprived, n = 14), and H-cells (C; replete, n = 11; calcium deprived, n = 8) of the replete and calcium-deprived groups. Only neurons that received the entire concentration series are included, so the values for 30–300 mM CaCl₂ vary slightly from those shown in Fig. 4. *P < 0.05, replete vs. calcium deprived.
present an entire concentration series for all of the compounds that we tested. Just as responses to only certain concentrations of CaCl₂ were affected by calcium deprivation, we may have found differences for other compounds if we had used other concentrations. It is also likely that calcium appetite involves the coordination of many different brain regions, and gustatory neural responding may show changes to a larger range of stimuli in areas rostral to the NST.

It is unlikely that S-cells increased their responding to CaCl₂ in calcium-deprived rats because of increased firing in the CT. The CT is not very sugar responsive in the rat (4, 17), and there are few sugar-best fibers (15), so S-cells in the NST most likely receive their input from other peripheral nerves. Also, the effects of calcium deprivation on whole nerve CT responding appear to differ from the NST results (20). In calcium-deprived rats, CT responses are significantly smaller to 30–300 mM CaCl₂ and 100 mM MgCl₂ and significantly greater to 30–300 μM CaCl₂ and 30–100 μM calcium lactate than in replete controls. It is surprising that effects similar to these were not observed in the NST, because the CT is one of the nerves from which it receives input. In part, this may be due to methodological differences between the studies, such as the area stimulated with taste solutions (whole mouth vs. anterior tongue). Another possibility is that whole nerve CT responses were affected in some cases because of a change in the percentages of different classes of fibers in the nerve, but not by changes in the mean response within each class of fibers. For example, the CT fibers that respond best to HCl also give large responses to CaCl₂ relative to the neurons that are narrowly tuned to NaCl (29). A reduction in the percentage of HCl-sensitive CT neurons could have been responsible for the decrease in whole nerve responses to 30–300 mM CaCl₂ in deprived rats. This explanation provides for a greater correspondence with the NST, where calcium deprivation was associated with a reduction in the percentage of HCl-sensitive neurons. However, we should also point out that the distribution of NST neurons into subgroups can vary widely between studies, even for control groups. When the percentage of H-cells of the calcium-deprived group was compared with that of control groups from four prior studies (18, 21, 23, 24), a significant reduction was found in only one instance (23). Thus the difference found relative to the replete group in the present experiment may not have been an effect of calcium deprivation.

In summary, CaCl₂ elicited significantly larger responses in sugar-oriented NST neurons (S-cells) in calcium-deprived than in replete rats. Neural response thresholds were also lower in S-cells of the deprived rats. Responding to other stimuli and in other neurons was similar in calcium-deprived and replete subjects.

**Perspectives**

Our demonstration that metabolic and oral information about calcium is integrated parallels other ingestive systems in the rat, including sodium appetite (21, 37) and sugar-calorie appetite (19). It is clear that the taste signal originates in the oral cavity, most likely the tongue, but the question arises as to where the metabolic signal is generated. For sodium and calories, a major signal arises from the liver (30, 44). Certain brain regions may also be affected directly, either by changes in blood glucose level (31) or by elevation of hormones associated with sodium depletion (26). For calcium deficiency, it is not known which signals stim-

---

**Table 2. Log thresholds (M) for neural responding to CaCl₂**

<table>
<thead>
<tr>
<th>Neural Subgroup</th>
<th>Replete</th>
<th>Calcium Deprived</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cells</td>
<td>-2.6 ± 0.2</td>
<td>-3.2 ± 0.2</td>
</tr>
<tr>
<td>S-cells</td>
<td>-1.9 ± 0.2</td>
<td>-3.1 ± 0.3*</td>
</tr>
<tr>
<td>N-cells</td>
<td>-2.2 ± 0.6</td>
<td>-3.3 ± 0.4</td>
</tr>
<tr>
<td>H-cells</td>
<td>-3.7 ± 0.3</td>
<td>-3.1 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.005, replete vs. calcium deprived.
ulate and satiate calcium intake, let alone where they may be acting. The liver may be involved, as it contains functional calcium-sensing receptors (5). Another possibility is that decreases in plasma calcium levels may directly influence neural activity in circumventricular organs (CVOs) such as the subfornical organ, which contains calcium-sensing receptors (34) and is involved in sodium appetite and thirst (40). However, it is also possible to create a need-free calcium appetite by chronic infusions of 1,25-dihydroxyvitamin D$_3$ ([1,25(OH)$_2$D$_3$]; Ref. 42). There are also receptors for [1,25(OH)$_2$D$_3$] and hormones that are elevated during chronic calcium deficiency, such as parathyroid hormone and calcitonin, in CVOs and in hypothalamic nuclei involved in feeding (39, 48, 49). The binding of these substances may provide the signal that stimulates calcium appetite and may influence gustatory responding, because some of the areas mentioned above send projections to the NST (47).

The authors thank D. Pilchak, L. Curtis, and S. Rabusa for excellent technical assistance.

Funding for this project was provided by National Institutes of Health Grant DK-46791.

REFERENCES