Neural Representation of the Taste of NaCl and KCl in Gustatory Neurons of the Hamster Solitary Nucleus

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Boughter, John D., Jr., Steven J. St. John, and David V. Smith. Neural representation of the taste of NaCl and KCl in gustatory neurons of the hamster solitary nucleus. *J. Neurophysiol.* 81: 2636–2646, 1999. NaCl and KCl are monovalent salts that can be discriminated behaviorally by hamsters on the basis of their tastes. We examined the effects of the passive Na^+ channel blocker amiloride on responses to both of these salts in 34 taste-responsive neurons of the nucleus of the solitary tract (NST) in the hamster. The effects of amiloride were assessed with two different, commonly employed stimulus protocols. Additionally, concentration-response functions for each salt were measured in 37 neurons. Cells were characterized by their best response to (in M) 0.03 NaCl, 0.1 sucrose, 0.003 HCl, 0.001 quinine hydrochloride, and 0.1 KCl. In neurons classified as NaCl-best, amiloride reversibly blocked responses to both NaCl and KCl. In neurons classified as HCl-best, amiloride had no effect on either stimulus. In sucrose-best neurons, amiloride blocked the response to NaCl but not KCl. These results support the hypothesis that both salts are transduced by at least two different receptor mechanisms. In the NST, information arising from these different inputs is maintained in discrete populations of neurons. In addition to differences in amiloride sensitivity, the cell types also differed in their responses to the salts across concentration. At midrange salt concentrations, NaCl-best neurons were far more responsive to NaCl than KCl, whereas HCl- and sucrose-best neurons responded equivalently to the two salts at all concentrations. Because NaCl- and HCl-best cells cannot by themselves distinguish NaCl from KCl, it is the relative activity across these cell types that comprises the code for taste discrimination.

INTRODUCTION

Inward movement of Na^+ through amiloride-blockable ion channels can depolarize taste receptor cells (Avenet and Lindemann 1988, 1991). Application of micromolar concentrations of amiloride to the anterior tongue inhibits the peripheral nerve response to NaCl in rats (Brand et al. 1985; Heck et al. 1984; Ye et al. 1993) and hamsters (Herness 1987; Hettinger and Frank 1990). In addition to inhibiting NaCl responses, amiloride reduces responses to citric and hydrochloric acid in hamster taste receptor cells (Gilbertson et al. 1992, 1993). Furthermore, lingual application of amiloride inhibits NaCl- and acid-evoked responses in second-order neurons of the hamster solitary nucleus, but only in certain physiologically defined cell types (Boughter and Smith 1998; Smith et al. 1996). Responses to sodium salts are blocked by amiloride in neurons classified as NaCl- and sucrose-best but not those classified as HCl- or quinine-best, whereas the responses to

acids are reduced by amiloride only in NaCl-best cells (Boughter and Smith 1998). Thus input from amiloride-sensitive and -insensitive transduction mechanisms remains segregated in the medulla, which may have implications for coding the taste of salts and acids in the CNS.

We examined the relative contributions of amiloride-sensitive and -insensitive activity to the neural representation of the taste of two salts, NaCl and KCl. Despite their chemical similarity, behavioral evidence suggests that both hamsters and rats can distinguish the taste of these two salts (Frank and Nowlis 1989; Morrison 1967; Nowlis et al. 1980; Spector and Grill 1992). Further, adulteration of NaCl and KCl with amiloride disrupts a previously learned discrimination between these two stimuli in rats (Spector et al. 1996). Previous analysis of hamster gustatory neural responses has shown that no one neuron type alone (N- or H-best) is sufficient to provide a discriminable neural signal between sodium and nonsodium salts (Smith et al. 1983). Because amiloride has a specific effect on N- but not H-best neurons (Boughter and Smith 1998; Hettinger and Frank 1990), we reasoned that examining the effects of amiloride on the neural responses to NaCl and KCl could reveal how activity in these gustatory neuron types contributes to the neural discrimination between these salts. Because equimolar KCl and NaCl do not stimulate N-best neurons equivalently, we also examined the responses of these cells to several concentrations of each stimulus to further clarify the contribution of each neuron type to the neural distinction between these two salts.

A portion of these results was presented at the annual meeting of the Association for Chemoreception Sciences in Sarasota, FL, April 1998.

METHODS

Animals and surgery

Twenty-seven young adult male hamsters, *Mesocricetus auratus*, weighing a mean of 163 ± 4.2 (SE) g, were deeply anesthetized with urethan (1.7 g/kg ip). Additional anesthetic was given as needed during the course of each experiment. The hamster was tracheotomized, and the hypoglossal nerve was transected bilaterally to eliminate tongue movements. The animal was secured in a nontraumatic headholder (Erickson 1966), and the snout was angled downward 27° from horizontal to minimize brain movement associated with breathing (Van Buskirk and Smith 1981). Body temperature was monitored and maintained at 37°C with a heating pad. A midline incision was made through the tissue overlying the posterior skull, and part of the occipital bone was removed to reveal the cerebellum. After the dura was removed, the posterior portion of the cerebellum was aspirated to expose the floor of the fourth ventricle for 3–4 mm anterior to obex.
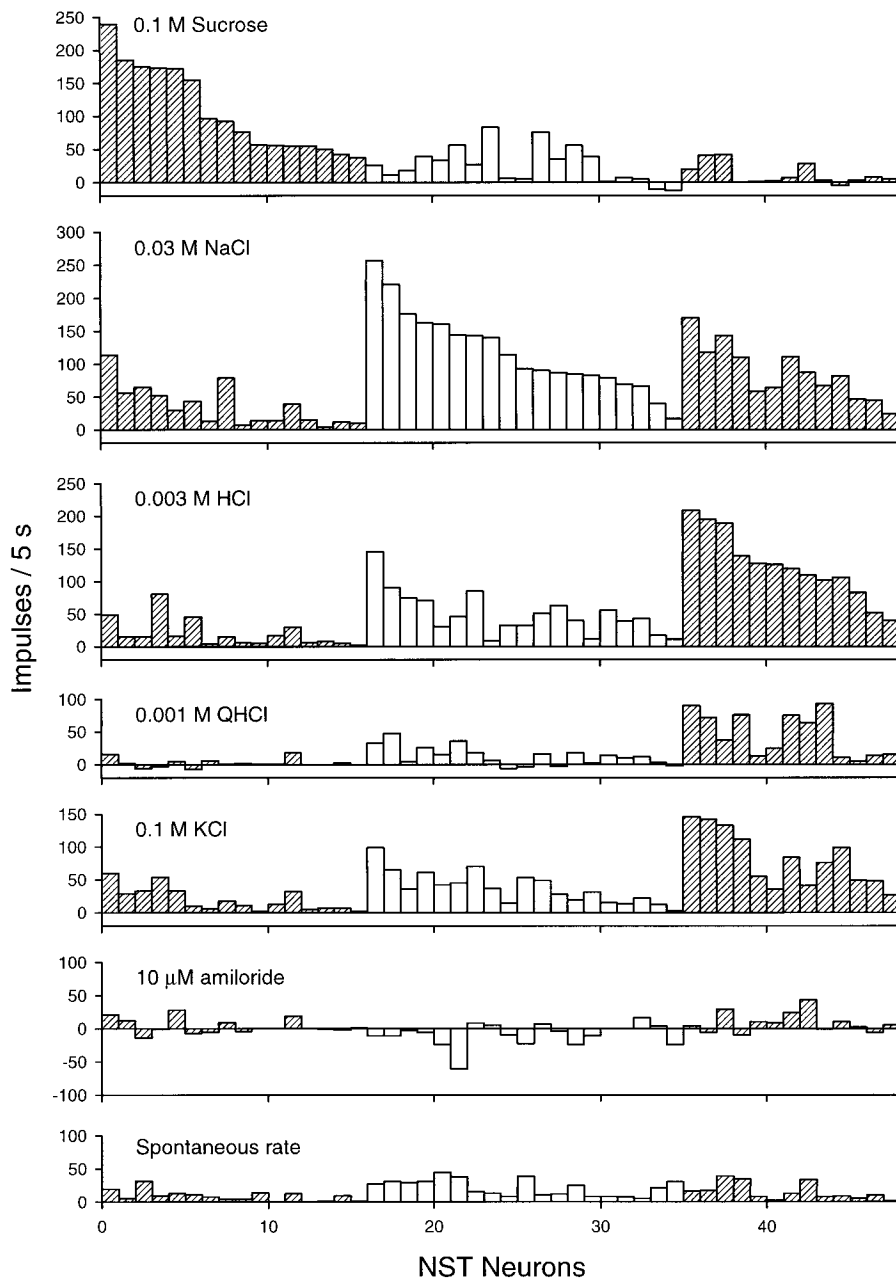
FIG. 1. Across-neuron patterns of responses to 6 taste stimuli. Responses are adjusted for spontaneous rate, shown in the figure. Neurons are grouped from left to right into best-stimulus classes and ranked within each group according to the magnitude of the response to the best stimulus. Response of any 1 neuron can be read vertically. Neurons 1–16 are S-best (▨), 17–35 are N-best (□), and 36–48 are H-best (▨).

Single-neuron recording and stimulation

Tungsten microelectrodes (0.2–2.0 MΩ) were used to record action potentials extracellularly from the nucleus of the solitary tract (NST). The mean (± SD) coordinates (in mm) for taste cell recordings were 2.19 ± 0.22 anterior to obex and 1.39 ± 0.19 lateral to the midline. Action potentials were amplified (Grass P511), discriminated with a dual time-amplitude window discriminator (Bak DDIS-1), displayed on a storage oscilloscope, and monitored with an audio monitor. The amplified action potentials were recorded along with voice cues on digital PCM-VCR tape. An IBM-compatible computer configured with a Lab Master DMA board (Scientific Solutions; Solon, OH) and custom software controlled chemical stimulus delivery and on-line data acquisition and analysis.

Taste-evoked NST activity was initially identified by a change in neural activity associated with the application of anodal current pulses (40 μA, 0.5 s) to the anterior tongue (Smith and Bealer 1975). Single units were identified and isolated while applying a taste stimulus mixture containing (in M) 0.1 sucrose, 0.001 quinine hydrochloride (QHCl), 0.03 NaCl, and 0.01 citric acid to the anterior tongue with a syringe. Stimuli were made from the following chemicals (Sigma Chemical; St. Louis, MO): NaCl, KCl, HCl, sucrose, QHCl, and amiloride-HCl (amiloride). The stimuli were dissolved in distilled water; for amiloride trials, the stimuli were dissolved in 10 μM amiloride. Stimuli were delivered to the anterior tongue at room temperature (21°C) by a gravity-flow system composed of two-way solenoid valves connected via Tygon tubing to a distilled water rinse reservoir and stimulus reservoirs; the flow rate was 2.6 ml/s. The animal's tongue hung vertically over the side of the mandible and was held in place with a small clamp attached to the ventral lingual epithelium. The stimulus delivery tube was positioned directly over the dorsal anterior tongue within 2 mm of the surface. The posterior tongue region containing the foliate and vallate papillae remained in the oral cavity and was not exposed to the stimuli, as demonstrated by the flow of methylene blue over the tongue surface.
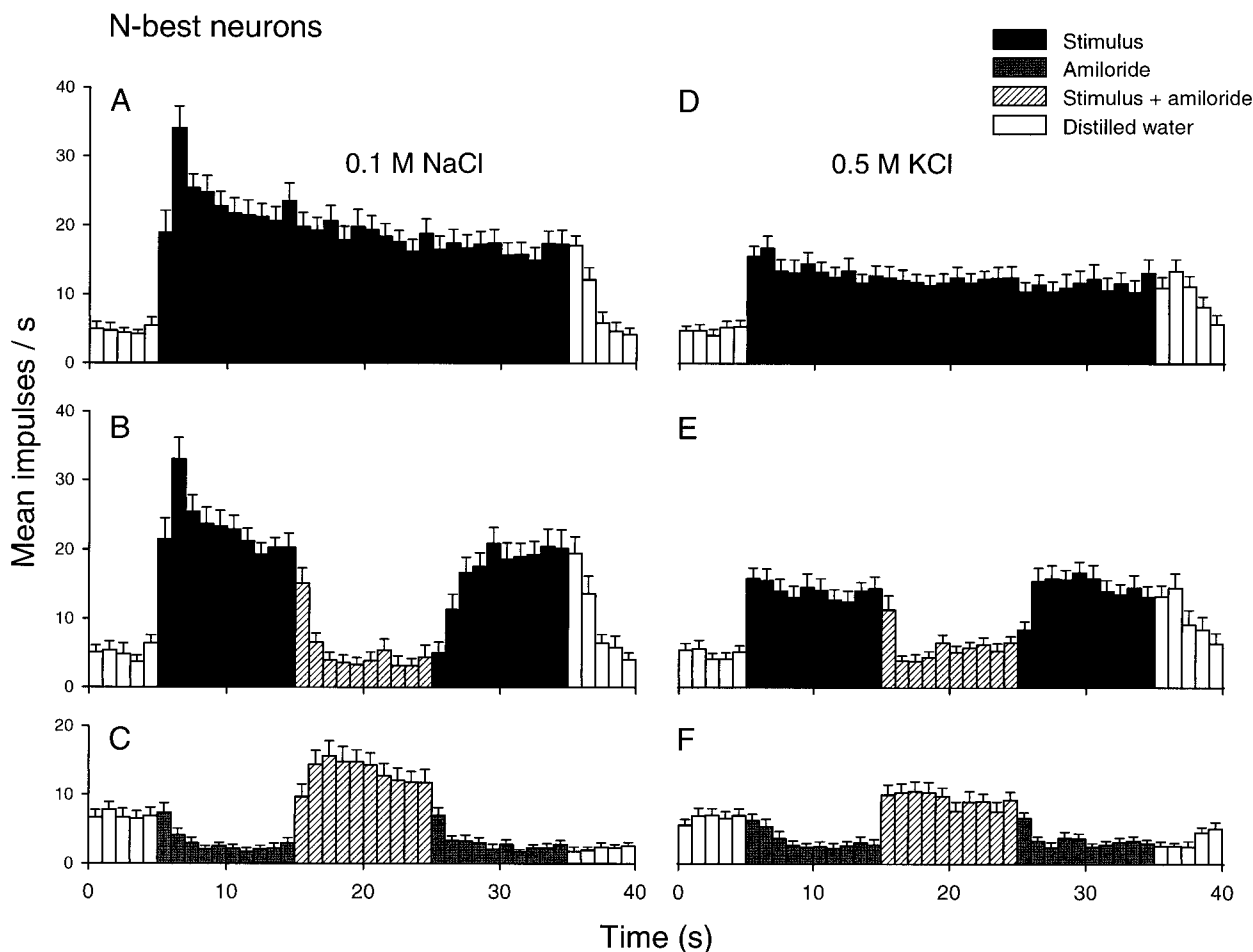
N-best neurons

FIG. 2. Mean peristimulus time histograms for N-best neurons. Fifteen neurons were tested with all trial types shown. Neurons were tested with 0.1 M NaCl alone (■, s 6–35; A), with amiloride added during the response (▨, s 16–25; B), or with amiloride flowing before (▥, s 6–15; C) and during (▨, s 16–25; C) the NaCl stimulation. Error bars show SE. When added to an ongoing NaCl stimulus, amiloride completely and reversibly blocked the response to NaCl in these cells (B). When the cell was adapted for 10 s with amiloride (C), the response to NaCl (▨, s 16–25) was diminished relative to the initial NaCl response (■, s 6–10; A or B). The effect of amiloride on the response to 0.5 M KCl was also assessed in these same 15 cells. KCl elicited a sustained response in these N-best cells (D); amiloride addition strongly attenuated this response (E). The KCl response was also diminished after amiloride pretreatment (F). See Fig. 5 for a statistical summary of these effects.

Best-stimulus classification

The taste response profile of a neuron was determined with (in M) 0.1 sucrose, 0.03 NaCl, 0.003 HCl, 0.001 QHCl, and 0.1 KCl, presented in random sequence. These concentrations elicit half-maximal integrated responses in the whole hamster chorda tympani (CT) nerve (Frank 1973). The stimulation sequence for these five trials, during which the computer collected data, was a continuous flow initiated by the delivery of 5 s of distilled water, followed by 10 s of stimulus, and then by 10 s of distilled water. After these five trials, a trial of 10 μM amiloride was presented. To avoid adaptation effects, the tongue was rinsed with an additional 50 ml of distilled water after each trial, and individual stimulations were separated by $\geq$2 min. Each neuron was classified as sucrose-, NaCl-, HCl-, QHCl-, or KCl-best by determining which stimulus evoked the greatest net response (calculated as the number of impulses during the first 5 s of stimulus application minus the number of impulses during the immediately preceding 5 s of distilled water).

Amiloride trials

The effects of 10 μM amiloride on responses to 0.1 M NaCl and 0.5 M KCl in single NST neurons were tested by two methods: amiloride addition and preadaptation. Amiloride-addition trials were initiated by the

delivery of distilled water (5 s), the stimulus alone (10 s), the stimulus mixed with amiloride (10 s), the stimulus alone again (10 s), and distilled water (10 s). Preadaptation trials were initiated by the delivery of 5 s of distilled water, followed by 10 μM amiloride for 10 s, the stimulus dissolved in amiloride for 10 s, amiloride alone again for 10 s, and distilled water for 10 s. Thus in a preadaptation trial the stimulus was presented mixed in amiloride and following amiloride adaptation, whereas in an amiloride-addition trial, amiloride was presented during an ongoing salt stimulus. A control trial consisted of distilled water (5 s), the salt stimulus (30 s), and distilled water (10 s). These three trial types were run with both 0.1 M NaCl and 0.5 M KCl for each neuron ($n = 34$). After each trial the tongue was rinsed with >100 ml distilled water, and trials were separated by $\geq$3 min to avoid possible adaptation effects. The concentrations of NaCl (0.1 M) and KCl (0.5 M) were chosen to produce robust responses in N-best neurons to both stimuli. The amiloride concentration of 10 μM produces a complete block of the integrated hamster CT nerve activity evoked by 0.1 M NaCl when applied during salt stimulation (Hettinger and Frank 1990).

Concentration series

Thirty-seven neurons were tested with concentration series of both NaCl and KCl (0.001–1 M, in 1-log steps). The NaCl concentration
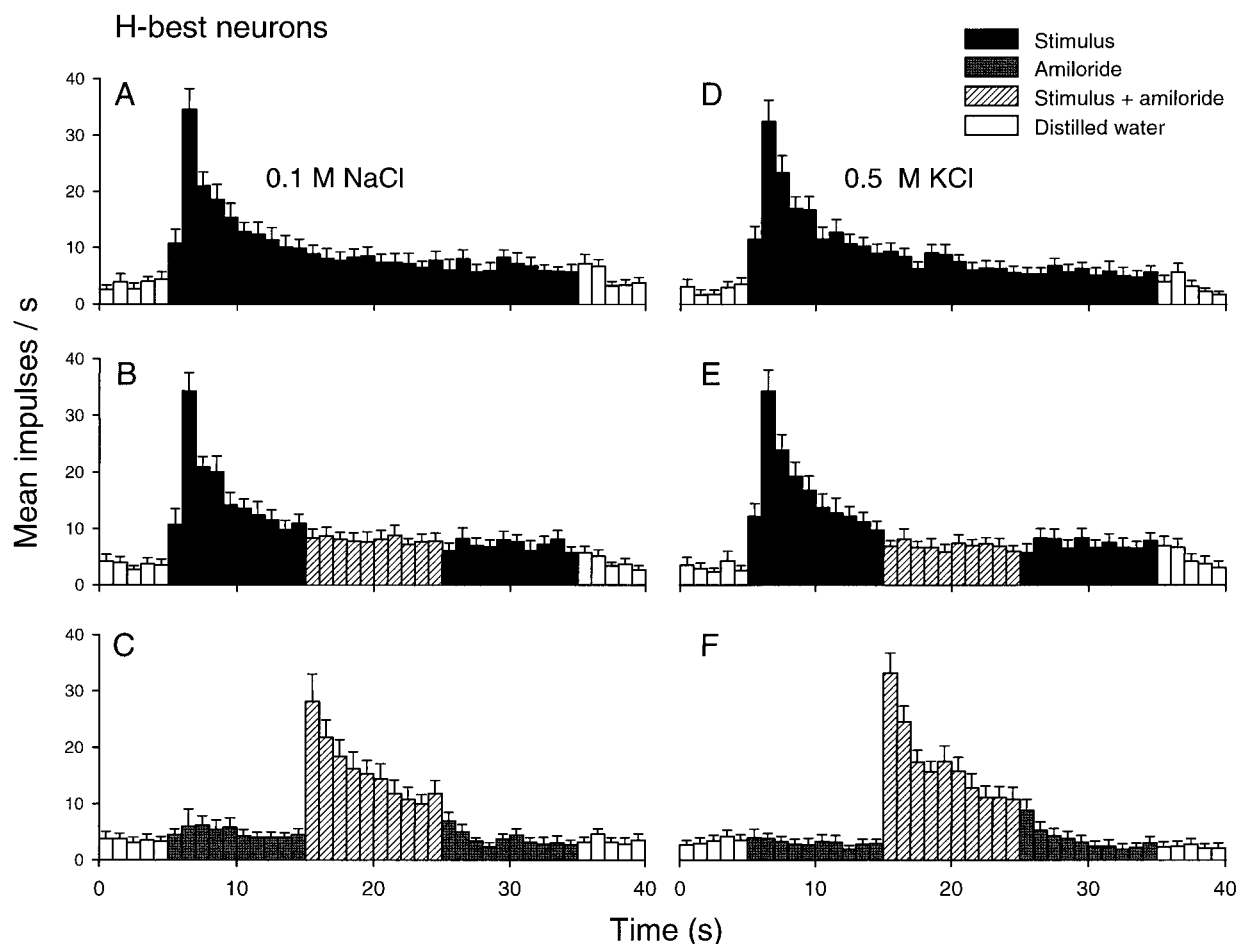
H-best neurons

FIG. 3. Mean peristimulus time histograms for 10 H-best neurons. Conventions and shading are the same as in Fig. 2. Cells responded equally to NaCl and KCl (*A* and *D*). However, responses to NaCl (*B* and *C*) and KCl (*E* and *F*) were not blocked by amiloride with either treatment method. See Fig. 5 for a statistical summary of these effects.

series was extended to 0.001–3 M (in ½-log steps) in a subset of these cells (*n* = 23). Each series was conducted in ascending order. Trials consisted of a continuous flow of distilled water (5 s), the salt (10 s), and distilled water (10 s). Trials were separated by ≥2 min during which the tongue was rinsed with ≥50 ml of distilled water.

Data analysis

The window-discriminated action potentials were converted into frequency counts (impulses/s); stimulus time histograms were displayed on-line, and the data were stored on the computer for off-line analyses. For the concentration series and the best-stimulus characterization, the primary response measure was the net impulses/5 s (the number of impulses during the first 5 s of stimulus presentation minus the number of impulses during the preceding 5 s of distilled water presentation).

For the amiloride-addition trials, the effects of amiloride on the responses to NaCl and KCl were assessed as follows. Because it typically took a few seconds for the addition of amiloride to reduce the NaCl response in N-best cells, we counted the net impulses/5 s during the last 5 s of amiloride treatment. This response was compared with the net response during an equivalent time period in the control trial, i.e., s 16–20 of the stimulus alone. In both cases, net firing rate was calculated by subtracting the firing rate to distilled water during the 5 s immediately preceding stimulus onset. This procedure ensured that changes in the neural response over time (i.e., adaptation) did not contribute to the measured effects of amiloride treatment.

For the amiloride preadaptation trials, the primary response measure was the number of impulses during the first 5 s of stimulus presentation (s 16–20) minus the number of impulses during the last 5 s of the amiloride adapting solution (s 11–15). This was compared with the averaged net response from the first 5 s of stimulus presentation (s 6–10) in the amiloride-addition and control trials.

Mean net response frequencies of amiloride versus control conditions were compared with two-tailed paired *t*-tests (with Bonferroni correction). Concentration-response functions were analyzed by ANOVAs.

RESULTS

Cell classification

Taste-evoked action potentials were recorded from 48 individual NST neurons. Cells were classified as responding best to sucrose (S-best; *n* = 16), NaCl (N-best; *n* = 19), or HCl (H-best; *n* = 13) on the basis of their net responses to these stimuli. During the course of the experiment, two cells responding best to QHCl were encountered, but neither was viable long enough to complete a stimulus protocol. No cell responded best to 0.1 M KCl. The responses of all 48 neurons to these five stimuli as well as to 10 μM amiloride are shown in Fig. 1. In this figure, the neurons are arranged from left to right along the abscissa in three groups: S-best (neurons 1–16),
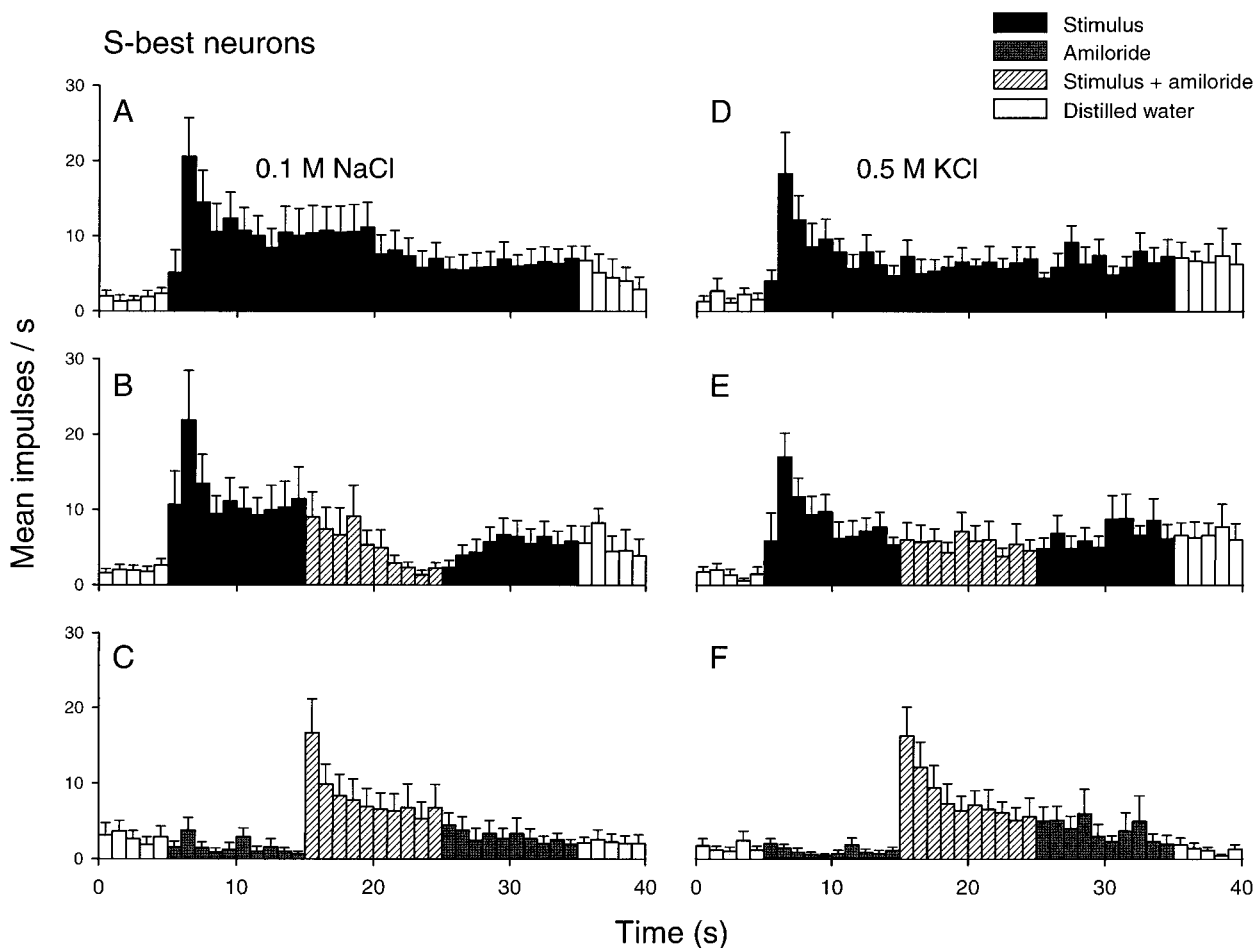
FIG. 4. Mean peristimulus time histograms for 9 S-best neurons tested with NaCl/amiloride trials and a subset consisting of 7 of these neurons tested with KCl/amiloride trials. Conventions and shading are the same as in Fig. 2. Responses to NaCl (A) were reduced by amiloride in both trial types (B and C). However, responses to KCl (D) were unaffected by amiloride (E and F). See Fig. 5 for a statistical summary of these effects.

N-best (neurons 17–35), and H-best (neurons 36–48). Within each group, the cells are arranged in order of the response magnitude to their best stimulus. Most neurons responded to more than one of these compounds. N-best neurons typically responded also to HCl and KCl, and H-best units responded well to NaCl, QHCl, and KCl. Application of 10 μM amiloride significantly inhibited the baseline firing rate of N-best neurons (Student's t-test vs. 0, $t = -2.29$, df = 18, $P < 0.05$), suggesting that much of the spontaneous activity of these central neurons may result from activity of the amiloride-sensitive channels in the receptor cells. Amiloride did not significantly alter the baseline firing rate of H-best ($t = 2.04$, df = 13, $P = 0.064$) or S-best ($t = 1.24$, df = 14, $P = 0.117$) neurons. The spontaneous rate (during the last 5 s of the prestimulus water rinse) is plotted at the bottom of Fig. 1. The N-best neurons had a significantly higher mean ($\pm$ SE) spontaneous firing rate (21.1 ± 2.9) than S-best cells (8.9 ± 2.1; two-tailed independent t-test, $t = 3.357$, df = 30, $P < 0.01$).

Amiloride treatment

Application of 10 μM amiloride had different effects on the responses of NST neurons, which related to the cell's response profile.

N-BEST NEURONS. Fifteen N-best neurons were tested with NaCl alone for 30 s (Fig. 2A, ■), with the addition of 10 μM amiloride following 10 s of NaCl stimulation (Fig. 2B, ▨), and with the addition of NaCl following 10 s of preadaptation to amiloride (Fig. 2C, amiloride = ▥, NaCl + amiloride = ▨). When added during NaCl stimulation (Fig. 2B), amiloride reversibly blocked NaCl responses in all N-best cells relative to control trials (see STATISTICAL ANALYSIS OF AMILORIDE EFFECT). Amiloride preadaptation, although not completely inhibiting the NaCl response, did reduce the magnitude of the response relative to the NaCl response following water (cf. Fig. 2C, 16–20 s, with Fig. 2, A or B, 6–10 s). The same 15 cells were also tested with 0.5 M KCl. KCl elicited a smaller response in these neurons (Fig. 2D); however, amiloride reduced the response to KCl relative to control trials in both the amiloride-addition (Fig. 2E) and preadaptation (Fig. 2F) trials.

H-BEST NEURONS. NaCl (0.1 M) produced a small response in H-best neurons (Fig. 3A; $n = 10$) relative to the N-best neurons (Fig. 2A). In contrast to the N-best neurons, amiloride did not reduce the response to NaCl in either the amiloride-addition or preadaptation trials in H-best cells (Fig. 3, B and C). Lingual application of 0.5 M KCl evoked a response in these neurons (Fig. 3D) that was similar in magnitude and time course to the
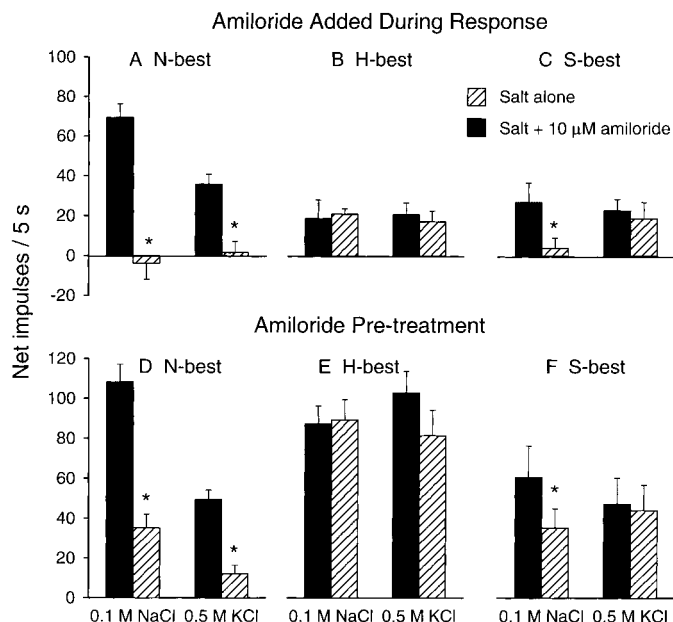
FIG. 5. Mean responses (net impulses/5 s) to stimuli and stimuli + amiloride for each gustatory neuron type and for both amiloride-addition and pretreatment trial types. For N-best neurons (A and D), $n = 15$; for H-best units (B and E), $n = 10$; for S-best units (C and F), $n = 9$ for NaCl, $n = 7$ for KCl. SE, +1 standard error; *: significant difference in the means of the stimulus alone vs. the stimulus mixed with amiloride (Bonferroni t, $P < 0.025$; see text for details).

NaCl response. Similarly, amiloride did not reduce the response to KCl in either amiloride trial type (Fig. 3, E and F).

S-BEST NEURONS. The effect of amiloride on the response to NaCl in S-best neurons was tested in nine cells (Fig. 4, A–C); its effect on KCl was examined in a subset of these cells ($n = 7$; Fig. 4, D–F). Application of 0.1 M NaCl for 30 s evoked a sustained response in these neurons (Fig. 4A); amiloride reversibly reduced this response in the amiloride-added trial type (Fig. 4B). Likewise, the NaCl response was reduced following amiloride preadaptation (Fig. 4C; see STATISTICAL ANALYSIS OF AMILORIDE EFFECT). Although 0.5 M KCl also elicited a response in S-best neurons (Fig. 4D), this response was unaffected by amiloride treatment (Fig. 4, E and F).

STATISTICAL ANALYSIS OF AMILORIDE EFFECT. In neurons classified as N-best (Fig. 5A), amiloride added during ongoing stimulation produced a complete block of both the 0.1 M NaCl (two-tailed paired t-test, $t = 8.65$; df = 14, $P < 0.00001$) and 0.5 M KCl ($t = 6.50$; df = 14, $P < 0.00001$) response. In contrast, NaCl and KCl responses in H-best neurons were unaffected by the addition of amiloride ($t < 1.96$; $P > 0.08$; Fig. 5B). In S-best neurons (Fig. 5C), the NaCl response was significantly reduced by amiloride ($t = 3.23$; df = 8; $P < 0.02$), whereas the response to KCl was unaffected ($t = 0.56$; df = 6; $P > 0.5$).

Similarly, in neurons classified as N-best (Fig. 5D), amiloride pretreatment significantly reduced the response of both NaCl ($t = 9.39$; df = 14; $P < 0.00001$) and KCl ($t = 5.03$; df = 14; $P < 0.001$). Responses to these stimuli in H-best neurons were unaffected by amiloride pretreatment ($t < 1.56$; df = 9; $P > 0.15$; Fig. 5E). In S-best cells (Fig. 5F), the response to NaCl was significantly diminished after amiloride

($t = 2.95$; df = 8; $P < 0.02$), but the response to KCl was not altered ($t = 0.76$; df = 6; $P > 0.4$).

Concentration-response functions

The responses of 37 NST neurons to concentration series of NaCl and KCl demonstrate that the various neuron types respond differently to these stimuli across concentration. A three-way ANOVA revealed significant main effects of neuron type ($F_{[2,34]} = 9.84$, $P < 0.001$), salt ($F_{[1,34]} = 46.88$, $P < 0.001$), and concentration ($F_{[3,102]} = 159.05$, $P < 0.001$). Concentration-response relationships differed as a function of neuron type, as indicated by a significant three-way interaction ($F_{[6,102]} = 8.50$, $P < 0.001$).

Examination of the concentration-response functions reveals that N-best cells ($n = 16$) responded very differently to NaCl and KCl (Fig. 6A), whereas these stimuli were roughly equally effective in stimulating H-best ($n = 9$) and S-best ($n = 12$) neurons across these salt concentrations (Fig. 6, B and C). Two-way ANOVAs for each neuron type support these conclusions. In N-best neurons, there was a significant main effect of salt ($F_{[1,15]} = 68.33$, $P < 0.001$) and concentration ($F_{[3,45]} = 79.47$, $P < 0.001$) as well as a significant interaction ($F_{[3,45]} = 20.33$, $P < 0.001$). In H-best neurons, however, only the effect of concentration was significant ($F_{[3,24]} = 56.67$, $P < 0.001$); there was not a main effect of salt or a salt ×
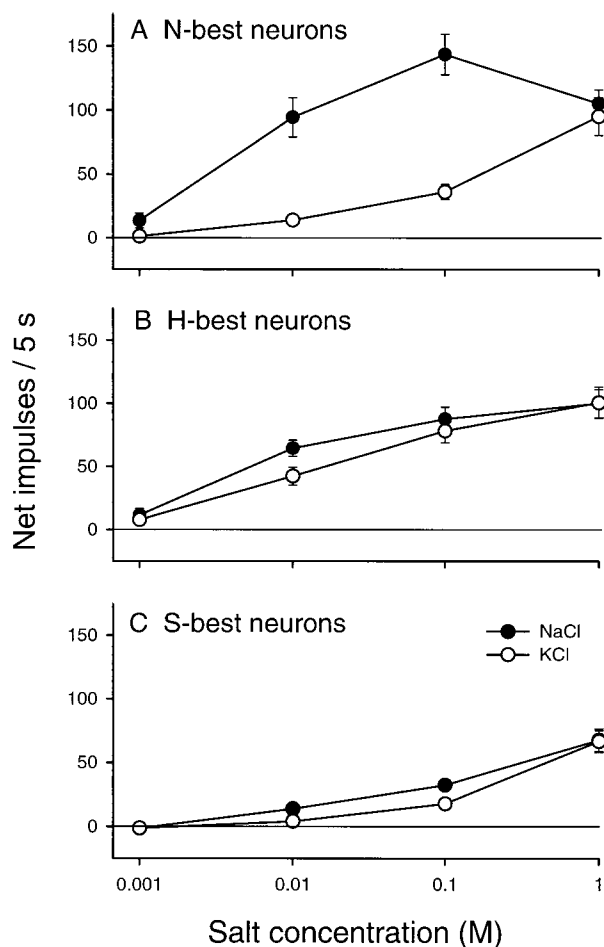

FIG. 6. Mean (± SE) concentration-response functions for NaCl (●) and KCl (○) in N-best (A), H-best (B), and S-best (C) neurons.
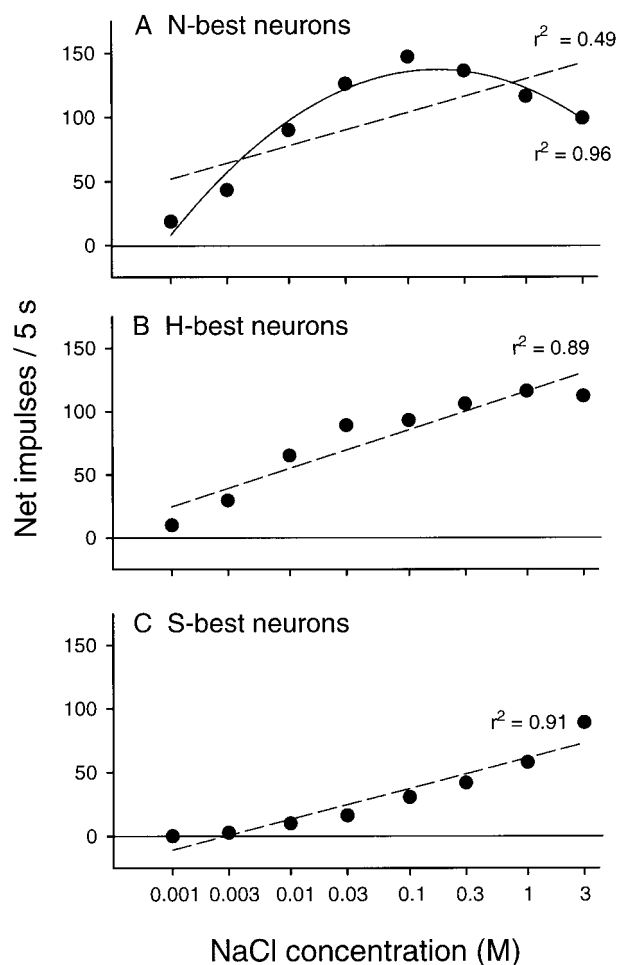
FIG. 7. Mean NaCl concentration-response functions for 10 N-best (*A*), 4 H-best (*B*), and 9 S-best (*C*) neurons that were tested with the extended NaCl stimulus array (0.001–3 M, half-logarithmic steps). A linear fit (- - -) accounted for ~90% of the variance in the H- and S-best concentration-response functions but only 49% in N-best neurons. A quadratic fit, in contrast, accounted for 96% of the variance (—, *A*).

concentration interaction. In S-best cells, a two-way ANOVA did reveal a significant main effect of salt ($F_{[1,11]} = 6.21$, $P = 0.03$) and of concentration ($F_{[3,33]} = 48.48$, $P < 0.001$) and a salt × concentration interaction ($F_{[3,33]} = 3.27$, $P = 0.03$), but it is clear that this latter effect was small (Fig. 6*C*).

In all cases, there was a significant effect of concentration. A closer inspection of the data, however, indicates that not all concentration-response functions were monotonically increasing with concentration (Figs. 6–8). There appeared to be a consistent nonmonotonic concentration-response relationship for NaCl in N-best neurons (Fig. 6*A*). This prompted us to extend the NaCl concentration range to 3 M in half-logarithmic steps in a subset of cells. In all, 10 N-best (Fig. 7*A*), 4 H-best (Fig. 7*B*), and 9 S-best (Fig. 7*C*) neurons were tested with this extended concentration series. The more complete curves retained the general shapes of those obtained with fewer stimuli (Fig. 6). When regression functions were fit to the mean data (for cases where all 8 NaCl concentrations were tested), linear fits (- - -) accounted for 89 and 91% of the variance for H- and S-best neurons (Fig. 7, *B* and *C*). In contrast, a linear fit accounted for only 49% of the variance in N-best neurons,

whereas a two-parameter quadratic fit (—) accounted for 96% of the variance (Fig. 7*A*).

Concentration-response functions for four representative neurons of each type are shown in Fig. 8. Although the concentration-response functions for NaCl in many H- and S-best cells were not strictly linear, in no case was the nonlinearity as striking as in N-best cells, each of which showed an inverted U-shaped concentration-response function. The response to KCl reliably increased as a function of concentration in all cell types. That 1 M NaCl and 1 M KCl are roughly equally effective at driving N-best cells (Figs. 6*A* and 8*A*) results from the relatively linear nature of the KCl concentration-response function and the nonlinear nature of the function for NaCl. Of the 10 N-best cells tested with the extended series of NaCl concentrations, 5 had a peak response at 0.1 M, 4 at 0.3 M, and 1 at 1.0 M. Of the other six N-best neurons that were tested only with the standard series, only one had a peak response at the highest concentration (1.0 M); the remaining neurons had a peak response at 0.1 M ($n = 4$) or 0.01 M ($n = 1$). To investigate whether this nonmonotonicity was due to adaptation, we tested several applications of 0.1 M and 1.0 M NaCl in random order on a subset ($n = 5$) of the N-best cells; 0.1 M consistently elicited the stronger response (data not shown). Although only approximately one-half of all H-best cells tested had a peak response at the highest tested concentration (Fig. 8*B*), there was never a case where the response to the highest concentration was greatly reduced relative to lower concentrations. In contrast, all S-best cells gave a peak response to the highest NaCl concentration tested.

DISCUSSION

We investigated the effects of lingual application of the passive Na$^+$ channel blocker amiloride on responses to NaCl and KCl in gustatory neurons in the hamster NST. Amiloride caused a significant decrease in responses to both NaCl and KCl in N-but not H-best neurons. Additionally, the response to NaCl but not KCl was diminished in S-best neurons. These effects occurred whether the amiloride was added to an ongoing salt response or used to preadapt the cell before salt stimulation. Thus, in a subset of NST gustatory neurons, amiloride clearly reduces the responses to both NaCl and KCl. Despite this apparent overlap in the transduction mechanisms for these salts, NaCl was a much more effective stimulus for N-best neurons than was KCl. The response to NaCl was considerably greater in these cells over most of a broad concentration range. In contrast, NaCl and KCl were roughly equally effective stimuli for H- or S-best neurons across several concentrations.

Effects of amiloride vary with neuron type

As suggested by previous studies, the effect of amiloride on responses to ionic substances can vary with neuron type as well as with the stimulus. In hamster NST neurons, amiloride can inhibit responses to salts of at least two different cations (Na$^+$ and K$^+$, this study) as well as responses to two acids (HCl and citric acid) (Boughter and Smith 1998). For all these stimuli, amiloride inhibits responses in N-best but not H-best neurons. The effects of amiloride are also restricted to N-best CT fibers in both the hamster (Hettinger and Frank 1990) and rat (Ni-
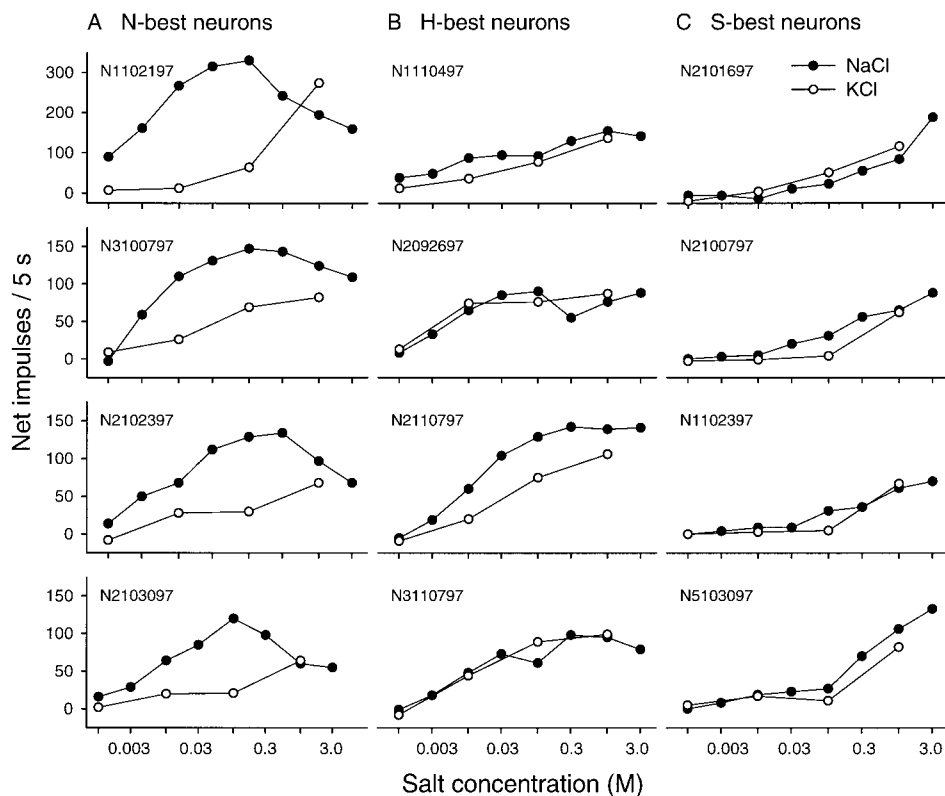
FIG. 8. Concentration-response functions for NaCl (●) and KCl (○) in individual N-best (A), H-best (B), and S-best (C) neurons. Note that the first row of functions is drawn to a different scale.

nomiya and Funakoshi 1988) and to cells responding most to NaCl in the rat NST (Giza and Scott 1991; Scott and Giza 1990).

In this study, amiloride blocked responses to NaCl but not KCl in S-best neurons. In previous experiments, we also found that amiloride inhibited responses to NaCl but not HCl or citric acid in S-best cells of the NST (Boughter and Smith 1998; Smith et al. 1996). Taken together, these findings suggest a pattern of convergence of CT nerve fibers onto NST neurons in which the S-best NST cells receive input from both N- and H-best CT fibers (see Boughter and Smith 1998). Because the responses of S-best NST neurons to KCl are not reduced by amiloride, the majority of the information provided to these cells about KCl must arise from input from H-best CT fibers, which do not have an amiloride-sensitive response to any stimulus (Hettinger and Frank 1990). Conversely, the amiloride-sensitive NaCl responses of S-best cells in the NST must originate via amiloride-sensitive N-best CT fibers. Of all the cell types, S-best cells show the greatest increase in breadth of tuning between the CT nerve and the NST (Travers and Smith 1979; Van Buskirk and Smith 1981), suggesting convergence from diverse CT fiber types. Convergence of peripheral taste fibers onto NST neurons has been shown directly in the rat (Travers et al. 1986), hamster (Sweazey and Smith 1987), and sheep (Vogt and Mistretta 1990). Such convergence has the effect of increasing the absolute sensitivity of the gustatory system but results in a decrease in stimulus specificity at the level of the brain stem.

Effects of amiloride vary with the stimulation protocol

The effects of amiloride on taste responses have been assessed typically either by pretreating the tongue with amiloride

(Brand et al. 1985; Heck et al. 1984; Smith et al. 1996) or by introducing the amiloride into the stimulating solution (Boughter and Smith 1998; Hettinger and Frank 1990). Invariably, greater inhibition is obtained with the latter method. Minear et al. (1996) compared these methods directly in rats with whole nerve CT recordings. They found that adding 100 μM amiloride to various concentrations of NaCl suppressed the integrated CT response by 65%, whereas pretreatment with 100 μM amiloride suppressed the response to NaCl by only
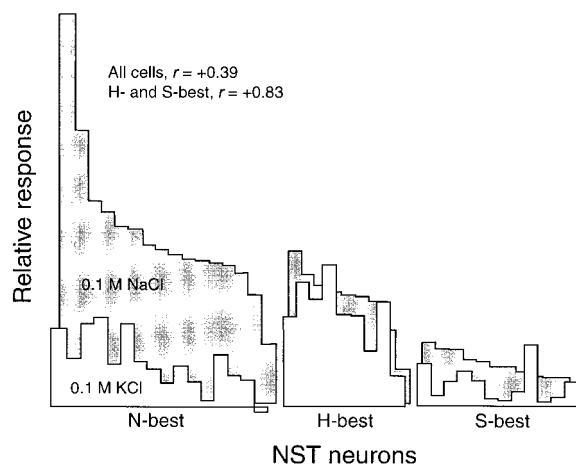

FIG. 9. Across-neuron patterns for 16 N-best, 9 H-best, and 12 S-best neurons for 0.1 M NaCl (▥) and 0.1 M KCl (□). Patterns of neural activity evoked by these 2 stimuli across all cells are distinct, resulting in a low across-neuron correlation (+0.39), indicative of 2 stimuli that are behaviorally discriminable (Erickson 1963; Smith et al. 1979). However, correlation across only the H- and S-best neurons is +0.83, suggesting that without differential input from N-best cells these 2 stimuli would be much more difficult to discriminate.

34%. The authors suggested two possibilities for this discrepancy. First, lengthy pretreatment (1–5 min) with amiloride may alter the taste receptor environment in such a way as to reduce the effects of amiloride on subsequent responses. However, we preadapted the tongue with 10 μM amiloride for only 10 s and still observed a smaller effect on NaCl or KCl responses relative to amiloride addition, which produced a complete (100%) suppression of the salt response in N-best neurons (Figs. 2, *B* and *C*, and 5, *A* and *D*). The second explanation offered by Minear et al. (1996) for these differences arises from the fact that they recorded integrated responses from the whole CT nerve. The integrated response was a combination of activity of amiloride-suppressible (N-fibers) and amiloride-insensitive (H-fibers) fiber types. N-fibers give a sustained response to NaCl, whereas H-fibers respond to NaCl with a brief burst of activity that subsides after a few seconds (the same pattern exists in NST cells; cf. Figs. 2*A* and 3*A*). The authors reasoned that the integrated CT response should therefore have a smaller amiloride-suppressible component early in the trial and a larger amiloride-suppressible component later in the trial because of these differences in the time course of responses in different fiber types. Indeed the amiloride pretreatment trial measures amiloride's effect immediately, whereas the amiloride-added trial measures the effect after 10 s of salt stimulation. Nonetheless, this explanation cannot account for the current data because we recorded the responses of single neurons, not multiunit activity as in the study of Minear et al. (1996).

The current single-neuron data suggest another possibility. Biophysical studies of amiloride indicate there is competitive inhibition between Na$^+$ and amiloride at the amiloride-sensitive sodium channel on the apical membrane of taste cells (Lindemann 1996). These data, in concert with electrophysiological and behavioral studies, indicate a trade-off between amiloride concentration and NaCl concentration that dictates amiloride's potency in inhibiting NaCl responses (Brand et al. 1985; Hettinger and Frank 1990; Spector et al. 1996). The ability of a fixed concentration of amiloride to block the response to NaCl depends on the Na$^+$ concentration gradient across the apical membrane, which in turn determines the driving force for Na$^+$. During continual NaCl stimulation, the concentration gradient for Na$^+$ across the taste cell membrane would gradually decrease as Na$^+$ moves inward through the amiloride-sensitive channel. Thus it is reasonable to conclude that the driving force for Na$^+$ after 10 s of NaCl stimulation would be less than at the onset of the NaCl stimulus. This would result in a more effective block of the NaCl response when amiloride is presented during the steady-state portion of the response (see Figs. 2 and 5).

Nonmonotonic NaCl response function

N-best cells differed significantly in responses to NaCl and KCl most strikingly at low- and midrange concentrations (Figs. 6–8). Because responses of these cells to both salts are completely blocked by 10 μM amiloride (Fig. 5*A*), these data suggest that K$^+$ readily penetrates the amiloride-sensitive channel only at higher concentrations. N-best neurons responded to NaCl with a nonmonotonic, inverted-U shaped concentration-response function. These cells did not show this response to KCl, although it is possible that such

a response would have occurred had higher KCl concentrations been used. The functional significance of the nonmonotonic function for NaCl is not readily apparent. Clearly, the responses of these neurons do not accurately convey the intensity of the stimulus, but they may contribute to some sort of hedonic code or other code that is behaviorally relevant. Such functional hypotheses await further experimental scrutiny.

A second question is the mechanism of the inverted-U concentration-response function. There is some indication that the responses seen in N-best NST cells may reflect the concentration-response relationships of N-best CT nerve fibers (Rehnberg et al. 1993). In taste receptor cells, Gilbertson and Zhang (1998) demonstrated self-inhibition of Na$^+$ at the amiloride-sensitive Na$^+$ channel that peaks at an extracellular Na$^+$ concentration of ~50–75 mM. How self-inhibition at such low concentrations could play a role in the inhibition we see in NST neurons at concentrations of >300 mM is not intuitively obvious. Yamamoto et al. (1984) reported that rat cortical neurons often show a descending concentration-response function for NaCl; had lower concentrations of NaCl been included in their stimulus array, it is conceivable that some of the functions they described as "descending" might have been classified as "inverted-U shaped." In the Yamamoto study, however, these descending functions were typically found in S-best, not N-best, neurons. Whether inverted-U shaped concentration-response functions for NaCl exist at other levels of the hamster gustatory system, and whether such responses have behavioral consequences, is an interesting question that demands further study.

Implications for taste quality coding

In hamsters, aversions conditioned to NaCl do not readily generalize to KCl or other nonsodium salts, and vice versa, suggesting that these two classes of stimuli can be discriminated by these animals (Frank and Nowlis 1989; Nowlis and Frank 1981; Smith et al. 1979). Our data provide a possible neurophysiological substrate for this behavioral discrimination at the level of the NST. Figure 9 shows the responses of all neurons tested with isomolar concentrations of NaCl and KCl; the neurons are ordered by responses to 0.1 M NaCl and grouped into N-units (*left*), H-units (*middle*), and S-units (*right*). The across-neuron patterns evoked by 0.1 M NaCl and 0.1 M KCl are clearly quite different across the entire array of neurons. Indeed the across-neuron correlation between these two stimuli is only +0.39, a correlation that is characteristic of highly discriminable stimuli (Erickson 1963; Erickson et al. 1965; Smith et al. 1979). However, if the N-units do not contribute to the across-neuron pattern, the interstimulus correlation between NaCl and KCl across the H- and S-best neurons rises to +0.83, a correlation characteristic of stimuli with similar tastes (Erickson 1963; Erickson et al. 1965; Smith et al. 1979). Blocking the activity of N-best neurons with amiloride prevents rats from discriminating midrange concentrations of NaCl and KCl (Spector et al. 1996). Although such behavioral studies have not been conducted in hamsters, the electrophysiological data presented here would predict that amiloride adulteration of salt stimuli should reduce the discriminability of sodium and nonsodium salts in hamsters by eliminating the differential input provided by the N-best cells.

Although the responses to NaCl and KCl did not correlate well at 0.1 M, the strongest KCl concentration (1.0 M) correlated highly with both 0.1 M NaCl and 1.0 M NaCl ($r = +0.72$ and $+0.74$, respectively). Generally, stimuli with high across-neuron correlations have been shown to generalize in behavioral experiments (e.g., Erickson 1963; Frank and Nowlis 1989; Nowlis and Frank 1981; Smith et al. 1979). Given such a relationship, these results predict that higher concentrations of KCl may actually be more difficult for hamsters to discriminate from NaCl than lower concentrations. This hypothesis has not been tested behaviorally.

Nevertheless, the current data strongly suggest that the behavioral discrimination between sodium and nonsodium salts depends on the differential input provided by N-best gustatory neurons (McCaughey and Scott 1998). Although the other cell types respond with similar magnitude to NaCl and KCl, the N-best cells respond differentially to these two stimuli; the distinct across-neuron pattern that results (Fig. 9) provides the neural substrate that allows these animals to detect and respond appropriately to sources of sodium in their diets. We have previously shown that activity in neither N-nor H-best neurons alone is sufficient to generate distinctive across-neuron patterns for sodium salts versus nonsodium salts and acids (Smith 1985; Smith and Frank 1993; Smith et al. 1983). The current data further support the hypothesis that N-best cells are necessary but not sufficient for this differentiation. Indeed within either N- or H-best cells the patterns of activity evoked by NaCl and KCl are correlated, but when the responses of all cells are compared these stimuli evoke uncorrelated patterns of activity (Fig. 9). Only by comparing the activity across these neuron types can a clear distinction be made between NaCl and KCl. Because the firing rate of N-best cells can be modulated by changing either the salt (NaCl and KCl) or its concentration, activity in this one cell type (or any other) is ambiguous with regard to both taste quality and intensity. Such ambiguity in the responses of single gustatory cells first led Pfaffmann (1955, 1959) to propose that taste quality is coded by an across-neuron pattern. Although it certainly can be argued that N-best cells are necessary for coding the taste of sodium salts (see McCaughey and Scott 1998), there are no data to show that they alone are sufficient to do so. Thus, although there are identifiable neuron types in the gustatory system and one type that is driven by an amiloride-sensitive receptor input, the unambiguous coding of taste quality depends on the relative activity across these cells (Erickson 1968, 1984; Erickson et al. 1965; Pfaffmann 1955, 1959).

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