Behavioral and electrophysiological responses to NaCl in young and old Fischer-344 rats

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Abstract. This study compared both behavioral and electrophysiological responses to NaCl in young and old Fischer-344 rats. These comparisons were made in the same individuals. Preference for NaCl solutions versus water was assessed using two-bottle preference tests. The integrated response of the chorda tympani nerve to NaCl was recorded. NaCl neural-response magnitude and corresponding behavioral sensitivity appear to decrease with age in the Fischer-344 rat at concentrations ≥ 0.15 M and neural-response magnitudes increase at lower concentrations.

Introduction

Recent human psychophysical studies indicate small changes in taste as a function of age. For example, small but statistically significant increases in taste threshold for NaCl with aging have been demonstrated (see, for example, Grzegorczyk et al., 1979; Weiffenbach et al., 1982; Spitzer, 1988; Bartoshuk, 1989). Perception of suprathreshold concentrations often appears to suffer little or no loss with aging (see, for example, Byrd and Gertmann, 1959; Bartoshuk, 1989; Chauhan, 1989; Murphy and Gilmore, 1989). Comparisons of elderly and young adults show that elderly adults demonstrate higher preferences for certain foods containing suprathreshold NaCl concentrations (Murphy and Gilmore, 1989; Zallen et al., 1990), although no significant difference in their ability to detect the saltiness of these concentrations was observed.

Electrophysiological recordings comparing young (6-month) and old (24- and 30-month) Fischer-344 rats indicate that the slope of the NaCl response-concentration functions of old rats is flatter than that of young rats (McBride and Mistretta, 1986). On the basis of these and developmental data, McBride and Mistretta (1986) predicted that electrophysiological response frequencies to NaCl might decrease as a function of age.

The present study was undertaken to compare both behavioral and electrophysiological responses to NaCl in young and old Fischer-344 rats. These comparisons were made in the same individuals.

Materials and methods

Thirty-two male, Fischer-344 rats (Rattus norvegicus) were used in this study. Sixteen were classified as young rats (5-7 months old during the course of the experiment) and 16 as old rats (24-27 months old). All rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN). A 12:12 h light:dark cycle was in effect throughout their lifetime. At the onset of testing, the young rats weighed from 315 to 330 g, and the old rats weighed from 400 to 490 g.
Behavioral methods

Details of the apparatus and solutions. Each rat was tested individually in a suspended stainless-steel cage and, unless stated, had access to Purina Laboratory Chow (No. 5001) and tap water ad libitum. The control (tap water) and tastant (tap water + NaCl) solutions were presented in 250-ml bottles (fitted with stainless-steel sipper spouts) mounted on the front of the cage. Reagent-grade sodium chloride (Sigma Chemical Co.) was used.

Details of the preference-testing procedure. Taste responses to sodium chloride were assessed during three consecutive, two-bottle preference tests lasting 48 h each. In the first test, 0.05 M NaCl was paired with water. The positions of the water and salt solutions were reversed at the end of the first 24 h as a control for position preferences. In a similar manner 0.15 and 0.20 M NaCl were tested in the second and third tests. For each 48-h test, the preference ratio was calculated as the total amount of salt water consumed divided by the total liquid intake. Preference and aversion were defined as median preference ratios that were significantly above or below the indifference ratio of 0.50.

For determination of preferences and aversions, the Wilcoxon signed-ranks test was applied as a one-sample median test (Zar, 1984). For direct comparisons of old and young rats, Mann–Whitney U tests (with normal approximation) were performed separately for each concentration on the preference ratios and total amount of NaCl solution consumed. For assessment of whether NaCl concentration influenced the amount of water ingested, Friedman’s ANOVA by ranks was performed on total fluid intake across the three NaCl concentrations, separately for each age group.

Electrophysiological methods

Electrophysiological recording was begun upon completion of all behavioral testing. Eight of the old rats and seven of the young rats previously tested behaviorally were also tested electrophysiologically. The experimenter was blind with respect to the results of the behavioral tests.

Details of neural recording. The rats were anesthetized with chloropent (3 cm³/kg body weight for young rats; 2 cm³/kg body weight for old rats) administered intraperitoneally. Supplemental doses of chloropent were administered throughout the entire surgical and recording procedure. Rats were placed on a heating pad that maintained their body temperature between 36 and 38°C. A small, midline incision was made over the trachea, which was visualized and cannulated. The cannula was held in place by sutures holding the wound closed around it.

Each rat was secured in anatraumatic head holder, and a lateral opening was made just rostral and ventral to the pinna. The chorda tympani nerve was visualized, sectioned close to its exit from the tympanic bulla, dissected free from surrounding tissues, and desheathed. It was placed on a platinum electrode, and an indifferent electrode was placed in nearby tissue. The nerve was then covered with mineral oil to prevent desiccation.

The electrical activity from the entire chorda tympani nerve was fed through a high-impedance probe and a.c. preamplifier (Grass, P5 series), and the output was fed into an integrator, with a time constant of 1.0 s. attached to a pen recorder. In addition,
output signals from the preamplifier were displayed on an oscilloscope and fed into an audio monitor.

Upon completion of the electrophysiological recording session, each rat was immediately overdosed with sodium pentobarbital and perfused intracardially with physiological saline followed by Bouin’s fixative. The tongues of these animals were processed to be used in another study of aging.

*Details of chemical stimulation.* Taste stimuli at room temperature were applied to the dorsal surface of the anterior two-thirds of the tongue via a free gravity-flow system. A sample of 20–30 ml of each taste stimulus was allowed to flow over the tongue continuously at a flow rate of ~6 ml/s. Each taste solution remained on the tongue for 20–30 s, after which it was followed by at least one distilled water rinse of equal volume and flow rate. Additional rinses were applied if necessary until the integrated activity returned to baseline level. The next taste stimulus was not applied until a minimum of 60 s after the last water rinse.

The taste stimuli reported here consisted of an ascending series of NaCl concentrations: 0.025, 0.05, 0.1, 0.15, 0.25, 0.5 and 0.75 M. Each concentration was presented at least twice. For standardization purposes (see below), 0.1 M NaCl was re-applied frequently throughout the entire recording session.

*Details of the neural-response analysis.* The integrated response of the chorda tympani nerve to NaCl has an initial phasic response followed by an adapted (or steady-state) response. The integrated response magnitude was measured as the average height of the steady-state response for the first 10–15 s after stimulus application.

Because variables of the recording session (e.g. length of nerve exposed in air, possible damage during dissection) influence the absolute magnitude of the neural responses from different individuals, the absolute magnitudes of responses of different individuals cannot be directly compared. This problem is alleviated if one compares instead the ratios of the absolute response magnitudes to responses by the same individuals to a standard stimulus, such as 0.1 M NaCl or 0.1 M NH₄Cl. In this experiment such normalization of the data is confounded by the possibility that any apparent differences between age groups are due to age-related changes in the neural response to the standard. Therefore, 0.1 M NaCl was chosen as the standard for normalization because it produced approximately one-half of the maximum response. The normalized, repeated measures of the response to each concentration of NaCl were averaged across the entire recording session.

**Results**

*Behavioral results*

Old rats exhibited a significant preference for the 0.05 M NaCl solution ($T = 3; \ p \leq 0.01$) and an aversion to the 0.15 and 0.20 M solutions ($T \leq 3$ in both cases; $p \leq 0.01$). Young rats were indifferent to the 0.05 and 0.15 M solutions ($T \geq 10$ in both cases; $p \geq 0.05$) and showed a significant aversion to the 0.20 M solution ($T = 0; \ p \leq 0.01$) (Figure 1). Thus, the lowest concentrations at which young and
Fig. 1. Median preference ratios (A) and NaCl intake (B) in young ($n = 16$) and old ($n = 16$) rats for three concentrations of NaCl. Mann–Whitney U tests (with normal approximation) were used to compare young and old rats, separately for each parameter. An individual rat's score is represented by a circle. (A) A ratio of 0.5 (dashed line) represents no preference for water or NaCl solution; ratios above 0.5 indicate preference for NaCl solution, and those below 0.5 indicate aversion. Old rats exhibited a significant preference for the 0.05 M NaCl solution ($T = 3; P \leq 0.01$) and an aversion to the 0.15 and 0.20 M solutions ($T = 3; P \leq 0.01$). Young rats were indifferent to the 0.05 and 0.15 M solutions ($T \geq 10; P \geq 0.05$) and showed a significant aversion to the 0.20 M solution ($T = 0; P \leq 0.01$). The preference ratios of old rats for the 0.05 and 0.20 M NaCl solutions were significantly higher than those of young rats ($z > 2.53; P < 0.02$). Preference ratios of the age groups for the 0.15 M solution were statistically indistinguishable ($z = 1.32; P > 0.05$). (B) Total fluid (NaCl solution + water) intake was not influenced significantly by NaCl concentration in young ($\chi^2 = 1.56; d.f. = 2; P < 0.05$) or old ($\chi^2 = 2.38; d.f. = 2; P > 0.05$) rats. Relative amounts of water and NaCl solution ingested differed; old rats consumed significantly more NaCl solution than young rats ($P < 0.05$) in all cases.

Old rats displayed differences in their preferences for tap water and NaCl differed; that concentration was 0.05 M for old rats and 0.20 M for young ones.

The preference ratios of old rats for the 0.05 and 0.20 M NaCl solutions were significantly higher than those of young rats ($z > 2.53$ in both cases; $P < 0.02$). The preference ratios of the two age groups for the 0.15 M solution were statistically indistinguishable ($z = 1.32; P > 0.05$) (Figure 1A).

The total fluid intake (NaCl solution + water) over the three 48-hour test periods was not influenced significantly by NaCl concentration in young ($\chi^2 = 1.56; d.f. = 2; P < 0.05$) or old ($\chi^2 = 2.38; d.f. = 2; P > 0.05$) rats.
Behavioral and electrophysiological responses to NaCl

Fig. 2. Example of the electrophysiological record of integrated neural responses of the chorda tympani nerve in an old rat across seven concentrations of NaCl.

Fig. 3. Normalized, integrated response—concentration functions from chorda tympani recordings of old (n = 8) and young (n = 7) rats to NaCl (mean ± SE).

Fig. 4. Response—concentration functions for NaCl in individual young and old rats. Note the greater variance in young rats.
2; $P < 0.05$) or old ($x^2 = 2.38; \text{d.f.} = 2; P > 0.05$) rats. Consequently, the 48-h tests differed only in the relative amounts of water and NaCl solution ingested; old rats consumed significantly more NaCl solution than young rats ($P < 0.05$) in all cases (Figure 1B).

**Electrophysiological results**

Robust responses were produced by 0.1 M NaCl in the chorda tympani nerve of both young and old Fischer rats (see example in Figure 2). Figure 3 shows the mean response–concentration functions of young and old rats. The chorda tympani of the old rats appears to be less responsive to NaCl than that of young rats at the higher NaCl concentrations ($\geq 0.15$ M) and more responsive at the lower NaCl concentrations (Figure 3). Furthermore, the variability among individuals is greater for young rats than for old rats (Figure 4) (a trend that was not apparent in the behavioral data, see Figure 1).

**Discussion**

The data show that young and old Fischer-344 rats show electrophysiological and behavioral differences in response to NaCl. Electrophysiologically, the old rats appear to be more responsive than the young rats at the lowest concentrations and less responsive at the higher concentrations of NaCl (Figure 3). Because low concentrations of NaCl are preferred and high concentrations of NaCl are avoided by most rat strains (see, for example, Midkiff et al., 1987), this result would be expected to appear behaviorally as a greater intake of low and high concentrations of NaCl by the old rats than by the young ones. These expectations were supported by the behavioral data (Figure 1).

It has been shown that Fischer-344 rats (~60 days old) do not prefer NaCl at concentrations that are preferred by other strains (Midkiff et al., 1987). Taste reactivity data indicate that these differences are related to taste rather than post-ingestional processes and appear to be mediated by the chorda tympani nerve (see, for example, Grill and Bernstein, 1988; Sollars et al., 1991). The preference ratios and intakes of the young Fischer-344 rats in this study are similar to those of Midkiff et al.'s (1987) Fischer-344 rats, but the preference ratios and intakes of the old Fischer-344 rats in this study are similar to those of the non-Fischer strains tested by Midkiff et al. (1987). Thus, the age-related changes occurring in Fischer-344 rats appear to result in behavioral responses to NaCl that are similar to those of most strains of rats and dissimilar to those of young Fischer-344 rats.

Studies have shown that the difference in behaviorally and electrophysiologically measured NaCl responses between young Fischer-344 and Wistar rats is abolished by pretreatment with amiloride hydrochloride (Bernstein and Longley, 1990). Therefore, young Fischer-344 rats may be different from other strains in their amiloride-sensitive sodium-transport mechanism. If so, then the old Fischer rats may be demonstrating an age-related change in their amiloride-sensitive sodium transport mechanism that makes their NaCl responses appear to be similar to those of most rat strains.

The taste equation proposed by Beidler (1954) provides a conceptual framework for electrophysiological comparisons between young and old rats. It is a mathematical description of the theory that the magnitude of the neural response is proportional to
Table I. Calculated values of \( R_s \), \( K \) and \( KR_s \) from the taste adsorption theory for NaCl in old and young Fischer rats (Beidler, 1954). Age-class comparisons are made with unpaired t-tests.

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Old</th>
<th>z-Value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R_s )</td>
<td>2.1 (±0.2)</td>
<td>1.5 (±0.2)</td>
<td>-2.78</td>
<td>0.005</td>
</tr>
<tr>
<td>( K )</td>
<td>8.7 (±1.5)</td>
<td>17.2 (±9.5)</td>
<td>3.00</td>
<td>0.003</td>
</tr>
<tr>
<td>( KR_s )</td>
<td>17.2 (±3.0)</td>
<td>27.8 (±9.4)</td>
<td>2.67</td>
<td>0.008</td>
</tr>
</tbody>
</table>

The amount of stimulus adsorbed to the receptors. The mathematical expression is:

\[
R = \frac{CKR_s}{1 + CK}
\]

where \( R \) is the response magnitude to a stimulus of concentration \( C \), \( R_s \) is the maximum response and \( K \) is the association constant (or 1 divided by the concentration that produces a response magnitude that is 50% of the maximum response), which is a measure of the strength of binding. Because the sodium current in a channel is proportional to the interaction of the sodium ion with the channel itself, the taste equation can be expressed as:

\[
i_{Na} = \frac{i_{maxNa}CK}{1 + CK}
\]

If one assumes that the magnitude of neural response is proportional to the sodium current, then

\[
R_{Na} = \frac{CKR_{maxNa}}{1 + CK}
\]

Thus, the original taste equation can be directly applied to the sodium response transduced by a sodium channel. According to this formula, \( KR_s \) is proportional to 1/threshold. Linear plots of \( C/R \) versus \( C \) were constructed for each age group, and the Beidler taste equation was applied to the data. The median \( R_s \), \( K \) and \( KR_s \) values of young and old rats are compared in Table I.

It is apparent from Table I that young and old rats significantly differ in \( R_s \), \( K \) and \( KR_s \). Because young and old rats significantly differ in \( KR_s \), they may differ in NaCl threshold as well. This electrophysiological prediction was corroborated by the behavioral data: old rats showed a significant preference or aversion to the NaCl solution at concentrations to which the young rats were indifferent (i.e. 0.05 and 0.15 M), but the old rats were less responsive electrophysiologically to concentrations of NaCl above 0.15 M and consumed concomitantly more NaCl at 0.20 M than did the young ones.

McBride and Mistretta (1986) predicted that the response to NaCl should decrease as a function of age in Fischer-344 rats, on the basis of developmental data and the shape of the response—concentration functions they obtained from their neural recordings. In addition, they reported obtaining higher \( K \) values for NaCl in older rats than in younger rats; the present study obtained similarly higher \( K \) and lower \( R_s \) values for NaCl in old rats than in young rats.
In conclusion, NaCl neural-response magnitude and corresponding behavioral sensitivity decrease significantly with age in the Fischer-344 rat at concentrations greater than or equal to 0.15 M, and neural-response magnitudes increase at lower concentrations. The differences between young and old Fischer-344 rats may be due to some change in the NaCl 'receptors' (see Table I), possibly a decrease in their number, an increase in affinity, or an age-related change in the amiloride-sensitive sodium-transport mechanisms. Both amiloride-sensitive and amiloride-insensitive pathways have been described for adult rat taste cells, the latter contributing about one third of the total (Mistretta, 1991); but the sodium response of 12–13-day-old rat pups is not altered by amiloride. Perhaps the differences in sodium responses seen in the present experiments are also due to a change in the relative or absolute population of amiloride-sensitive channels. Bernstein and Longley (1990) proposed such a possibility to account for differences between the Fischer-344 and Wistar rat sodium preferences. Furthermore, rats appear to be different than humans in their NaCl discriminations in that the old rats are better at discriminating lower concentrations than are young rats, but worse at discriminating higher concentrations. Elderly humans, on the other hand, appear to be worse at threshold discriminations than young humans, but often equal young humans at suprathreshold concentrations.

References

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