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CHILDREN'S SENSITIVITY TO ODOR OF TRIMETHYLAMINE

EGIL H. SOLBU,[1] FINN KONOW JELLESTAD,[1] and KNUT OLAV STRÆTKVERN[2]

[1]*Department of Physiological Psychology*
[2]*Department of Biochemistry*
University of Bergen
Bergen, Norway

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Abstract—Findings in this paper show a strong correlation between subjects' age and their olfactory sensitivity to the "fishy" odor of trimethylamine, with youngest subjects being most sensitive and adult subjects least sensitive to this odor. This was due to a high percentage of highly sensitive subjects in the youngest age groups; this percentage decreased with age. Data further support the notion that trimethylamine sensitivity is independent of sex. The sensitivity to trimethylamine per se showed no significant covariations with the subjects' preferences for or aversions against fish as food and is probably of minor importance for fish food acceptability.

Key Words—Odor sensitivity, trimethylamine, children.

INTRODUCTION

A recent survey of a representative sample of Norwegian households has shown that 19% of the included children strongly rejected fish dinners, while adult household members on the average were more willing to eat fish (Jellestad and Solbu, 1987). These results call for both psychological and physiological explanations.

Trimethylamine (TMA) might be one significant olfactory cause for fish food rejection. It has a typical "fishy" odor in low concentrations (Standsby, 1962) and is a substantial contributor to the smell of dead fish (Jones, 1967). TMA occurs in dead saltwater fish as a bacterial degradation product, increasing in concentration as fish muscle decomposes (Huss, 1983; Smith et al., 1980).

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In living fish TMA is found as odorless TMA oxide (TMA-O). TMA-O may represent as much as 7% of the dry weight of some fish species, depending on the season and location of catch (Huss, 1983, p. 18). Certain species of bacteria split TMA-O into TMA and oxygen by anaerobic respiration. During the degradation process, free TMA accumulates in the muscle tissues, enabling the fish industry and fish food control authorities to use TMA as an indicator of fish freshness and quality (Shewan et al., 1971; Castell et al., 1974; Ritskes, 1975).

The average threshold of adults to the smell of TMA is very low. Leonardos et al. (1969) has found the TMA recognition threshold to be 0.00021 parts TMA to one million parts of air; this is the lowest threshold of the 53 commercially important odorants in their study, including hydrogen sulfide and methyl mercaptan. Amoore and Forrester (1976) have reported similar results using a more easily available sniff-bottle method, the average TMA threshold for adults being 0.0005 ppm (0.0005 parts TMA to one million parts of water).

As far as we know, children's sensitivity to the odor of TMA has not been published previously. Children are often claimed to have a more "sensitive nose" than adults. This postulate is not satisfactorily documented. On the other hand, the sense of smell is weakened during adulthood and may be considerably deteriorated in the elderly (Murphy, 1987, p. 253). If this developmental tendency begins in early childhood, the sensitivity of children to TMA should be measurably higher than for adults when these two groups are tested under similar conditions. This also opens the possibility that children might detect TMA in lower concentrations than adults when TMA is a deterioration product in raw fish muscle or a gas set free during cooking.

With this background, a study of children's sensitivity to the smell of TMA was deemed useful as a possible physiological contributor to the cause of fish food rejection and a possible documentation of the impression that children in general are more odor-sensitive than are adults.

METHODS AND MATERIALS

Subjects. This study originally included 356 subjects. Five were excluded because of major data loss or errors; the final sample consisted of 322 pupils and 29 adults. The children's group consisted of 168 girls (52.3%) and 153 boys (47.7%), their ages ranging from 6 to 16 years, the average being 11.0 years (SD 3.1). The sex classification for one subject was lost. Pupils from nine schools were represented in the sample.

The adult group consisted of 15 teacher-training students, eight psychology students, and five employees at the Institute of Physiological Psychology; 19 were women (65.5%) and 10 were men (34.5%). The age of the adult group was 20–42 years with a mean age of 24.5 years (SD 5.3).

All 351 subjects were tested during daytime in their respective school or work environments. No subjects were paid for their participation in the study.

Equipment. Glass tubes with an inner diameter of 15 mm and length of 18 cm were used for the odor sensitivity study. These were filled with 10 ml of liquid, raising the liquid level to 11.5 cm from the tube rim. All tubes had odorless airtight screw-caps permitting odorants to be prepared some hours in advance.

Four tube-rack sets of odorants were used, each of these containing 40 tubes and being easily transportable. The use of four sets made simultaneous testing of four subjects possible. Twenty tubes in a set contained TMA diluted in a buffer solution controlling the pH value, the remaining 20 tubes contained buffer solution only. Tubes were arranged pairwise with one TMA tube and one buffer tube. The pairs were numbered from 18 to 37 corresponding with the step of dilution of the TMA in the TMA tube, and the tubes were also coded with either a V (left) or an H (right), depending on their position in the tube pair.

Commercially available crystalline trimethylamine hydrochloride (Sigma) was used in the experiment. Numbered polypropylene beakers were used for the dilution series, one for every dilution step.

Dilution Steps and Buffer. Buffer was made by dissolving 20.02 g $KHCO_3$ in 1000 ml distilled water. This solution was adjusted with 2 M KOH to pH 9.74, the 50% ionization point (pK_a) for TMA. Bicarbonate buffer then was diluted to 0.1 M by adding 1 liter of distilled water.

As a starting point for the dilution series, TMA hydrochloride saturated in buffer was used, representing dilution step 0. Purified TMA has a solubility of 410,000 ppm (w/v) in water (Freier, 1976; Amoore and Forrester, 1976), while the chemical literature gives contradictory information as to the solubility of TMA hydrochloride.

Fifty microliters of saturated solution were added to 204.8 ml buffer, corresponding to dilution step 12. Four milliliters of step 12 was pipetted into 28 ml buffer (step 15). Twenty milliliters of step 15 was added in 140 ml buffer (step 18). A 50% dilution procedure was then used down to step 37 by taking out 50 ml and diluting this in 50 ml buffer in a numbered beaker, sealed with laboratory film, and mixed. Ten milliliters were then pipetted into four tubes which were immediately capped. Then 50 ml was taken out of the beaker and added to 50 ml buffer in a new beaker; the procedure with sealing, mixing, and pipetting being repeated down to dilution step 37.

Procedure. At the most, four test administrators were collecting data simultaneously, one in each corner of the room in use. Each test administrator had one set of tubes and a flask with buffer solution. Subjects were first asked to smell the buffer solution (this allowed an olfactory reference and an exchange of gas in the nasal cavities) and were told to take "one good sniff" of each

tube (this was demonstrated). They also were told that the two tubes smelled different and were instructed to point out the tube in each pair with the stronger odor. If they did not recognize any difference, they should report that the tubes smelled the same.

Tube racks were arranged so that the TMA concentration in the TMA tube was doubled every time a new pair was presented to the subject. Tubes were uncapped, the caps being placed on left or right marked paper sheets to prevent exchanging the caps by accident. The placing of TMA tubes in relation to buffer tubes in the racks was taken from a published randomization table (Gellermann, 1933). When presenting the tubes, the test administrator held one tube in each hand, keeping them at a good distance from each other. Subjects first smelled the left tube, then the right. Tubes were kept at a 1- to 2-cm distance from beneath the subject's nose, and the subject sat with arms at rest when not pointing out the tubes. Subjects were not allowed to try more than once at each dilution step.

Establishing Sensitivity. The criteria for establishing the subjects' sensitivity to TMA was five correct choices of tubes in a row, the sensitivity measure referring to the first and most diluted TMA concentration of these five correct choices.

Because of the time factor and the subjects' limited attention span, we could not allow subjects to smell more tubes than necessary. A code on the scoring sheet enabled the test administrator to end the test when a sensitivity measure was attained. For this reason the procedure was not double-blind. A rack with only buffer tubes was used to control for the lack of a double-blind procedure. Neither test administrator nor subjects were informed about this control set in advance. Four of 34 subjects (11.8%) got a "sensitivity measure" established with this set, despite both parties' expectation of the tubes being odorous. The chance of meeting criteria by randomized choices was 3.1%.

Test Localities. The experiments were mostly carried out in classrooms. Test conditions were determined by tube temperature (20–22°C), room temperature (19–22.5°C), facilities for handwashing, and ventilation. No rooms were smaller than ordinary classroom size. A subjective background odor evaluation was made, resulting in two occasions when background odor was easily detected when first entering these rooms. Results from subjects tested in these rooms were statistically compared with results from other subjects in the same age groups, revealing no significant differences, allowing these subjects to be included in the data analyses.

Additional Data. Immediately before the TMA sensitivity measurements, subjects were given a questionnaire or a structured interview concerning their fish food preferences and aversions, as well as questions more directly related to the sensitivity experiment. This was done to study possible relations between sensitivity measures and food habits.

Data Analysis. In the statistical analyses, the programs *t* test, Mann-Whitney U test, ANOVA, Kruskal-Wallis ANOVA by ranks, and correlation from the statistical packet CSS (Complete Statistical System) (StatSoft Inc., 1987, 1988) were used.

RESULTS

Results are based on 322 pupils. Thirty-four subjects were tested with the control set, leaving 288 subjects in the TMA sensitivity test. Of these, 33 (11.5%) failed to get a sensitivity measure according to current criteria and were excluded from the calculations, leaving 255 subjects (88.5%) with established sensitivity measures.

Figure 1 presents the children's frequency distribution on the dilution series. The children's group as a whole got an average score of 25.5. Thirty-one children and one adult obtained a TMA sensitivity score of 31 or higher. These "highly sensitive" subjects affected the age groups' average scores to a different degree, as presented in Figure 2.

In Figure 2 the sensitivity to TMA is expressed in dilution steps. Estimation of the real TMA concentrations (ppm; w/v) in the dilution series was difficult because of contradictory findings in the chemical literature, and, for the sake of simplicity, only dilution step results are presented.

The youngest age group obtained an average score of 28.2: TMA halved

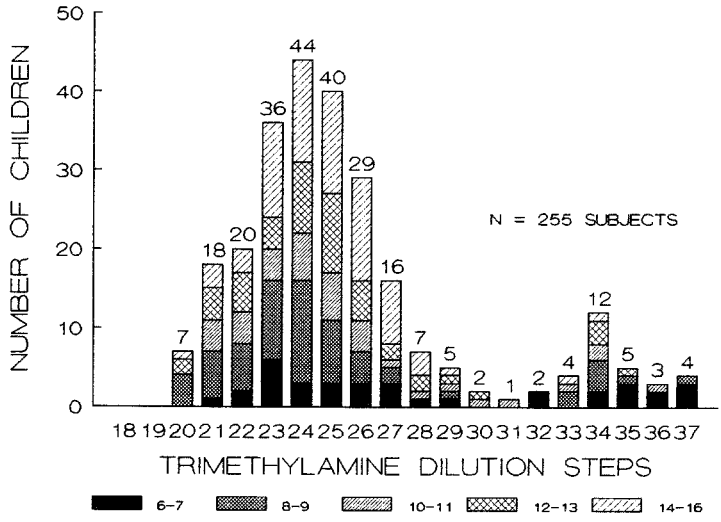

FIG. 1. Sensitivity of children to trimethylamine odor: frequency distribution.

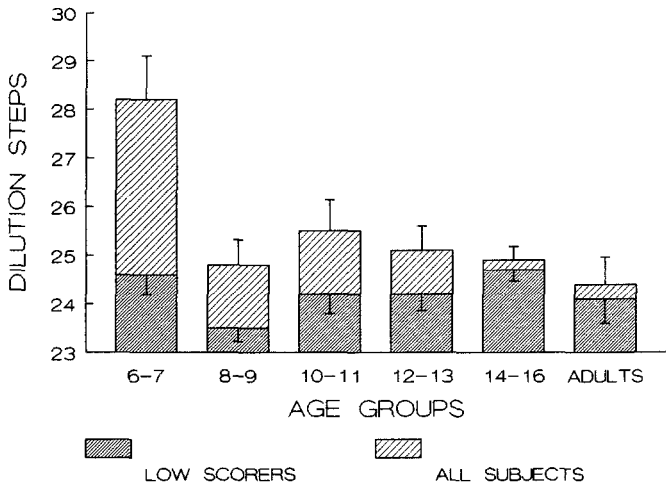

FIG. 2. Sensitivity of several age groups to trimethylamine odor: group means and standard error.

28.2 times. When subjects ages 6–7 with scores of 31–37 were taken out of the analysis, the average score dropped to 24.6, corresponding to the TMA saturated dilution halved 24.6 times.

The TMA sensitivity for the sample as a whole showed a significant negative correlation with age ($r = -0.16$, $P = 0.008$). A one-way analysis of variance showed a strong relation between age groups and the ability to smell TMA ($F = 5.40$, $P = 0.001$). Since rather large group standard deviations were found, a nonparametric Kruskal-Wallis analysis also was carried out. This supported the previously reported results ($H = 13.02$, $P = 0.01$).

The entire age group 6–7 years was significantly different from older pupils ($F = 20.99$, $P = 0.000$), and from the adult group ($F = 11.09$, $P = 0.002$). Children as a whole, however, were not significantly different from the adult group. Subjects ages 8–16 showed no statistically significant differences from adults in their sensitivity to TMA. With the high sensitivity group removed, no significant age differences were found exept for the subjects ages 8–9, who scored significantly lower than the 6- to 7-year-old group ($T = 2.3$, $P = 0.02$) and the 14- to 16-year-old group ($T = 3.6$, $P = 0.001$, two-tailed).

A closer view of the 32 subjects with scores 31–37 revealed a high percentage of "highly sensitives" in the 6- to 7-year-old group, the percentage decreasing with age. This is shown in Figure 3.

No significant sex differences were found concerning the subjects' ability to sense TMA. This is shown in Table 1 which also presents means, standard deviations, and standard errors for all age groups.

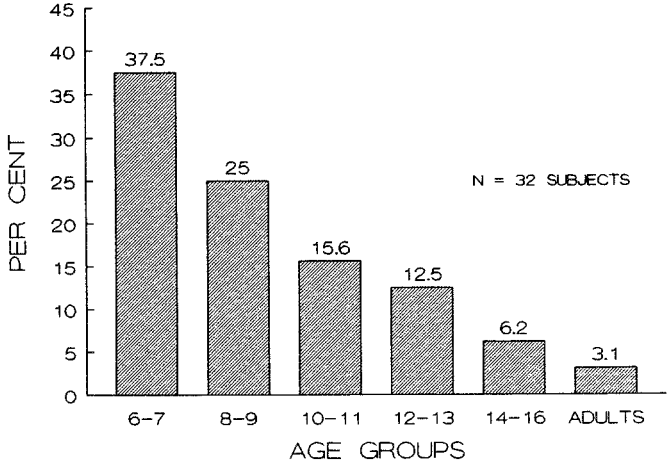

FIG. 3. Distribution of highly sensitive group (scores 31-37) by age.

TABLE 1. CHILDREN'S SENSITIVITY TO ODOR OF TRIMETHYLAMINE[a]

		All subjects				Low-sensitives only			
Sex	Age	M	SEM	SD	*N*	M	SEM	SD	*N*
Female	6-7	28.1		5.2	18				
Male		28.2		5.4	17 NS*				
Both		28.2	0.91	5.3	35	24.6	0.41	2.0	23
Female	8-9	24.5		3.7	26				
Male		25.1		4.3	36 NS*				
Both		24.8	0.52	4.1	62	23.5	0.27	1.9	54
Female	10-11	25.7		4.5	19				
Male		25.3		3.0	18 NS*				
Both		25.5	0.64	3.9	37	24.2	0.40	2.2	32
Female	12-13	24.9		3.2	32				
Male		25.3		4.0	17 NS*				
Both		25.1	0.50	3.5	49	24.2	0.34	2.3	45
Female	14-16	24.9		1.9	39				
Male		25.0		2.8	33 NS*				
Both		24.9	0.28	2.3	72	24.7	0.22	1.9	70
Female	Adults	24.1		3.0	18				
Male		24.9		2.7	10 NS*				
Both		24.4	0.56	2.9	28	24.1	0.50	2.6	27
Female	6-16	25.4		3.7	134				
Male		25.6		4.0	121 NS*				
Both		25.5	0.24	3.9	255				

[a] Arithmetic means (M), standard errors of the mean (SEM) and standard deviations (SD) for different age groups in the sample. *: *t* tests showed no significant sex differences.

Of the 288 pupils in the experimental group, 33 subjects failed to reach a TMA score. These were given the score 18, representing the strongest TMA concentration in this study and were included in the analyses of possible effects of food intake, spice, smoking cigarettes, and influenza on the sensitivity to the odor of TMA. None of the included variables were found to have a significant effect on TMA sensitivity.

Questions about smoking were only given to subjects who filled out a questionnaire on fish food preferences and rejections. Children given these questions at an interview normally were less than 13 years of age and were considered too young for smoking to be a relevant factor. One hundred twenty-six subjects (28 adults and 98 pupils ages 12–16) were asked about smoking habits, including smoking the last 60 min before testing. No significant interactions between smoking habits and TMA sensitivity were found. Adult smokers did not differ from nonsmokers concerning TMA sensitivity.

Food spice habits, as reported by the subjects, showed no significant variations with TMA sensitivity, nor did the use of lozenges, chewing gum, or food and fluid intake the last 60 min before testing. Quite surprisingly, the same lack of significant data was obtained when the sensitivity of children reporting a stuffy nose was compared with the sensitivity of subjects reporting not having stuffy noses.

Data on fish food preferences and aversions, as well as data on the frequency of fish dinners and amount of fish consumed, showed no connections with the TMA sensitivity data and will be published elsewhere.

DISCUSSION

The present study was carried out as a field experiment to ensure the accessibility of an adequate number of child subjects. The procedure was neither time consuming, frightening, nor too dependent on equipment. The chosen sensitivity criteria made similar experimental conditions available to both children and adults. The use of halving steps in the dilution series was chosen because this logarithmic scale gives the smell sensitivity distribution a nearly Gauss-shaped curve in a normal population without anosmic persons (Amoore et al., 1968).

We do not claim to have found optimal sensory thresholds, but a measure of the lowest concentration detectable under the given conditions. The lack of laboratory conditions and a significant difference in the dilution series procedure makes a direct comparison with the data reported by Amoore and Forrester (1976) difficult. A rather large part of the discrepancy in reported sensitivity measures between these studies is supposed to be due to the use of a saturated solution of TMA hydrochloride as the starting point for the dilution series in

the present study, while Amoore and Forrester (1976) refined their TMA by first extracting the free base from the hydrochloride with mineral oil.

Tereshchenko and Chemeris (1975) have reported the solubility of the hydrochloride to be 214 g/100 g water (corresponding to 1.3 million ppm TMA), while the Merck Index (1983) indicates a lower solubility than TMA without quoting values. Personal communication with Fluka Chemie AG, Switzerland, has supported the latter reference, indicating a solubility of 1 g TMA hydrochloride in 10 ml water (62,000 ppm TMA). Judged on a qualitative basis, TMA hydrochloride appears as less odor-intense than the free base (Merck Index, 1983).

The size of the air–liquid interface area will most likely also be of some importance to the degree of gas exchange and the level of gas concentration in the testing vessels. Amoore and Forrester (1976) presented their odorants in triangular shaped flasks which provided an estimated 15-fold larger gas evaporation surface compared to our test tubes. The distance from liquid level to the rim was roughly the same (9–11 cm), although the evaporated TMA contained in an Erlenmeyer flask will distribute in an air volume about four times that of a tube. The differences in testing vessel geometry could therefore account for differences in detection threshold ppm, given that the TMA concentration in the gas phase might have appeared more intense in the previous study. Nonconstant factors such as temperature fluctuations and shaking of the vessel will also influence the gas concentration, since TMA is a rather volatile substance in solution.

The present results show a strong correlation between age and TMA sensitivity, the youngest being most sensitive and adults least sensitive to the TMA smell, supporting a general notion within psychology and medicine that olfaction is weakened with age. Venstrom and Amoore (1968) reported a 50% decrease in sensitivity to 18 odorants over a 22-year adult age span in a cross-sectional study. Another cross-sectional study including 1.5 million readers of the *National Geographic* magazine also showed the smell sensitivity to six odorants to decrease with age (Gilbert and Wysocki, 1987).

Our data suggest that the TMA sensitivity only shows minor changes through the age group 8–16 years. This group was not significantly different from adults, although a decreasing tendency in sensitivity could be seen. These data indicate a similar tendency as those presented in *National Geographic* (Gilbert and Wysocki, 1987), where odor sensitivity only showed a slight fall for the ages 10–30 years.

When subjects with sensitivity scores 31–37 were removed from the analyses, age differences disappeared, showing that an unequal distribution of "highly sensitive" subjects in the different age groups accounts for most of the TMA sensitivity differences in this study. The mechanism for this unequal distribution, with a substantial proportion of highly sensitive subjects in the young-

est age groups, deserves investigation. One hypothetical explanation might be that a general wear-and-tear of the rhinal mucosa occurs with increasing age, which again might decrease the number of odor-sensitive persons and therefore lowers the average group sensitivity. Little is known about what is actually causing olfactory sensitivity changes in children and adolescents. Long-term studies should be carried out controlling for neurophysiological changes in the olfactory sense as well as for environmental and personal variables.

Our 8- to 9-year-old age group obtained a somewhat lower, although not significantly different, TMA sensitivity measure than did older groups of children. After removing high scorers from this group, the results became significantly different from the 6- to 7- and 14- to 16-year-old age groups. The 8- to 9-year-old age group consisted of pupils from both different schools and different classes, as did the other age groups. No significant differences between the classes in the 8- to 9-year-old year group were found.

Smoking was not found to interfere with the sensitivity to TMA. The lack of effects from smoking in general and smoking before sensitivity testing in particular have also been reported by Pangborn et al. (1967), Venstrom and Amoore (1968), and Vierling and Rock (1967). Whether or not smoking has an effect depends on the amount and duration of the smoking habit (Gilbert and Wysocki, 1987; Murphy, 1987). It is unlikely that smoking has had any significant effects on the results of our age groups.

Of the child subjects, 11.5% failed to have their sensitivity measure established. Some of these may be anosmic to the TMA odor, as reported by Amoore and Forrester (1976). Others may have lost their attention or interest in the test situation.

Our data clearly showed that the sexes do not differ in TMA sensitivity in any of the age groups studied. Neither Leonardos et al. (1969) nor Amoore and Forrester (1976) have reported results showing sex differences in TMA sensitivity. Our data do not support the possibility that TMA has a sexual signal function for humans as has been indicated for the red fox (Albone and Fox, 1971). The possibility remains that TMA has other signal functions perhaps tied to food poisoning. If man has a special sensitivity for TMA, as suggested by Amoore and Forrester (1976), it also may have a function in relation to avoiding contaminated fish food.

Norwegian fish food control authorities have established criteria for TMA-nitrogen concentrations in fish, allowing no more than 3 mg/100 g of cooled raw muscle and 5 mg/100 g of frozen muscle (Fiskeridirektoratet, 1986, pp. 41–42). Nitrogen constitutes 27.3% of the TMA molecular weight (The Merck Index, 1983), this being equivalent to 12.7 and 21.1 mg TMA (including minor amounts of mono- and dimethylamine) in 100 g of fish, respectively. This maximum concentration corresponds to 127 ppm (w/w) TMA in fish muscle. A considerable amount of the TMA will escape during meal preparation, either to

the air or to the water in the pan. If 1 kg of saltwater fish is cooked, the TMA concentration in the air most likely will exceed the average TMA odor detection threshold of both children and adults.

No relations between TMA sensitivity scores and frequency of fish dinners or fish food preferences and aversions were found in this study. Further studies should be carried out concerning children's subjective ratings of the TMA odor, this variable most likely being more important for fish food acceptability than the sensitivity per se.

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