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Effect of Temperature Change on Saccular Nerve Fiber Response in Goldfish

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by

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I would like to especially thank Dick Fay for all that he taught me about the real world of Science and experimentation; and for the encouragement that resulted in this thesis's completion. I would like to thank Bill Yost for all the work he has done over the years, both scientific and administrative, that has made the Parmly Hearing Institute the great institution that it is. Finally, I would like to thank Toby Dye for always extending his helpfulness and his friendship.

VITA

The author, Timothy J. Ream, was born on December 28, 1961 in Aurora, Illinois.

He attended Oswego Community High School in Oswego, Illinois; graduating in 1979.

In September of that year he enrolled at Creighton University in Omaha, Nebraska. In May, 1983, Mr. Ream graduated with honors. The degrees of Bachelor of Science in chemistry and Bachelor of Arts in Psychology were conferred.

In September, 1984, Mr. Ream was granted a research assistantship by the Parmly Hearing Institute of Loyola University of Chicago. After two years of study he took a leave of absence to travel to St. Monica's, Lesotho, Africa, where he taught high school for two years as a member of the United States Peace Corps. Upon his return in 1989, he was awarded the degree of Master of Arts.

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INTRODUCTION

Manipulation of metabolism is a useful method for studying normal functioning of the inner ear. Portions of the ear have been removed, fluids altered, toxins applied and oxygen supply reduced. In addition, the temperature of the ear has been altered as a means of exploring the system. Temperature manipulation is especially useful in that it is both non-invasive and reversible. This type of an experiment has been performed in every class of vertebrates, except fishes. These manipulations have produced results that are class-dependent.

A number of studies have measured the effects of temperature on the responses of primary afferent nerve fibers. The conclusions are largely the same regardless of the class of the specimen: temperature decreases are accompanied by decreases in spontaneous rate and increases in threshold values. Temperature does not affect the sharpness of tuning of a fiber. Such experiments have been performed on the pigeon (Schermuly and Klinke, 1985), the caiman (Smolders and Klinke, 1984), the gecko (Eatock and Manley, 1981; Klinke and Smolders, 1977), the toad (where such effects were observed for the amphibian papilla but not the basilar papilla; Moffat and Capranica, 1976) and frogs (where multi-unit activity in the torus semicircularis was measured; Hubl and Schneider, 1979; Mohneke and Schneider, 1979).

In these non-mammalian species, the additional effect of a shift in a fiber's best frequency was observed. Tuning curves (frequency threshold curves) for these fibers move up and down the frequency scale as temperature is increased or decreased respectively.

Studies on two mammalian species, the cat (high frequency fibers only; Klinke and Smolders, 1977) and the guinea pig (primary-like neurons in the ventral cochlear nucleus; Gummer and Klinke, 1983), however, have revealed similar effects, except that no significant effect of temperature on best frequency has been observed for mammals.

The difference in the way vertebrate auditory systems are affected by temperature suggests that tuning may be achieved in varying manners. The effect cannot be ascribed to the differences between poikilothermy and homoiothermy, since the warm-blooded pigeon behaves like the reptiles and the amphibians. Nor can the results be due to differences in middle-ear mechanics (Werner, 1983; Eatock and Manley, 1981). The different effects may be due to differences between the mammalian cochlea and non-mammalian hearing organs; at the level of the hair cells or more peripherally (Werner, 1983; Smolders and Klinke, 1984).

Eatock and Manley (1981) suggest that temperature may affect the electrical tuning of hair cells, possibly by altering channel-gating kinetics. This possibility would neatly account for the class difference with respect to temperature-induced best frequency shift. Crawford and Fettiplace (1981) demonstrated an electrical tuning mechanism in reptilian cochlear hair cells. Since then, electrical tuning has also been demonstrated in the amphibian sacculus (Lewis and Hudspeth, 1983) and the amphibian papilla (Pitchford and Ashmore, 1987)

and the bird cochlea (Fuchs, Nagai and Evans, 1988). Experimental evidence suggests that electrical tuning of hair cells is not present in mammalian species (Patuzzi and Robertson, 1988). Therefore, temperature-induced frequency shifts have been observed for the three classes of vertebrates where electrical tuning has been observed, and mammals, which do not possess electrical tuning, do not exhibit shifts in tuning curves with temperature changes.

As of yet, temperature manipulation has not been performed in fishes and the basis of tuning has not yet been determined. Among fishes, the hearing of the goldfish has been studied more than that of any other species (Fay 1988). In an effort to gain insight into the processes that govern the responsiveness of fibers in the goldfish auditory periphery, extracellular responses of primary afferents from the saccule of the goldfish were measured. The saccule, a vestibular organ in most vertebrates, responds to sound in fish. The goldfish is one of a number of fish species referred to as otophysans. Otophysans possess a specialized set of bones, the Weberian ossicles, which transmit sound vibrations from the swimbladder to the saccule (von Frisch, 1938).

Goldfish saccular fibers, like other primary auditory afferents, show frequency selectivity throughout the frequency range of hearing, at least up to one kilohertz. Saccular fibers show great variation in best sensitivity, best frequency, degree of tuning and spontaneous rate (Fay and Ream, 1986).

In light of the previous studies, it might be expected that the results obtained with goldfish in the present experiment should be

similar to the results obtained with other vertebrates. With decreasing temperature, it is expected that spontaneous rate would drop, sensitivity would decrease and degree of tuning would remain the same. With the lack of information regarding the basis of neural tuning in the goldfish, there is no basis for predicting the presence or absence of a frequency shift.

METHOD

A. Animals

Sixteen saccular fibers were sampled from 6 common goldfish (Carassius auratus), that were about 9 to 13 cm in length from snout to tail. The animals were obtained from local suppliers and maintained in large communal tanks in the lab for up to a year.

In preparation for recording, an animal was immobilized with a 1 μg/g body weight intramuscular injection of flaxedil and anesthetized by respirating with 1:3000 MS-222. The animal was placed in a small cylindrical (20 X 20 cm) water tank, and its head clamped onto a brass tube through which water flowed (0.25 l/min) into the mouth and over the gills for respiration (described in Fay, 1978). The animal was positioned in the center of the tank with its head tipped up at about 30 degrees, with only the dorsal surface of the skull above the water. The skull overlying the hind-brain was removed, the medulla and cerebellum retracted, and the saccular nerve exposed. A micropipet filled with 3 M KCl was positioned over the nerve under visual control, and was advanced during the experiment with a hydraulic microdrive. The electrode output was amplified by a Grass P511 preamplifier with a line filter, within a 300 to 3000 Hz band. Spikes were converted to standard 0.2 msec TTL pulses by an Ortec voltage-level discriminator.

B. Sound field and calibration

Sinusoids were created by a Wavetek 184 function generator. Frequency was programmed by a DC voltage from a digital-to-analog converter to the voltage-controlled generator. Amplitude was controlled by a Coulborn programmable attenuator, and envelope determined by a Coulborn electronic switch. Electronic signals were amplified by a Crown power amplifier, and transduced by a University UW-3 underwater loudspeaker. The speaker was placed at the bottom of the cylindrical water tank and covered with coarse sand. The sand smoothed out the tank's frequency response.

Sound-pressure levels were measured at the beginning of the experiment using a Gould CH-17 calibrated hydrophone suspended in the tank in the approximate position of the fish. Calibration was done automatically by sequentially presenting frequencies from 100-1300 Hz in 10-Hz increments and digitally recording the amplified hydrophone output. The rms voltage was calculated from the digital records. The average of six calibration curves, each obtained with the hydrophone in a different position within the space normally occupied by the fish, defined the standard tank calibration used for all animals.

The water tank rested on a steel plate, which was on a 6-cm thick limestone slab suspended in an IAC 400 series room on pneumatic pistons.

C. Temperature Manipulation

The saccule, the primary auditory receptor organ of the goldfish, is located a few millimeters ventral and posterior to the saccular nerve as described above. There is a hole in this bony shelf which allows passage of the saccular nerve. The location and small size of the sacculus make direct temperature manipulations and measurements impossible. For these reasons, temperature of the head region was altered by manipulating the temperature of the respirator water. Temperature was measured in the cranial cavity. The heat-conductive brass respirator tube, which articulated with the roof of the mouth, and the respirator water, which flowed across the gills, were able to change the cranial temperature over a range of 10°C. Temperature was constant in all parts of the cranium. It is assumed that the temperature of the closely located sacculus was equivalent to the temperature throughout the cranial cavity.

Throughout most of the experimental procedure the animal was immersed in water at the same temperature as the tank in which it was housed prior to testing. This ambient temperature varied slightly on a daily basis but remained within the range of 18 to 20°C. The respirator flow water was also at this temperature. After a fiber was identified and its frequency-intensity response area was mapped (as explained below), the respirator water was heated or cooled. Heating was accomplished by passing current through heat-conducting tape which was wrapped around a glass condenser pipe that was a part of the respiration apparatus. Cooling was accomplished by directing a larger

portion of the respirator water through a radiator which sat in a tank of ice and water. Neither process altered flow rate. Once measurements were complete (usually eight to twelve minutes) the temperature of the respirator water was returned to ambient, thus preventing significant changes in the experimental tank temperature.

An experiment performed on three animals demonstrated that once respirator flow temperature stabilized at either the hot or cold extreme or ambient, a wait of two minutes was necessary for the cranial cavity temperature to stabilize. However, it can be seen in Fig. 1 that the stabilized temperature of the cranial cavity never exhibits as great a change from ambient temperature as that of the respirator water.

D. Frequency and Intensity Response Mapping

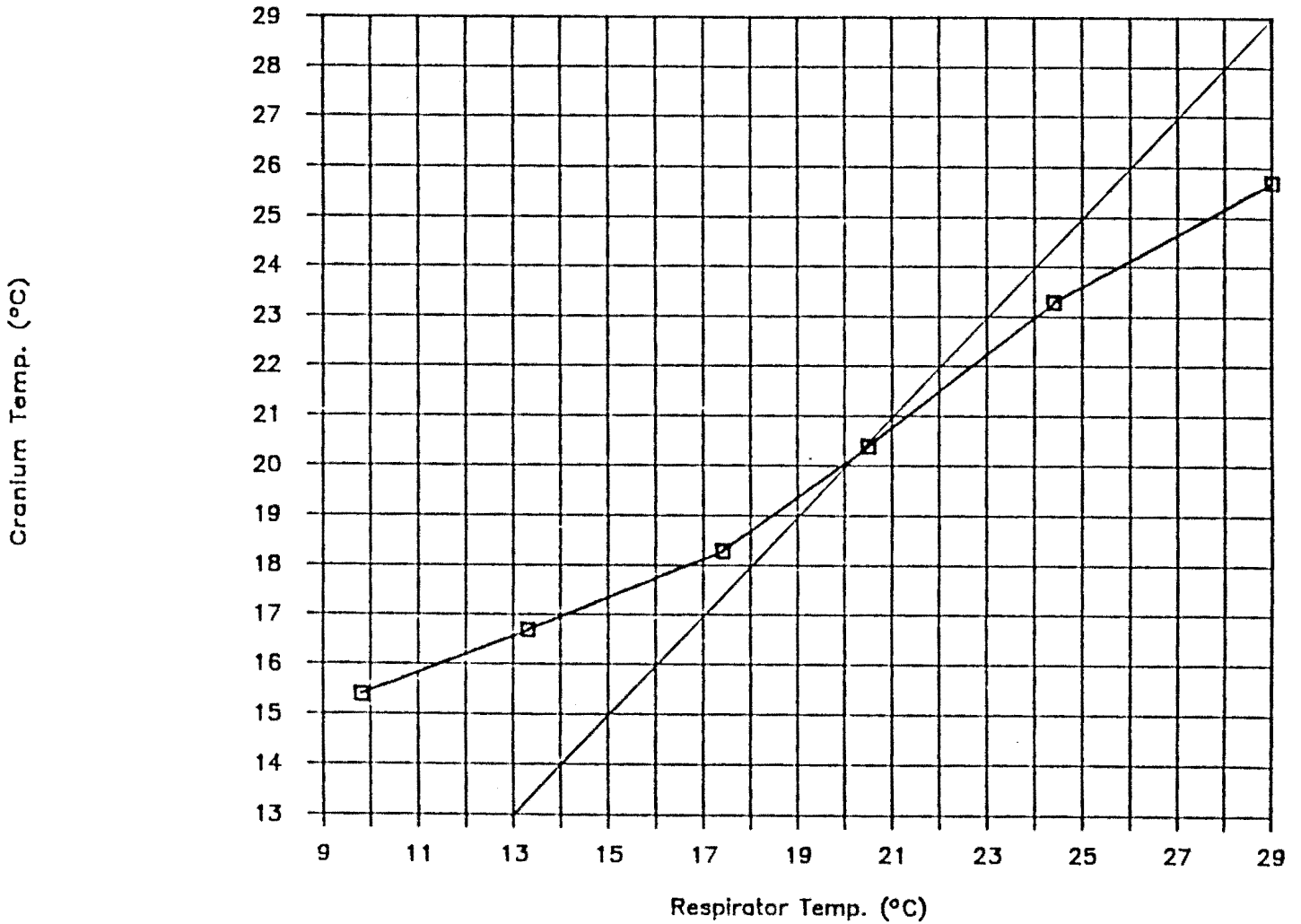
The search for fibers was performed at ambient temperature. The search stimulus was a 50 msec tone burst of 300 Hz alternating with one of 600 Hz presented twice per second at about 50 dB (re: 1 dyne/cm^2).

Once a fiber was contacted, the first measure taken was the rate of spontaneous activity. Spikes were counted in two-hundred 25 msec bins for a total sample duration of five seconds. From this sample, the mean and standard deviation of spontaneous rate were determined.

Next, calibrated signals at 20 frequencies, from 100 to 1300 Hz, evenly-spaced by one-seventh of an octave steps, were randomly presented at a level below the suspected threshold at best frequency. Signals were 50 msec in duration with 10 msec rise-fall times. Spikes were counted in two 25 msec windows during signal presentation and counts

Figure 1. Temperature of the cranium as a function of respirator water temperature. Temperature of the ear could not be measured directly but was instead inferred from the relationship of the temperature in the cranium to the respirator flow temperature. The straight line represents the hypothetical situation where cranium temperature follows respirator temperature perfectly.

Temperature Calibration Curve



were graphically displayed on-line. After presentation of a frequency series, the intensity level was incremented by 5 dB and the process of random frequency presentation was repeated. This continued until an upper level, usually 35 dB (re: 1 dyne/cm^2) was reached. This process was repeated a second and occasionally a third time when spike definition was poor or spontaneous rate was high.

The respirator flow temperature was then lowered or raised. The new temperature stabilized near either 10 or 27°C in three to six minutes. This was followed by a two minute wait before neural responses were measured. The measurement procedure was then repeated two or three times. Upon completion, temperature was returned to ambient, and, after the requisite wait, a new set of measures were taken. This procedure was then repeated using a temperature change in the opposite direction. A full cycle of measurements could be characterized as ambient-extreme-ambient-opposite-ambient. It should be noted that this is a somewhat ideal description, as units were frequently lost during the procedure. Many of the data that follow were gathered from units that only lasted through an ambient-extreme or ambient-extreme-ambient portion of the cycle.

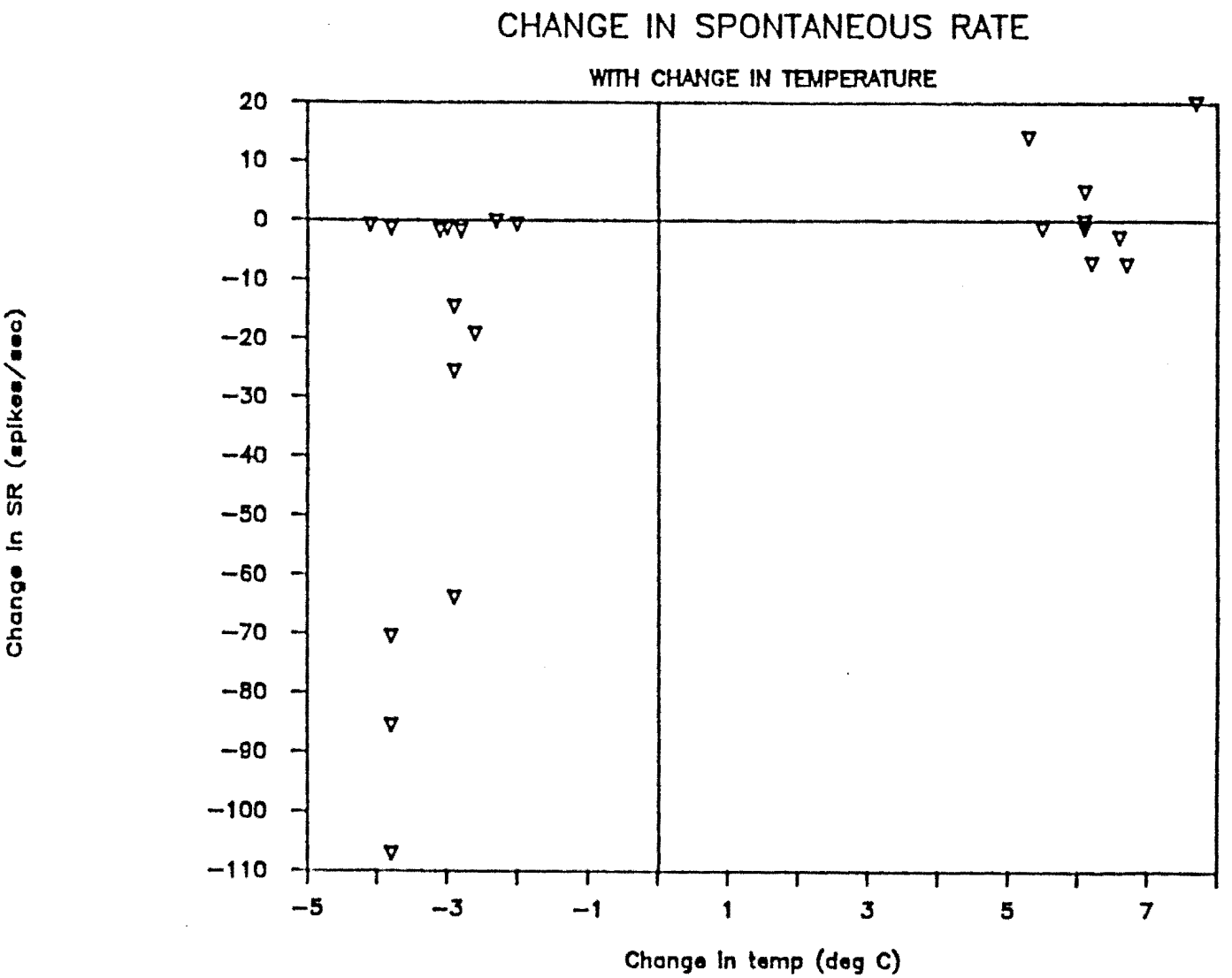
RESULTS

The temperature effects reported below all seem to be reversible. Ambient temperature response areas mapped after a temperature manipulation are similar to those initially mapped. Therefore all results obtained at a specific temperature were averaged.

An initial analysis of the data showed that the effects of raising the temperature from ambient were not simply the opposites of lowering it from ambient. These may have been genuine differences or may have resulted from differences among those fibers that were only measured at one temperature extreme or the other. To sort out this problem, the six fibers measured at both extremes were separately evaluated. This evaluation suggested that the magnitude of the spontaneous rate and sensitivity changes were different for the ambient-cold and the ambient-hot manipulations. The degree of change in best frequency, frequency at best sensitivity, was constant throughout the 15°C to 25°C temperature range.

Spontaneous rate was reduced with decreasing temperature. For the ambient to cold temperature change, the rate was reduced an average of 9.0 spikes/sec/°C. In addition, spontaneous rate increased slightly with increasing temperatures. At temperatures higher than ambient, spontaneous rate increased an average of 0.33 spikes/sec/°C.

Figure 2. Changes in spontaneous rate as a function of the change in temperature. Spontaneous rate increases with temperature. Temperature is expressed as a change from ambient (approximately 19o C). Many of the values near zero spike rate are from units with low spontaneous rates that dropped to zero with cooling.


CHANGE IN SPONTANEOUS RATE
WITH CHANGE IN TEMPERATURE
Change in SR (spikes/sec)
20
10
0
−10
−20
−30
−40
−50
−60
−70
−80
−90
−100
−110
−5
−3
−1
1
3
5
7
Change in temp (deg C)

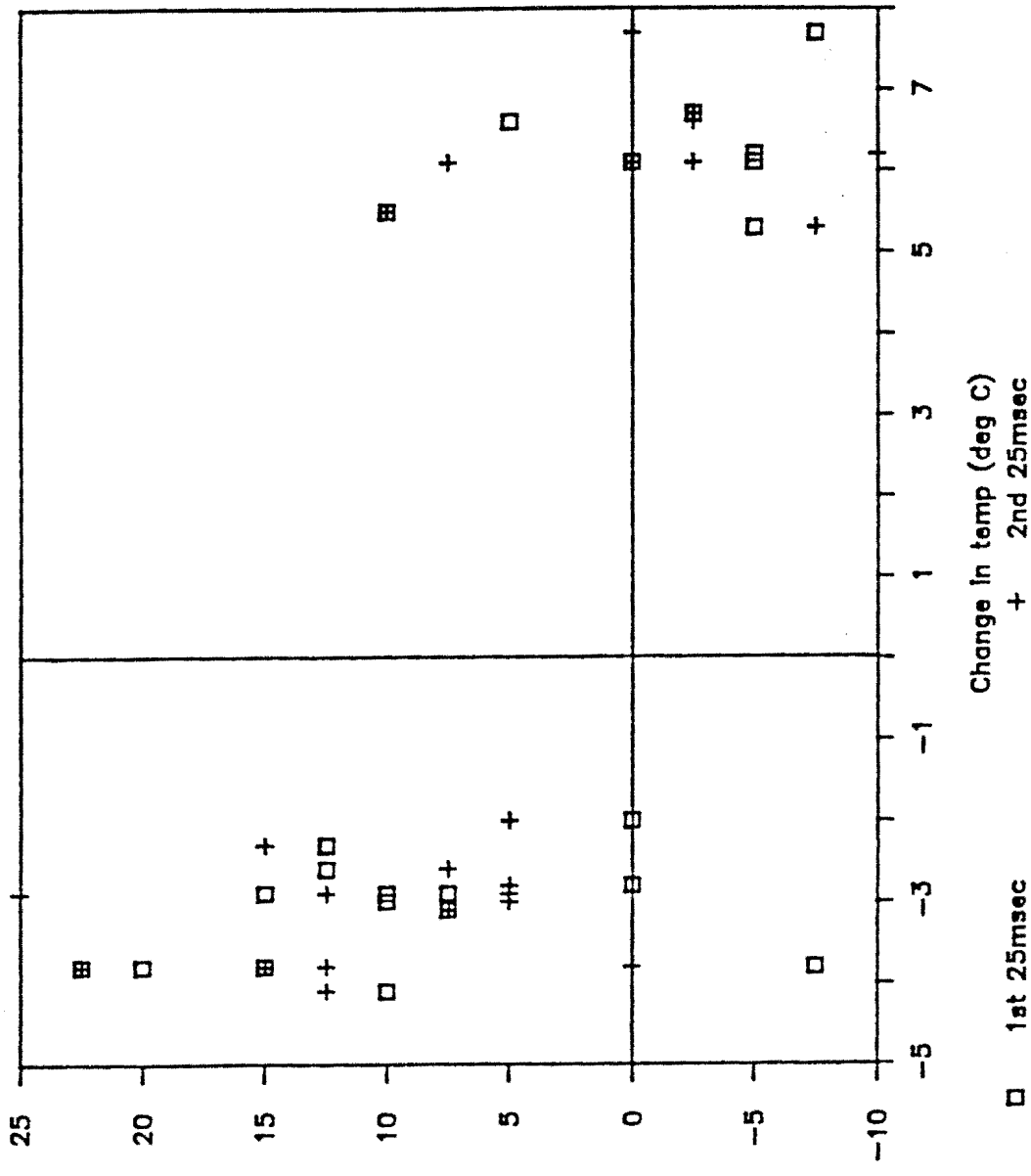
Best sensitivity was defined as the lowest intensity value at which a spike rate of two standard deviations above the mean spontaneous rate was evoked. Using this criterion, a frequency-intensity response area could be mapped. At any given frequency-intensity point outside the area of significant response, there was a statistical possibility of a spike rate occurring at a rate higher than this two standard deviation criterion. Such points were not considered part of the response area unless the point one seventh of an octave higher or lower, or the point 5 dB higher also showed significant response. In other words, a lone point of significant response was not considered significant.

Best sensitivity values decreased with decreasing temperatures. For the 16 fibers measured, an average threshold increase of 3.1 dB/°C was observed for temperatures below ambient. Fibers became only slightly more sensitive as temperatures were raised above ambient. An average threshold decrease of 0.2 dB/°C occurred at the higher temperatures.

Best frequency was defined as the frequency at best sensitivity. The values of best frequency, measured during the first 25 msec of the stimulus, decreased as temperature dropped. The average drop was 10.4 Hz/°C. As explained above, the effect was the same for both increases and decreases form ambient within the 10°C temperature span.

The degree of tuning was assessed with Q_{10dB} values. Q_{10dB} is the ratio of best frequency divided by the bandwidth at 10 dB above best sensitivity. There was no significant change in the values of Q_{10dB} as a function of changing temperature.

Figure 3. Best sensitivity thresholds as a function of the change in temperature. Sensitivity increased with temperature. Temperature is expressed as a change from ambient. Data are shown for estimates taken from both the first half and second half of the 50 msec stimulus.


25
20
15
10
5
0
−5
−10
−5
−3
−1
1
3
5
7
Change in temp (deg C)
+ 2nd 25msec
□ 1st 25msec
Change in BS (dB)

The responses of goldfish primary auditory afferents are somewhat different than the responses of other vertebrates. Relative to the other species studied, the goldfish has fibers with higher spontaneous rates, broader tuning, no tuning curve "tips," and a large set of fibers which have an essentially low pass tuning characteristic. For these reasons four fibers were selected from the sixteen measured and a more complete analysis was performed. The four fibers were selected to represent the full range of observed effects.

Figures 4 through 7 represent raw data on these four fibers. Figures of this type for additional fibers are included in Appendix A. The panels on the left of the figure show measurements made during the first 25 msec of the stimulus. The panels on the right show measurements made during the second 25 msec. The lower sections represent lower temperatures (15.1 to 16.3°C). The upper sections represent higher temperatures (23.3 to 25.9°C). The middle section refers to data taken at ambient temperatures (18.3 to 20.4°C). Notice that for two of the four fibers, only ambient and one other temperature were measured.

The ordinate represents stimulus intensity in dB (re: 1 dyne/cm^2). The abscissa is a logarithmic frequency scale. The linear dimensions of the squares that are the data points represent average spike counts for a given frequency-intensity point. Note that this serves to somewhat "square" the visual effect as spike rate increases. The number of spikes/sec can be approximated by looking at the five-point scale provided at the bottom of the figure. In the bottom left-hand corner of some of the boxes is a tiny "tick mark". These marks have been

Figure 4. This and the following three figures show spike rate plotted as a function of frequency and intensity of a tonal stimulus. Plots of Unit 6 response areas were made at three different temperatures. Warmest temperatures are the uppermost panels. Right and left-hand panels represent the first and second 25 msec of the stimulus. Linear dimensions of the small boxes indicate average spike counts for a specific frequency and intensity point. Estimates of these rates can be made with the Spikes/second scales located at the bottom of the diagram.

FREQUENCY THRESHOLD CURVES

UNIT # 6

Portion of the Tonal Stimulus

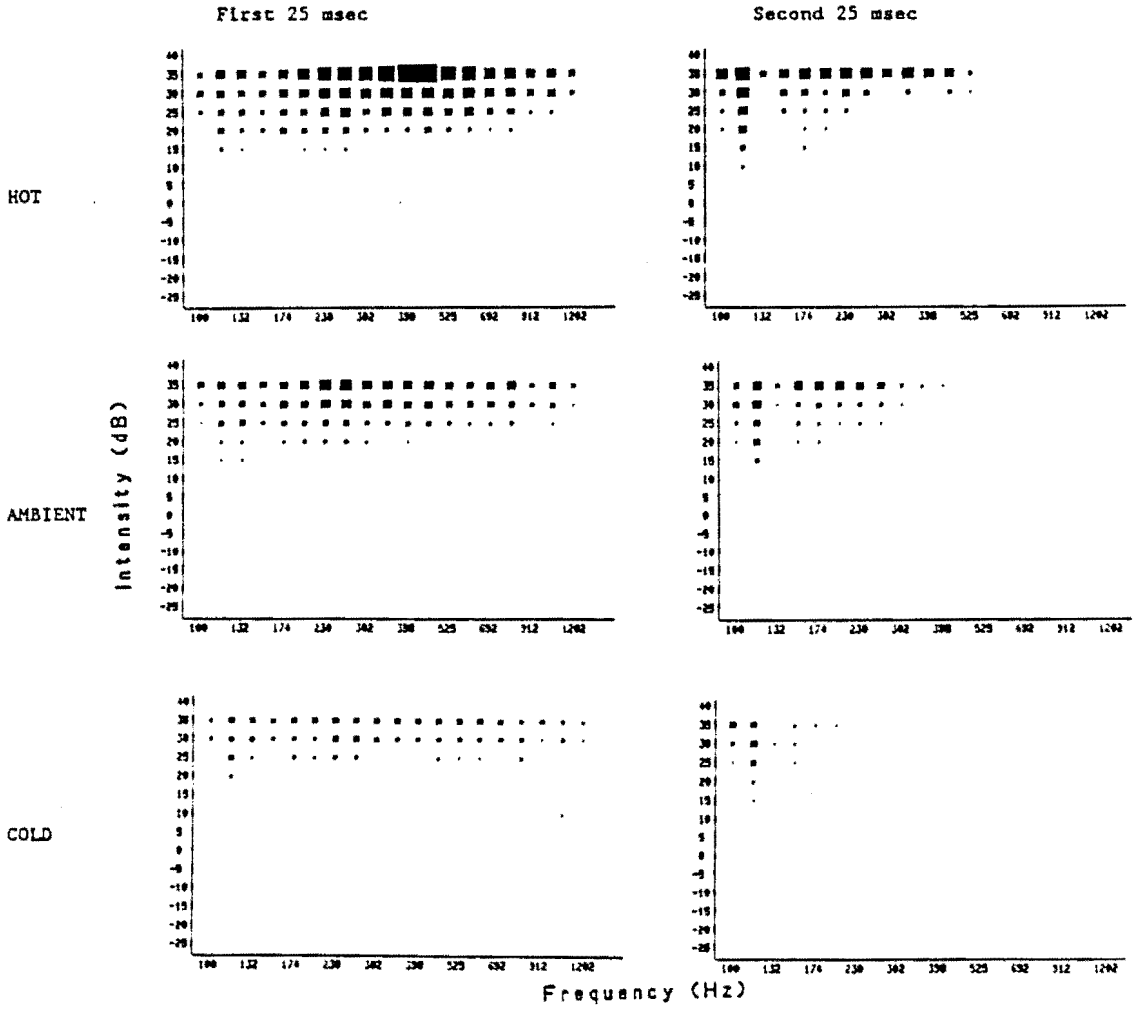
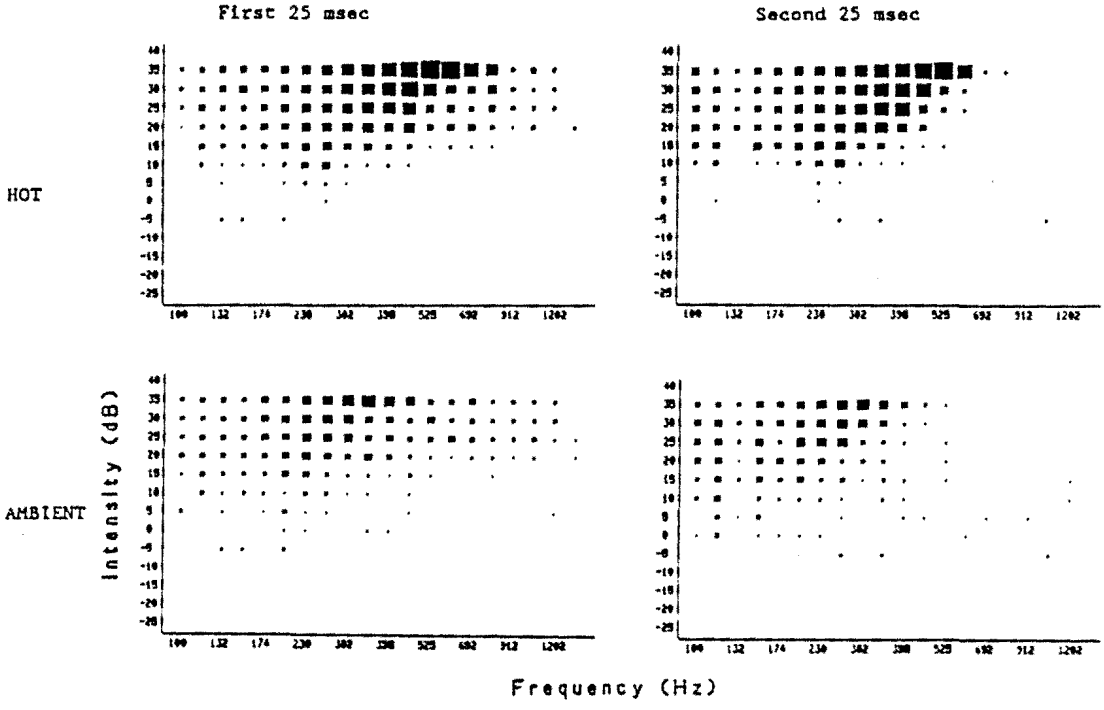


Figure 5. Plots of Unit 21 response areas taken at two different temperatures.

FREQUENCY THRESHOLD CURVES

UNIT # 21

Portion of the Tonal Stimulus



placed on all boxes which have spike counts at least two standard deviations above the mean spontaneous rate. Note that for a silent fiber such as Fiber 6 in figure 4, all boxes have marks.

The area of responding for Fiber 6 shows it to be a broadly tuned low-pass fiber. Looking at the right-hand panels one can note the lack of sustained firing at the higher frequencies. Note also the difficulty in defining a best frequency. As temperature declines, the primary effect is a large reduction in the rates of firing. This reduction is greatest at the higher frequencies.

Fiber 21 in figure 5 is another fiber of the low-pass type, tuned to about 200 Hz. Its spontaneous rate is 2.8 spikes/sec. When spontaneous rates are above zero there may be areas of responding in which rates drop below spontaneous. Such areas can be observed in the following three cases by looking at the higher frequencies in the right-hand panels.

For Fiber 21, as temperature changes, a 45 Hz change in best frequency, from 247 Hz to 202 Hz, can be observed during the first half of the stimulus. An even greater change is evident during the latter portion of the stimulus. However, the more striking change is the change in the sound frequency producing a maximum response at the highest intensity measured. This value changes from 525 Hz to about 350 Hz as the temperature drops from 25.2°C to 18.6°C.

Due to higher levels of spontaneous activity, Fiber 40 (fig. 6) provides an instance where the response area can not be easily visually identified. Only by connecting the lowest intensity tick marks at each frequency can one identify the response area. Fiber 40 is considered

Figure 6. Plots of Unit 40 response areas taken at three different temperatures.

FREQUENCY THRESHOLD CURVES

UNIT # 40

Portion of the Tonal Stimulus

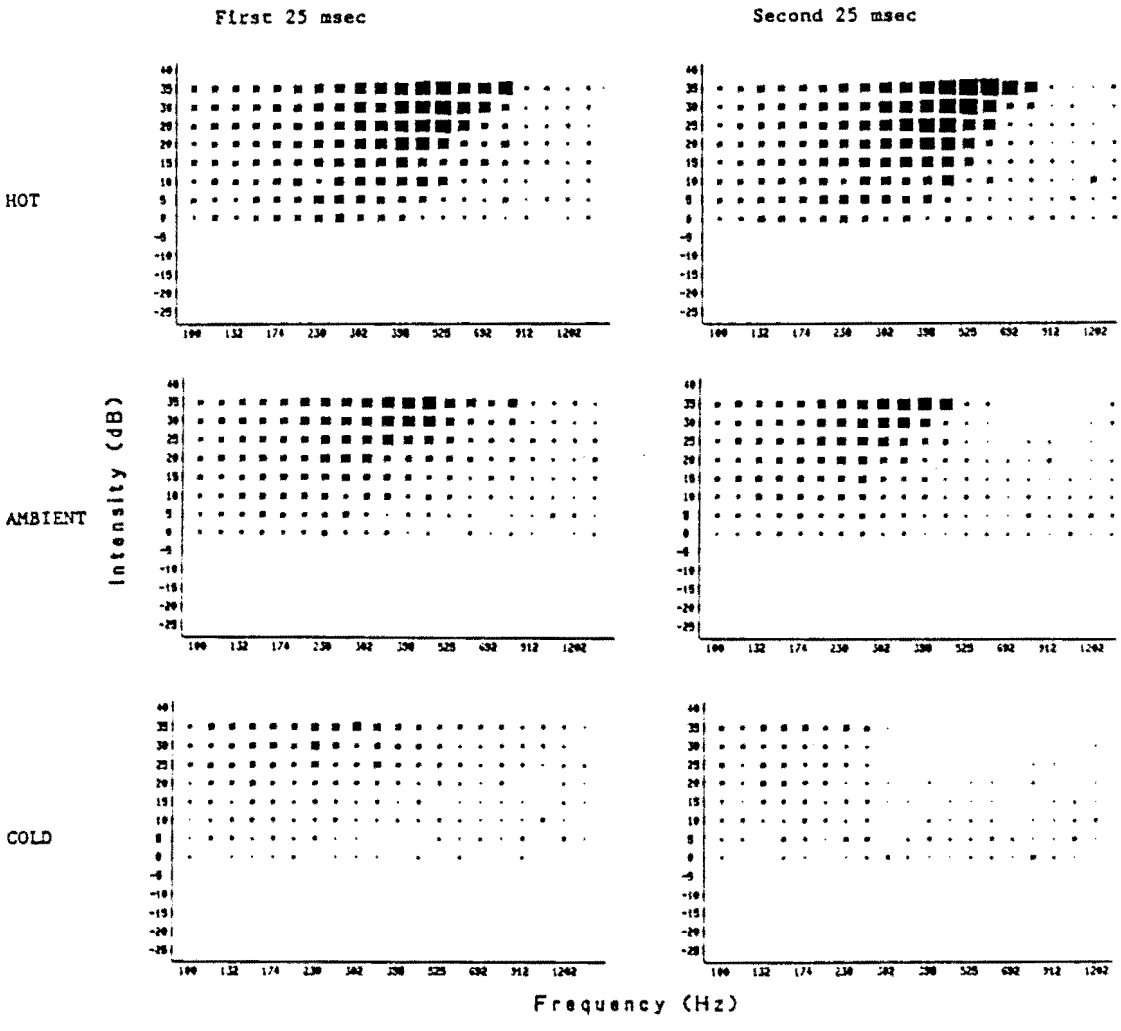


Figure 7. Plots of Unit 47 response areas taken at two different temperatures.

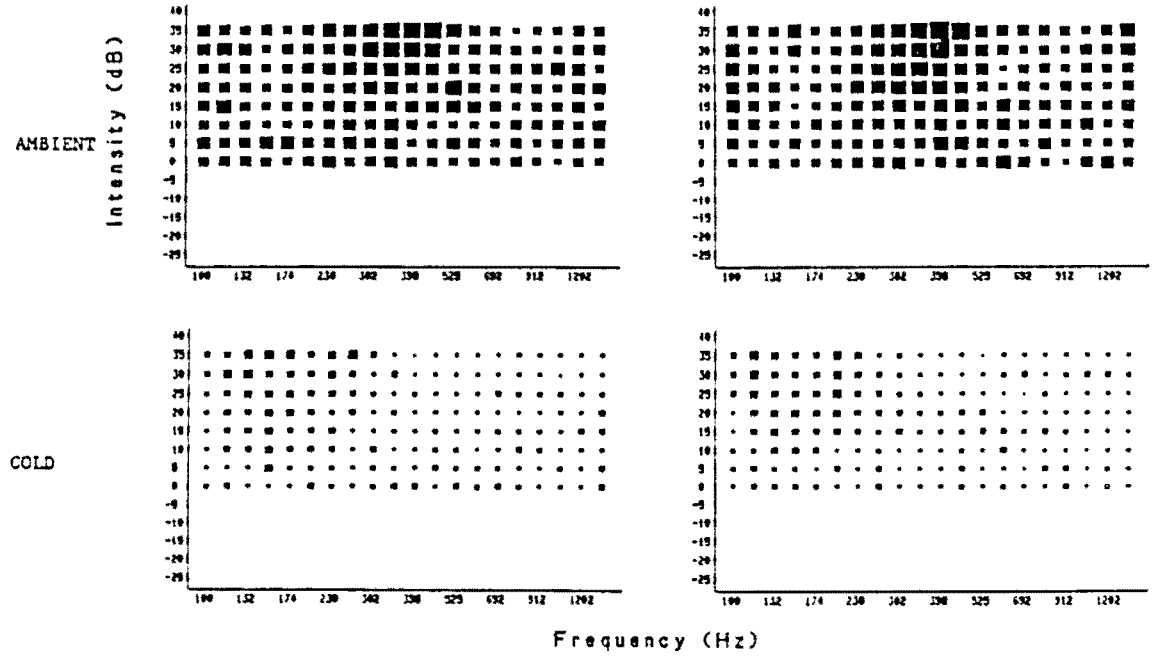
FREQUENCY THRESHOLD CURVES

UNIT # 47

Portion of the Tonal Stimulus

First 25 msec

Second 25 msec



a low-frequency, "bowl-shaped" fiber at the ambient and hot temperatures. Its spontaneous activity ranges from 60 to 27 spikes/sec as the temperature drops. The most striking feature about this fiber is the extent to which a signal produces firing below the level of spontaneous rate, especially for the higher frequencies at the lower temperatures. Note that the area of greatest sustained firing in the hot condition is an area of little or no response at the cold condition. A few frequency-intensity pairs which could produce sustained firing at rates exceeding 400 spikes/sec can evoke no more than a few spikes after cooling by 7.9°C.

Fiber 47 (fig. 7) produced the largest change in best frequency in the sample. Cooling 3.8°C produced a 189 Hz change, from 151 Hz to 340 Hz. This fiber, at ambient temperature, can be classified as a mid-frequency bowl-shaped fiber. This fiber is a high spontaneous fiber, with an ambient spontaneous rate of 200 spikes/sec. It demonstrates the difficulty of using an "increment above spontaneous rate" criterion when recording from high-spontaneous, low-frequency fibers. At the higher intensities, this fiber is responding below its spontaneous rate in the 150 to 200 Hz range. The fiber is simply following the stimulus with one spike per cycle. Unfortunately, any rate criterion would reject such a fiber as "insensitive" to the tone. Below 150 Hz, the rate of firing rises to the spontaneous rate. This type of a problem makes identifying a response area or even selecting a best frequency difficult. When the fiber is cooled, the spontaneous activity drops to 92 spikes/sec and this problem disappears.

For each of these fibers the best frequencies, as defined above, changed as temperature changed. To decide whether this was actually a frequency shift equivalent to those reported in non-mammalian vertebrate studies, figures 8 through 11 were produced.

These graphs (figs. 8-11) plot spike rate versus frequency for a given intensity and different temperatures. As many as three equally spaced intensities were plotted for each fiber. There is a striking generalization that can be made from such graphs. There is no instance where, for a given frequency and intensity, a fiber's spike rate is incremented as temperature is lowered. As temperature decreases, fibers become less responsive overall. However, this decrement in responsiveness is greatest at higher frequencies. It will be shown below that this is not what studies involving other non-mammalian vertebrates have demonstrated.

Figure 8. An iso-intensity series for Unit 6 showing spike rate as a function of frequency at three different temperatures.

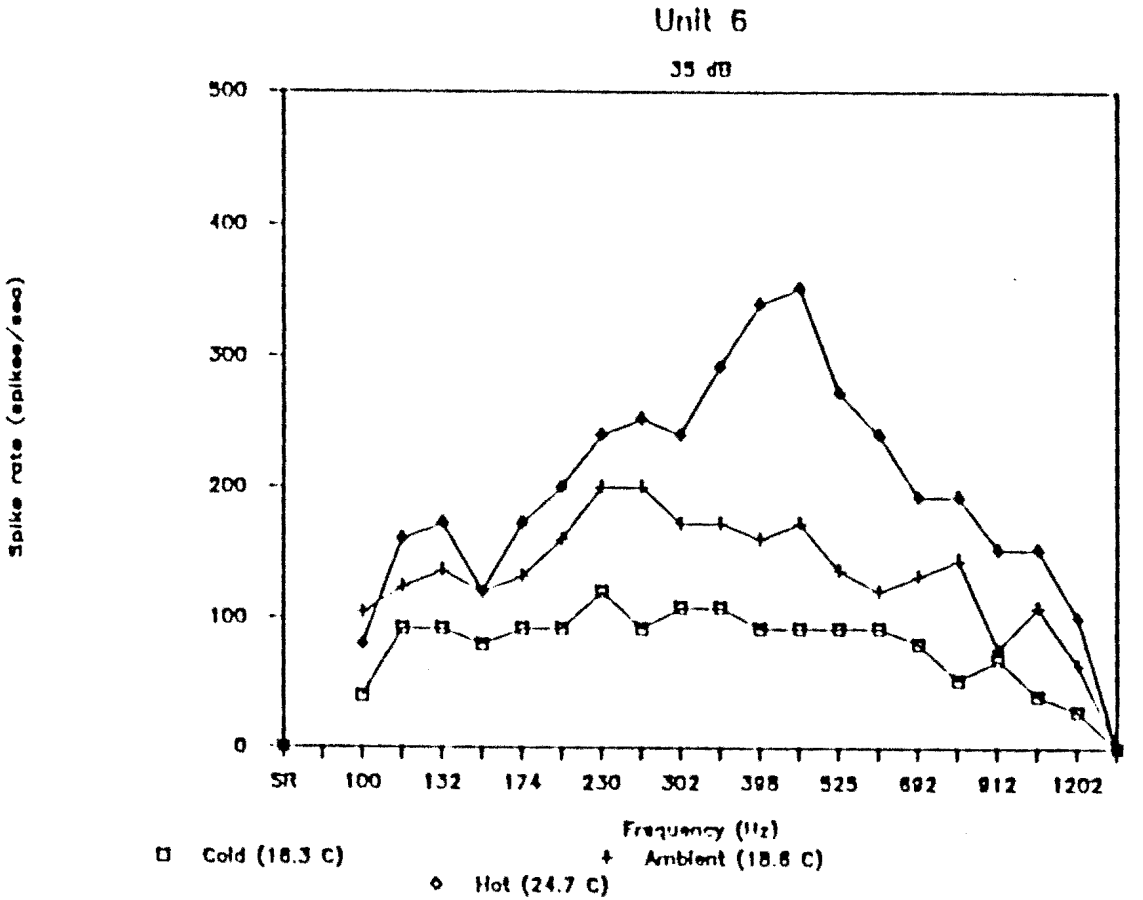

Unit 6
35 dB
Spike rate (spikes/sec)
500
400
300
200
100
0
SR
100
132
174
230
302
398
525
692
912
1202
Frequency (Hz)
Cold (16.3 C)
Ambient (18.6 C)
Hot (24.7 C)

Figure 9. Two iso-intensity series for Unit 21 showing spike rate as a function of frequency at two different temperatures.

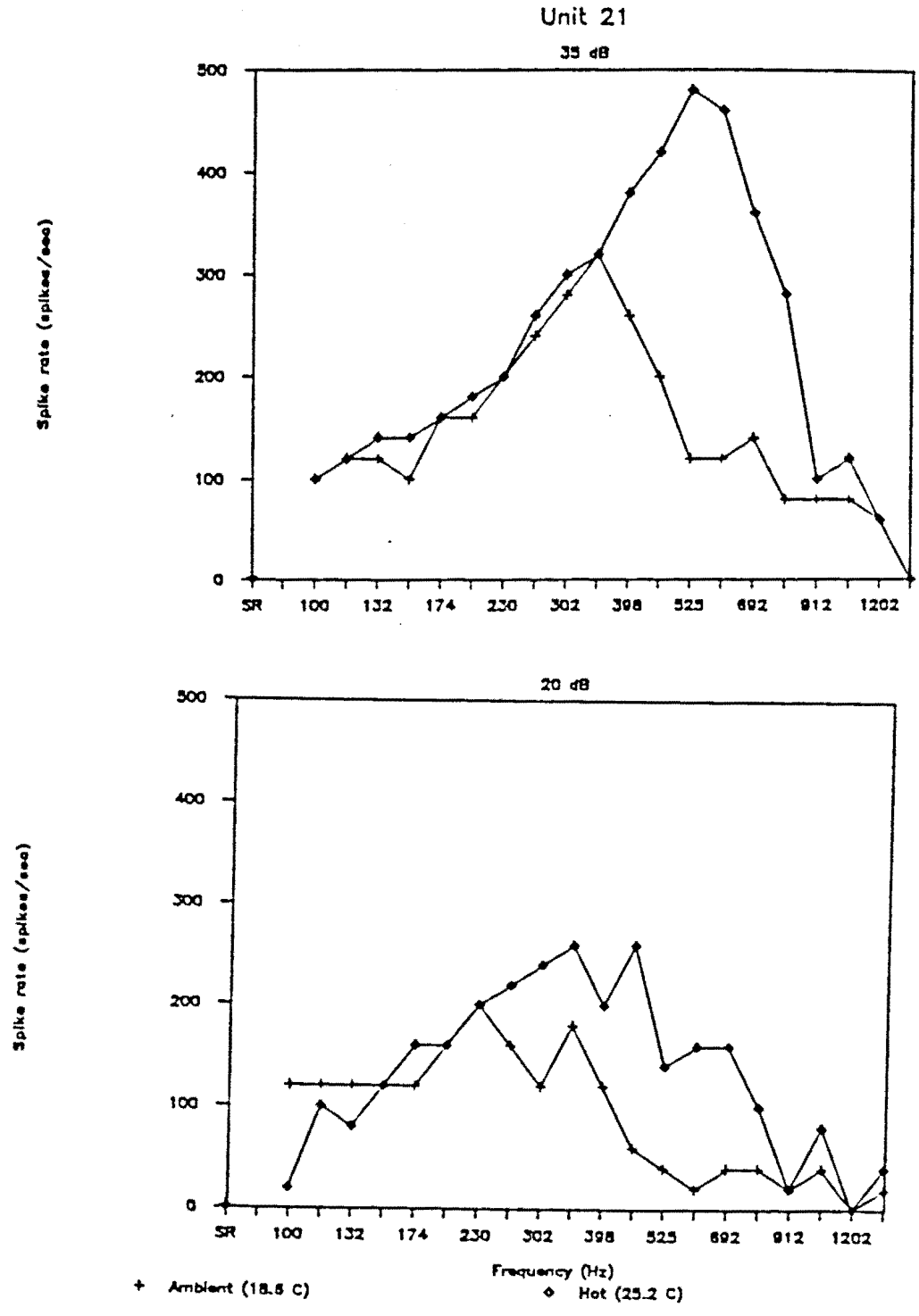

Unit 21
35 dB
20 dB
Spike rate (spikes/sec)
500
400
300
200
100
0
SR
100
132
174
230
302
398
525
692
912
1202
Frequency (Hz)
+ Ambient (18.6 C)
◇ Hot (25.2 C)

Figure 10. Three iso-intensity series for Unit 40 showing spike rate as a function of frequency at three different temperatures.

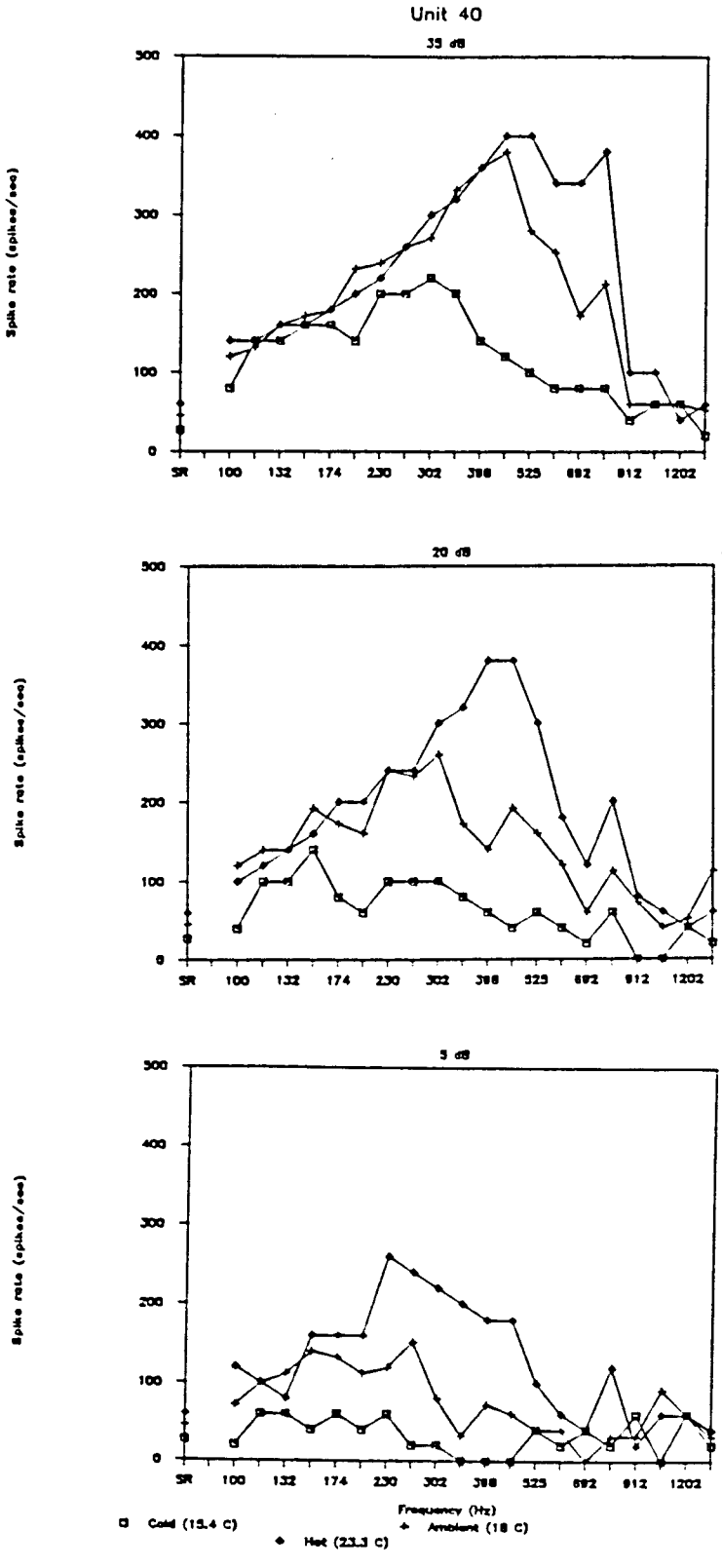
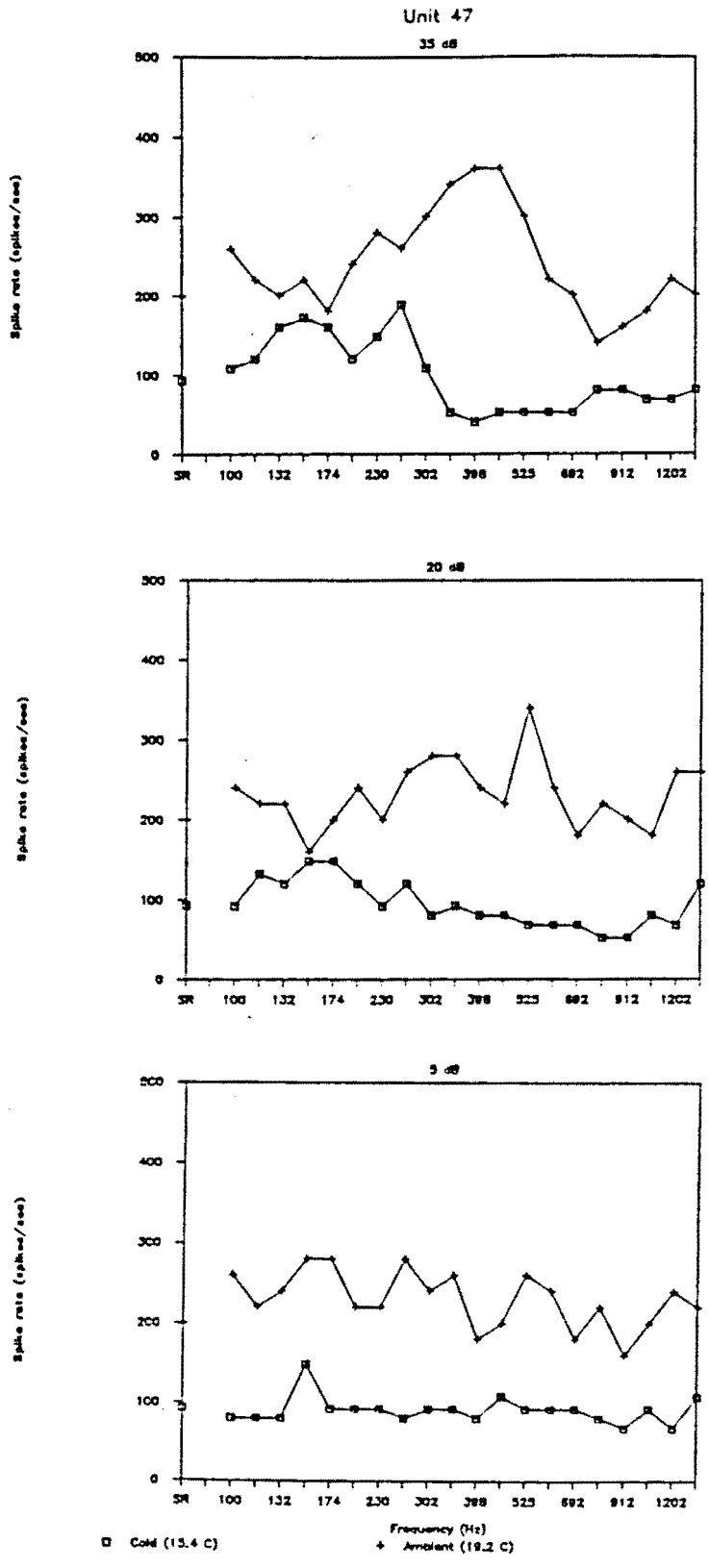

Unit 40
35 dB
20 dB
5 dB
Spike rate (spikes/sec)
300
400
300
200
100
0
SR
100
132
174
230
302
398
525
692
912
1202
Frequency (Hz)
Cold (15.4 C)
Ambient (18 C)
Hot (23.3 C)

Figure 11. Three iso-intensity series for Unit 47 showing spike rate as a function of frequency at two different temperatures.


Unit 47
35 dB
20 dB
5 dB
Spike rate (spikes/sec)
0
100
200
300
400
500
SR
100
132
174
230
302
398
525
692
912
1202
Frequency (Hz)
Cold (15.4 C)
Ambient (19.2 C)

DISCUSSION

Fay and Ream (1986) attempted to characterize the response properties of a large sample of goldfish saccular fibers. Relative to this previous study, the present sample of 17 fibers can be considered as fairly representative of the variety of response characteristics that can be found in the saccular nerve.

The 17 fibers showed spontaneous activity ranging from 0 to 200 spikes/sec. Nine fibers (53%) had spontaneous rates less than or equal to 10 spikes/sec. The average rate was 39.8 spikes/sec. In the previous study 90% of the fibers fell in the 0 to 140 spikes/sec range; 39% with rates of 10 spikes/sec or below. The average rate was 21.5 spikes/sec. This present sample contains proportionally fewer silent or very high spontaneous fibers.

The present sample of neurons did not include any highly sensitive fibers (sensitivity at best frequency less than -10 dB re: 1 dyne/cm^2). The average sensitivities between the two studies differed by only 2.8 dB; the previous study finding slightly greater mean sensitivity.

Fay and Ream (1986) found that 6% of the measurable fibers were not tuned (i.e. showed a change in threshold of 10 dB or less from 100 to 1000 Hz). Seventy-five percent of the tuned fibers had best frequencies below 300 Hz. Six percent of the fibers were tuned above 700 Hz. In the present study, 3 of the 17 fibers were untuned, while

71% of the tuned fibers fell into the low frequency group. The highest best frequency encountered was 602 Hz. As in Fay and Ream (1986), the sample can be considered to be comprised of mainly low frequency type fibers.

Two hundred and fifty-five of the 256 fibers in the Fay and Ream (1986) study had Q_{10dB} values that ranged from 0 to 1.6. In the present study, 16 of the 17 fibers had Q_{10dB} values that ranged from 0.2 to 1.3. The one extreme value was 3.8. This value occurred for a high-spontaneous fiber which, using the Fay and Ream (1986) methodology, would have been considered immeasurable. If this value is ignored the average Q_{10dB} value of 0.7 compares favorably with the value of 0.54 from the previous study.

The values compared above were all derived from data collected at ambient temperature; temperatures similar to those during the previous survey (Fay and Ream, 1986). However the method of data collection and analysis in the two studies differed slightly and may account for some of the above differences. The previous study used much finer frequency (10 Hz vs. 1/7 of an octave) and intensity (3 vs. 5 dB) step sizes in mapping the frequency-intensity response area. If there was an inherent bias present in this study relative to the previous one, it came from the necessity of measuring response characteristics for longer periods of time. Fibers which remained measurable for only 15 minutes would have been included in the previous sample but not in the present one. In addition, the longer measurement times created a tendency to remain in the dorsal portion of the nerve. Finally, no effort was made in this study to sample fibers from the outside edges of the nerve.

The important question that must be answered about these data is: are the temperature-dependent effects observed here equivalent to the effects seen in other non-mammalian vertebrates? Casual examination of the response areas seems to indicate that there is a temperature-dependent shift in best frequency. However, such shifts are accompanied by shifts in sensitivity. Furthermore, the shift is not uniform across frequency. A uniform shift in response area is precisely what has been observed in other vertebrate classes. In the caiman: "The response area shifts uniformly with temperature" (Smolders and Klinke, 1984). In the pigeon: "The properties are very much like those of the caiman" (Schemurly and Klinke, 1985). Eatock and Manley (1981) describe the effects of temperature on one of their gecko fibers as "fairly representative: a 1°C increase in temperature shifted the tuning curve of the fiber...toward higher frequencies without greatly affecting the shape of the curve."

In these experiments the "response area truly shifts and the new tip is always outside the tuning curve" measured at the initial temperature (Smolders and Klinke, 1984). Although goldfish response areas do not exhibit "tips", one would expect significant responding at some frequency-intensity points outside the response areas measured at other temperatures, if these data were to resemble previous non-mammalian vertebrate results. Nowhere in figures 8-11 is there an instance of increased responding after a decrease in temperature. Cooler temperature curves always fall within warmer ones.

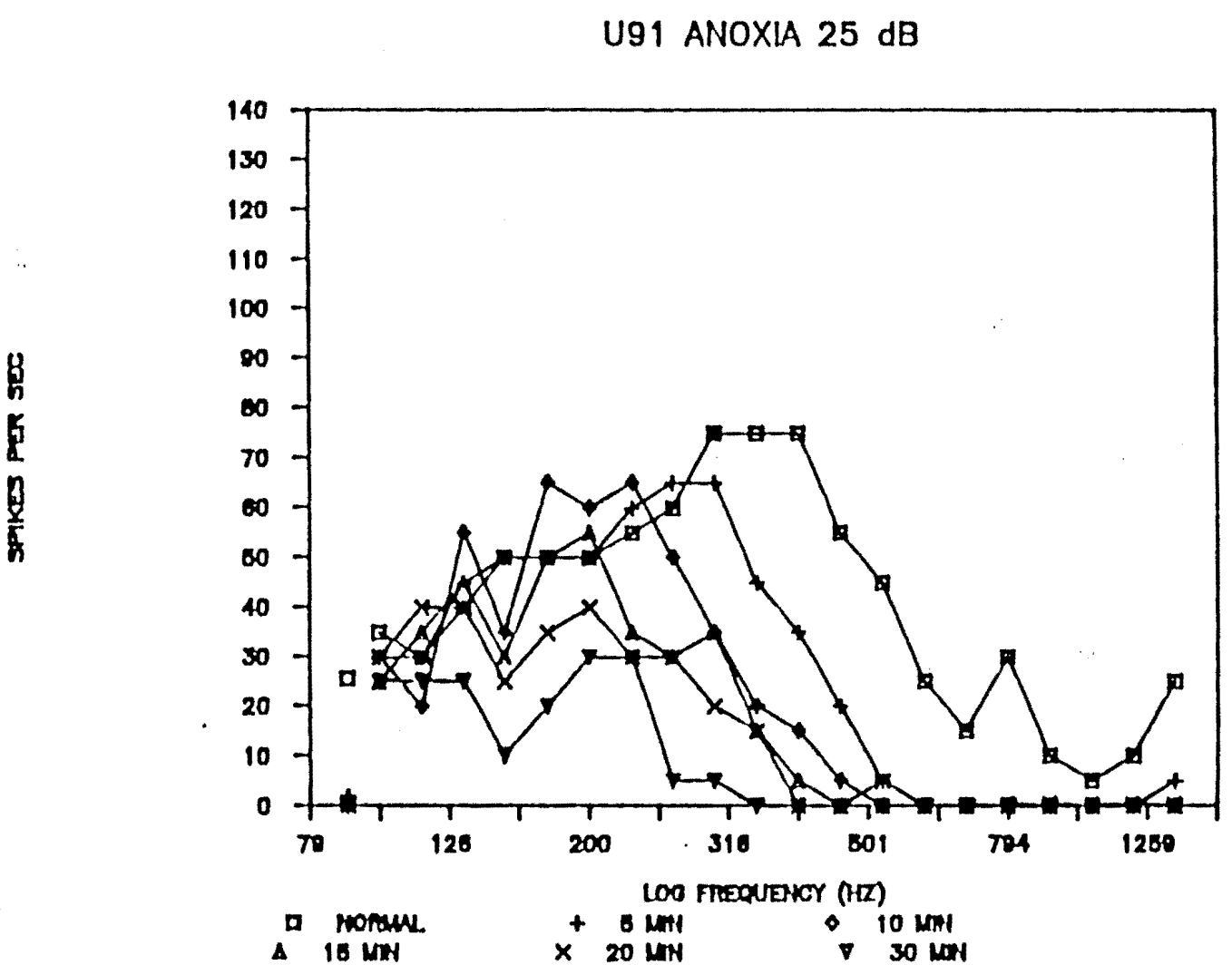
This sort of effect mimics the effects of other types of interventions on inner ear metabolism. Outer hair cell damage (Dallos

and Harris, 1978; Robertson and Johnstone, 1979), acoustic trauma (Cody and Johnstone, 1980), anoxia (Evans, 1974) or poisoning with cyanide, tetrodotoxin (Evans and Klinke, 1982a), and furosemide (Evans and Klinke, 1982b) all produce similar effects; new response areas fall within the original curves.

One difference between the data from these invasive procedures and the present temperature data is the effect on spontaneous rate. In these invasive experiments, a reduction in spontaneous rate was never reported. However, there is reason to believe that the reduction in spontaneous rate is caused by an effect on a process other than the one which results in a shift of the response area. In the caiman, best frequency thresholds reach a minimum at 28°C (for an animal acclimated to 27°C). A temperature change in either direction leads to increased thresholds. However, as temperature increases, spontaneous rates continue to increase without reaching a maximum (Smolders and Klinke, 1984). This seems to suggest that temperature has an effect on at least two separate processes. It is possible that a temperature-sensitive physiological process is in operation in all vertebrates and is responsible for the spontaneous rate change. This temperature dependent process is not affected by the invasive metabolism interventions listed above.

In addition to the manipulations listed above, the temperature effect observed here is quite similar to the effects of hypoxia on the goldfish (Fay, unpublished data) as can be seen in figure 12. As with temperature, this effect is reversible.

Figure 12. This series shows the changes in spike rate as a function of frequency for various periods of oxygen deprivation. The effect produced by hypoxia looks similar to that produced by lowering the ear temperature. This figure was produced using unpublished data gather by R. R. Fay.


U91 ANOXIA 25 dB
SPIKES PER SEC
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
79
126
200
316
501
794
1259
LOG FREQUENCY (HZ)
NORMAL
5 MIN
10 MIN
15 MIN
20 MIN
30 MIN

It should be pointed out that the notion that the present data are due to a temperature-induced hypoxia is counter-intuitive. At colder temperatures, the goldfish's oxygen demands decrease and oxygen solubility in water increases. So, in the cold condition, a fish needs less oxygen and has as much or more oxygen available as in the ambient condition, yet fibers respond similarly to those which have been oxygen-deprived.

Temperature effects on goldfish hearing are not similar to the effects observed in other non-mammalian species, nor are they like the temperature effects observed in mammals. The temperature effects in goldfish do, however, bear resemblance to the effects of the invasive types of interferences on the mammalian cochlea and especially to the effect of hypoxia on goldfish. These effects can be summarized as a loss in responsiveness which differentially affects higher frequencies.

Temperature may affect the electrical tuning of hair cells in a manner which shifts their area of responsiveness along the frequency scale. Species which do not display a temperature-induced frequency shift, may have auditory systems without electrically tuned hair cells. Based on the present data, one would predict that the hair cells of the goldfish saccule would not exhibit electrical tuning.

Finally, it should be noted that extrapolation of these results to effects on behavior should probably not be made. This is due to the fact that in this study subjects never became acclimated to the temperature extremes, but acclimation probably has an influence on temperature effects in poikilotherms (Werner 1983).

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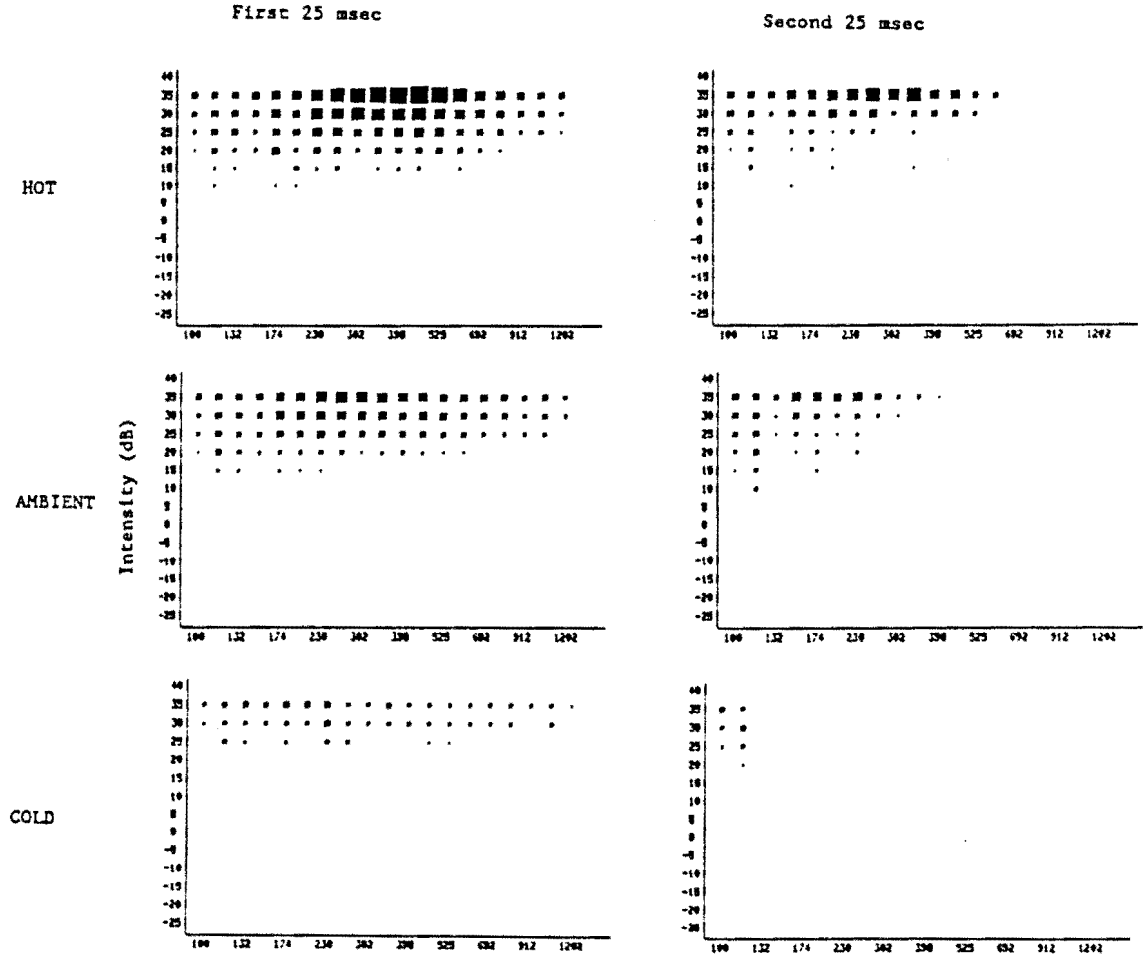
APPENDIX A

FREQUENCY THRESHOLD CURVES

UNIT # 7

Portion of the Tonal Stimulus



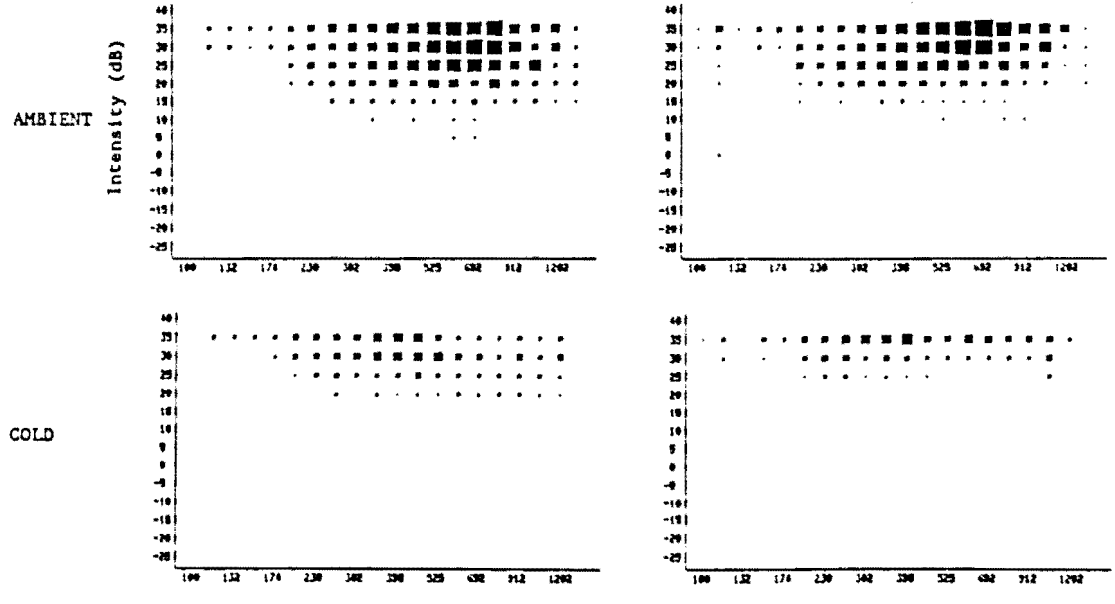
FREQUENCY THRESHOLD CURVES

UNIT # 22

Portion of the Tonal Stimulus

First 25 msec

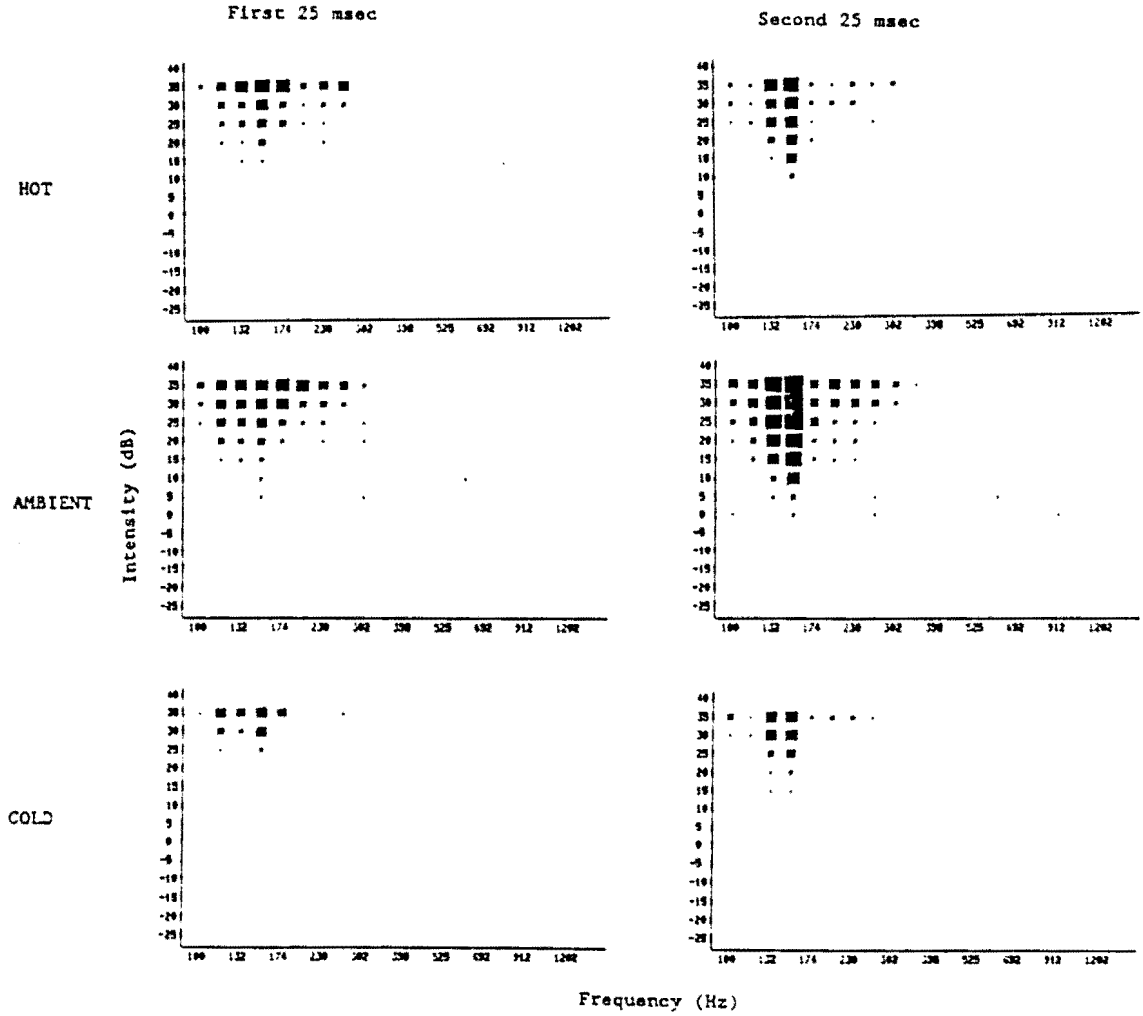
Second 25 msec



FREQUENCY THRESHOLD CURVES

UNIT # 34

Portion of the Tonal Stimulus



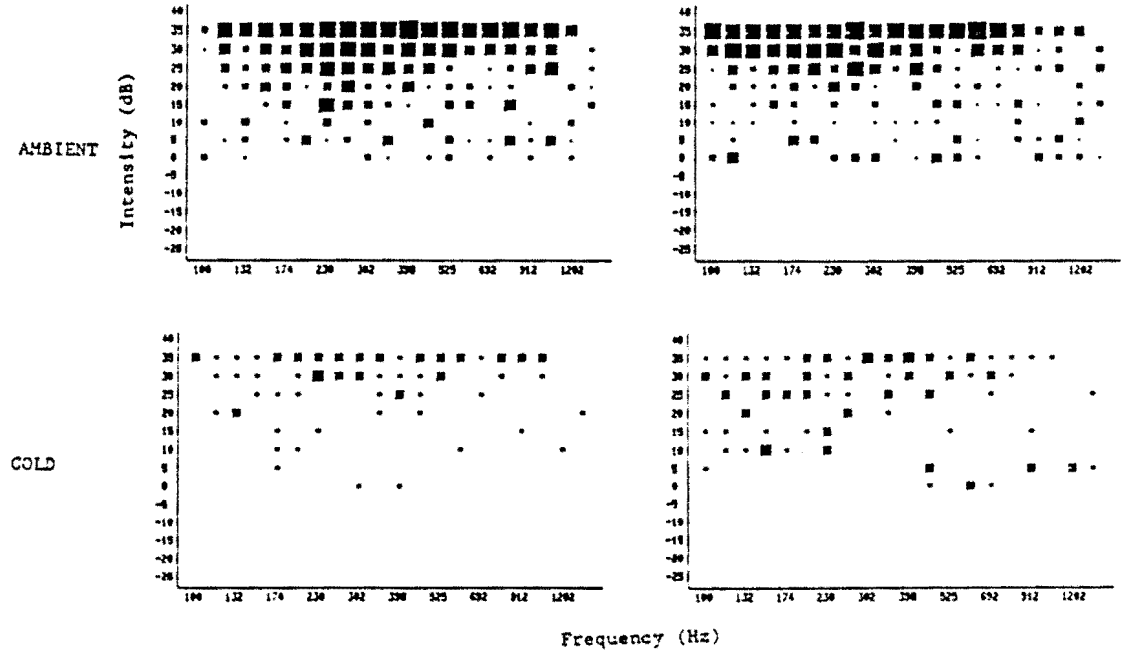
FREQUENCY THRESHOLD CURVES

UNIT # 36

Portion of the Tonal Stimulus

First 25 msec

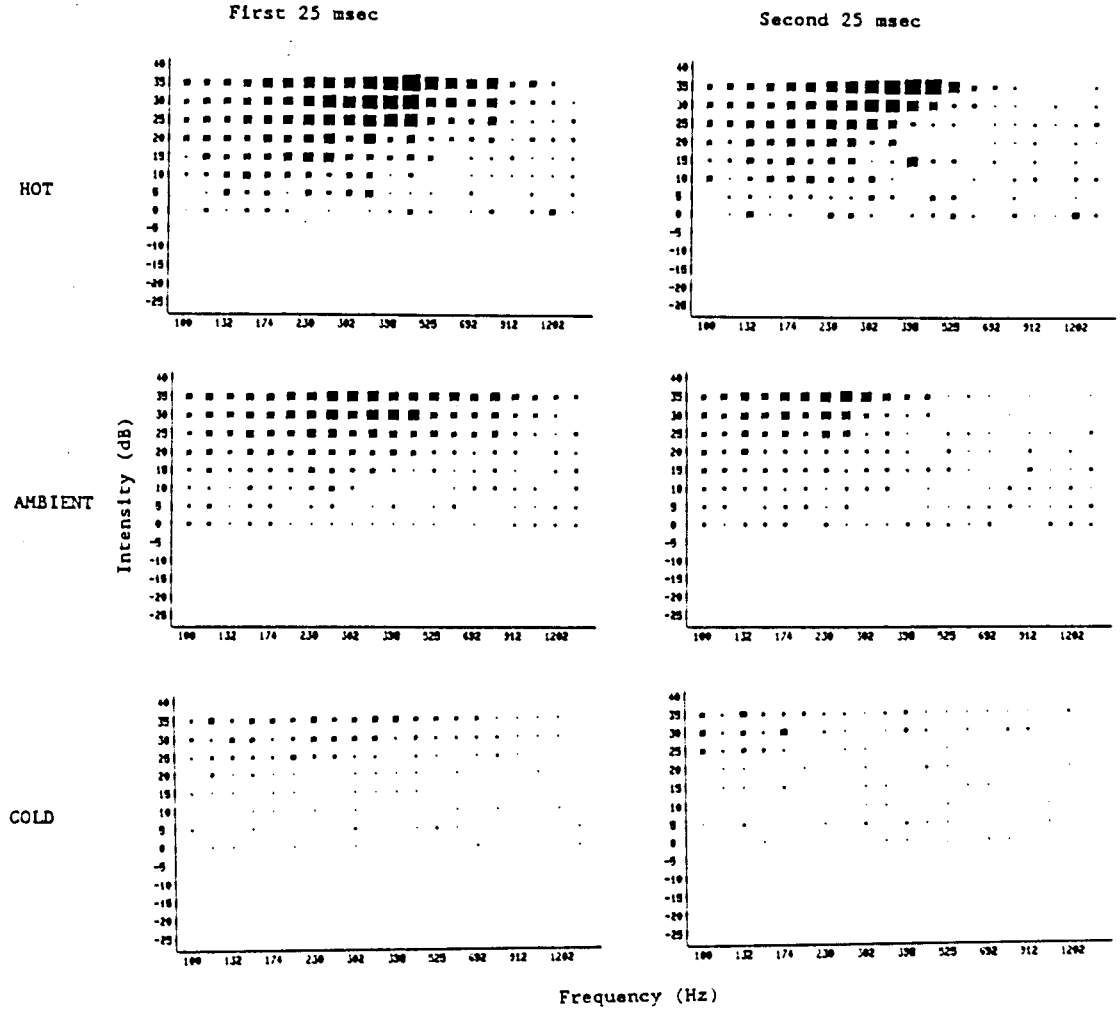
Second 25 msec



FREQUENCY THRESHOLD CURVES

UNIT # 37

Portion of the Tonal Stimulus



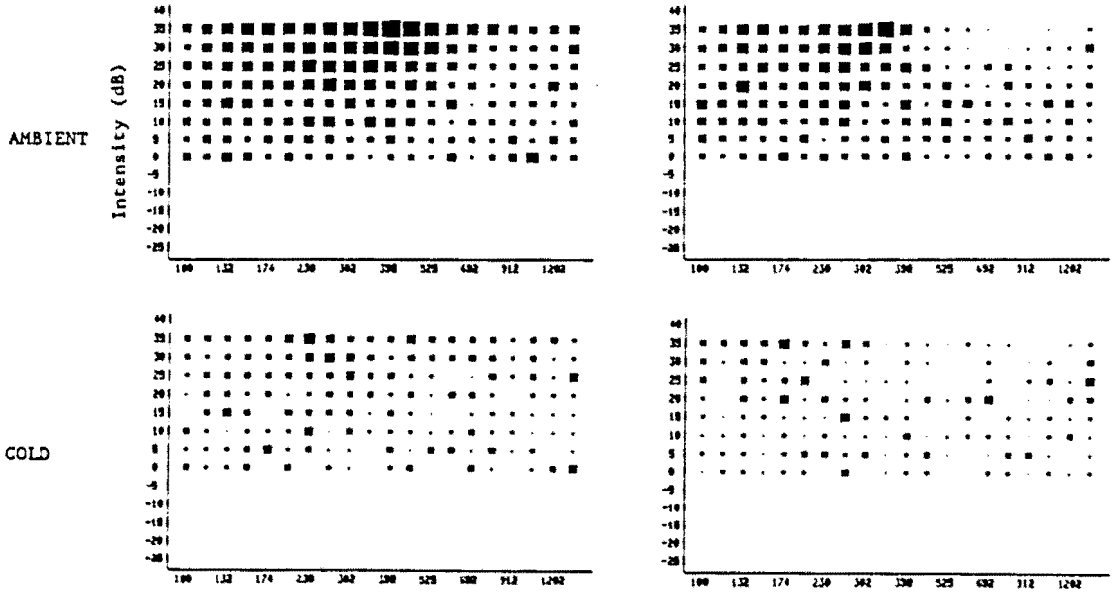
FREQUENCY THRESHOLD CURVES

UNIT # 39

Portion of the Tonal Stimulus

First 25 msec

Second 25 msec



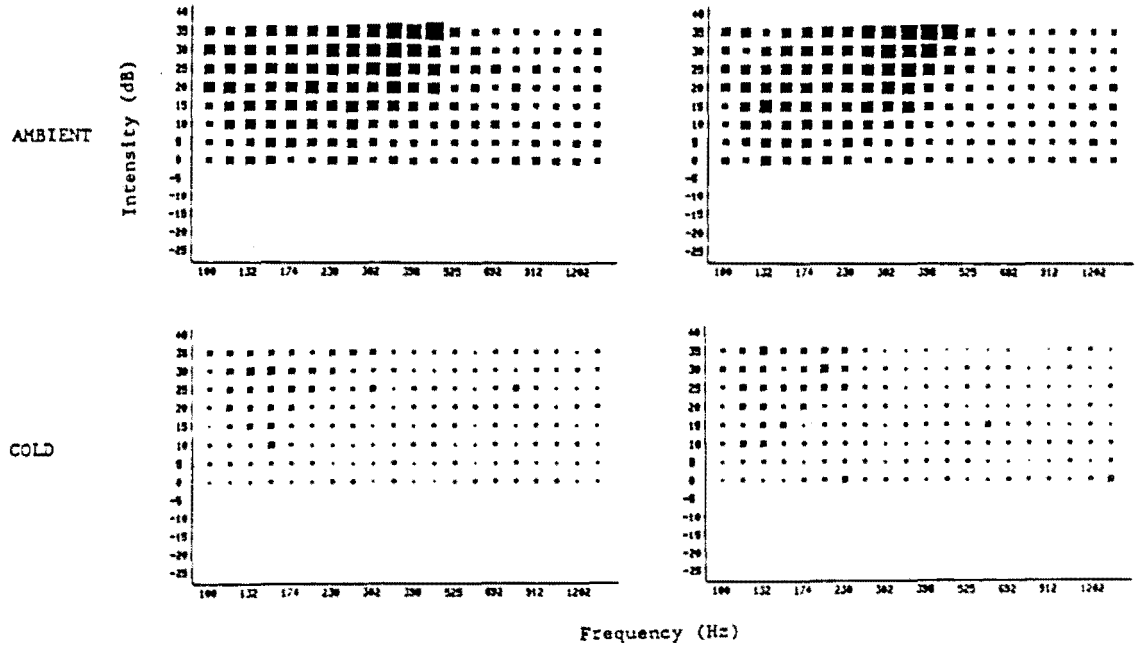
FREQUENCY THRESHOLD CURVES

UNIT # 46

Portion of the Tonal Stimulus

First 25 msec

Second 25 msec



APPROVAL SHEET

The thesis submitted by Timothy J. Ream has been read and approved by the following committee:

Dr. Richard R. Fay, Director
Professor, Psychology, Loyola University of Chicago

Dr. William A. Yost
Professor, Psychology, Loyola University of Chicago

Dr. Raymond H. Dye, Jr.
Associate Professor, Psychology, Loyola

The final copies have been examined by the Director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorpor-ated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Arts.

Sept. 19, 1989
Date

Richard R. Fay
Director's Signature