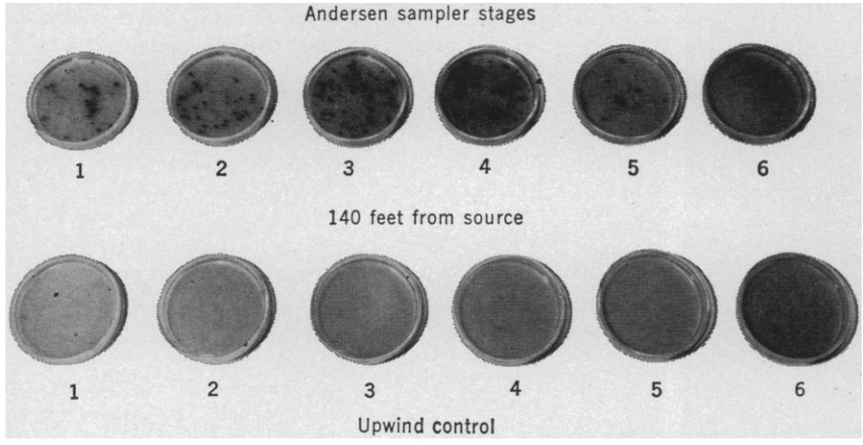

Fig. 2. Petri dishes containing Endo's medium, from various stages of the Andersen sampler. Note high concentration of coliform colonies on the upper set which were located downwind from the trickling filter bed shown in Fig. 1 (1 foot = 0.3 m).

greatest recoveries, both close in and at greater downwind distances. Relative humidity is known to have a pronounced effect on the survival of airborne *E. coli*; usually, the humidity during these studies was low. Low humidities were shown by Brown (*8*) to have a strongly adverse effect on survival of aerosolized *E. coli*. Positive recoveries of coliform organisms were made at night up to a distance of 0.8 mile from the source (which was the maximum distance sampled). Greater distances of downwind travel may be expected under more ideal conditions. Only a qualitative investigation of aerosol emission has been made to date, and Table 1 shows the number of coliform colonies and total number of bacterial colonies that were recovered under the various conditions of the study.

The counts presented in Table 1 are corrected for positive hole count as reported by Andersen (*7*). It should be noted that counts reported are derived from aerosol particles collected on the various stages of an Andersen sampler. Each particle collected theoretically gives rise to one colony; however, most of the particles collected contained more than one bacterial cell. Andersen (*7*) estimated that particles on stage 5 contained 1 to 4 cells; stage 4, 3 to 10 cells; stage 3, 9 to 25 cells; stage 2, 22 to 200 cells; and stage 1, 150 or more cells. In any event, the particle count presented is probably only a fraction of the total cell count. The heaviest counts were observed on stages 2, 3, and 4, with lower counts on stages 1 and 5. Few if any colonies were observed on stage 6. Particles recovered on stages 3 and below are known to be in the respirable size range; hence, if pathogens were present, they would be most infective in this size range. Particles larger than 5 μ in diameter (that is, those collected on stages 1 and 2) would be deposited in the upper respiratory tract but also may be swallowed and enter the gastrointestinal tract where many enteric pathogens are effective.

Since *E. coli* and other coliforms are the universal indicator of fecal pollution, it is apparent that the discovery of aerosolized coliform organisms arising from sewage treatment plants may portend a public health concern. Investigations should be conducted to attempt to identify other bacterial, fungal, and viral aerosols generated by sewage treatment facilities.

Note added in proof: After our report was submitted for publication, it was called to our attention that C. R. Albrecht had performed research of a somewhat similar nature. Albrecht submitted a thesis to the University of Florida in 1958 entitled "Bacterial Air Pollution Associated with the Sewage Treatment Process." We hereby acknowledge Albrecht's work.

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Temperature-Sensitive Neurons in the Brain of Brook Trout

Abstract. *The brain temperature of trout* (Salvelinus fontinalis) *was altered by perfusion of the gills with warm or cool water. Neuronal activity was recorded with microelectrodes. Twelve neurons responded to an increase in temperature with increased activity, and five neurons responded to a decrease in temperature with an increase in activity.*

There is substantial experimental evidence which indicates that ectothermic vertebrates can sense temperature (*1*). For instance, when tested in suitable temperature gradients they have been observed to select a definite range of temperatures within that gradient [for example, fish (*2*), amphibian larvae (*3*), and reptiles (*4*)].

Hammel *et al.* (*5, 6*) have shown in two ectotherms that at least part of the temperature-detecting mechanism is centrally located. They found that alteration of the anterior brain temperature of the Australian lizard (*Tiliqua scincoides*) affected its thermoregulatory behavior. Warming the brain delayed exit of the animal from a cold environment until the colonic temperature was 1° to 2°C below that at which the animal would normally have moved if the brain had not been heated. The converse was also observed when the brain was cooled while the animal was in a warm environment. Similarly, the Artic sculpin (*6*) responded to experimental alteration of anterior brain temperature with a change in thermoregulatory behavior.

Temperature-sensitive neurons have been found in the preoptic area of the brain of the Australian lizard (*7*) and in the hypothalamus of some endotherms (*8, 9, 10*). We have evidence for temperature-sensitive neurons in the brain of the brook trout (*Salvelinus fontinalis*).

The experimental fish were anes-

thetized by immersion in MS-222 (1:18,000 dilution) and secured in a restraining apparatus. The period of anesthesia lasted only until completion of the dissection. The top of the skull was removed to expose the optic lobes, which were then separated along the midline and displaced laterally. This permitted access to the floor of the third ventricle. This procedure ruptured small blood vessels lying along the midline of the brain. Hemorrhage was controlled with small pieces of absorbant paper tissue. The valvulla cerebelli, which extends rostrally into the ventricle, was held to one side of the midline of the brain with a stationary glass hook. Any movements by the fish caused loss of the recording site. Consequently the fish were immobilized by section of the spinal cord. The gills were continuously perfused with water. Ventilatory movements of the opercula, which also caused movement of the brain, were arrested with curare. The temperature of the brain was changed by perfusion of the gills with either cold or warm water, which in various experiments ranged from 8° to 20°C. Glass microelectrodes filled with Wood's metal (*11*) or with 2*M* KCl were used. The tip diameter of the metal-filled electrodes was about 10 μm; that of the KCl-filled electrodes was about 3 μm. The electrode resistance of both types was 1 to 5 megohm.

With the brain maintained at an initial constant temperature by the water perfusing the gills, the diencephalon was probed with an electrode until a neural unit that had a constant frequency of action potentials (activity) was found. The temperature sensitivity of the unit was then tested by changing the temperature of the perfusing water. If a unit changed in activity during warming (or cooling) of the brain, it was designated temperature sensitive. The activity was recorded on tape and monitored by oscilloscope and loudspeaker. The average frequency of action potentials during successive 30-second periods was calculated. The temperature sensitive units were found 0.1 to 1.1 mm below the floor of the third ventricle. They were distributed throughout an area which, in length, extended 1.5 mm posteriorly from the caudal border of the posterior commissure and which, in width, extended laterally 1 mm on either side of the midline.

Table 1. Temperature sensitivity of six warm-sensitive units from trout. For each unit is shown the temperature above which the activity increased beyond the steady-state activity as the brain was warmed from the initial constant temperature.

Initial temperature (°C)	Steady-state activity at initial temperature (impulses sec^{-1})	Temperature above which unit increased activity (°C)	Temperature sensitivity (impulses sec^{-1} deg^{-1})
8.7	35	13	+18
10.5	1–2	13	+16
11	20	11.5	+9
11.5	17	12.5	+3
13	0–1	16	+14
10	3	10	+2

Within any one fish, most of the units observed did not respond to temperature changes of the brain. The activity of some units remained relatively unchanged with time, whereas the activity of other units varied with time, but not with changes in brain temperature. However, units were found which did show a correlation between the level of activity and a changing brain temperature. Twelve units responded with increased activity as the brain was warming, and five units responded with increased activity as the brain was cooling. Units which responded in this manner are referred to as warm-sensitive and cold-sensitive units, respectively. Figure 1 shows the results from two typical experiments in which units showed activity as a function of temperature. Histogram A shows the response of a warm-sensitive unit; B shows the response of a cold-sensitive unit.

These data do not permit a decision to be made on the site of the sensory input as we changed brain temperature by peripheral warming or cooling. It could be located either peripherally (for example, in the mouth or gills) or centrally in the brain. Results from experiments on the lizard (*7*), dog (*8*), and rabbit (*9*), but with local warming

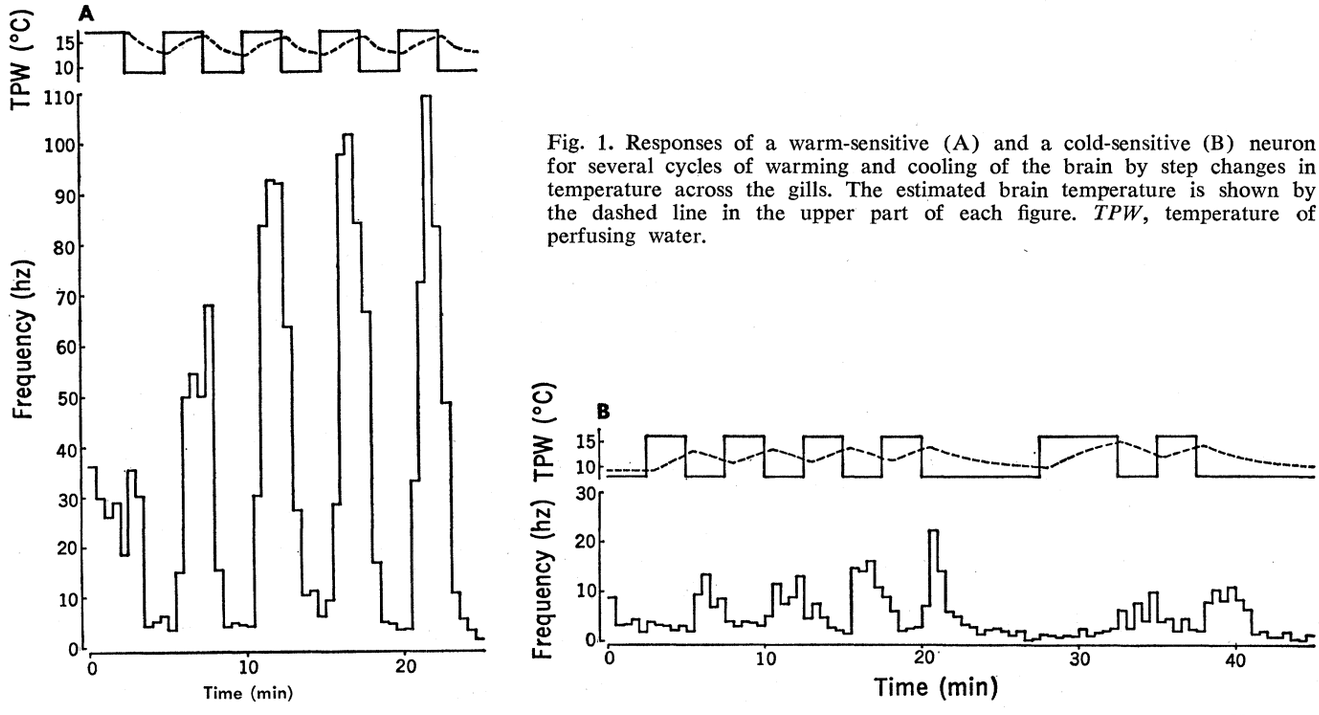

Fig. 1. Responses of a warm-sensitive (A) and a cold-sensitive (B) neuron for several cycles of warming and cooling of the brain by step changes in temperature across the gills. The estimated brain temperature is shown by the dashed line in the upper part of each figure. *TPW*, temperature of perfusing water.

and cooling of the brain, suggest that the temperature changes are detected centrally in those animals. Under the same conditions as those for the experimental data shown in Fig. 1, we examined the time course of the change in brain temperature as a function of the temperature of the perfusing water. A thermocouple was implanted in the brain 2.5 mm from the midline and at the level of the posterior commissure. The changes in brain temperature lagged behind the perfusion temperature by 20 to 25 seconds; these have been added to Fig. 1, A and B, as a dashed line. Increased activity of both the warm- and cold-sensitive neurons therefore tended to be synchronous with a changing brain temperature, rather than the temperature step across the gills. This result is consistent with the interpretation that the sensory site is in the brain. Furthermore, preliminary experiments in which neural activity and brain temperature were monitored simultaneously indicate that activity does follow brain temperature (Table 1).

Although the initial constant brain temperature was different for each unit tested, it appears that the mean steady-state activity of different units can be quite variable. For example, during the initial phase of warming the brain, the temperature 11.5°C was common to the first four units in Table 1 but the mean steady-state activities were dissimilar. In addition, the temperature of the brain above which the activity increased with warming varied between units. The range of temperature sensitivities of +2 to +18 impulses sec^{-1} deg^{-1} for the trout is above the range of +0.09 to +1.0 for the warm-sensitive units in the lizard (*7*), but is within the range of +1 to +21 impulses sec^{-1} deg^{-1} for the dog (*8*). The variation in the temperature sensitivity of individual units, their steady-state frequency, and the temperature of the brain at which they switched on with warming, suggests that the brain might have a population of temperature-sensitive neurons consisting of individual units that function over a specific temperature range.

Cabanac *et al.* (*7*) have already noted the similarities between mammalian and reptilian brains with regard to temperature-sensitive units. Now, with identification of temperature-sensitive neurons in the fish it appears that the ability to centrally detect changes in temperature could be common to all vertebrates. Though one cannot necessarily infer a close association between the activity of these units in the brain and some behavioral thermoregulatory response of the fish, one might speculate that the beginnings of a central thermostat exist in the lowest class of vertebrates.

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Auditory Frequency-Following Response: Neural or Artifact?

Abstract. *An electrical response which reproduces the waveform and frequency of the sound stimulus can be recorded from the central neural pathway for audition. Controversy has existed for some years over whether this frequency-following response (FFR) is neural or an artifact such as remote pickup of the cochlear microphonic or cross talk in the recording system. Two experiments resolve this issue by demonstrating that the frequency-following response depends upon functionally intact neural pathways. The frequency-following response, as well as auditory evoked potentials, is abolished by section of the eighth nerve; it is reversibly abolished by cooling of the cochlear nucleus.*

Some 40 years ago Wever and Bray (*1*) observed sound-evoked electrical activity in the eighth nerve, which reproduced the frequency and waveform of low-frequency sound stimuli. This response, which we have termed a frequency-following response (FFR), was observed at frequencies well beyond the limits of response of individual neural units. To account for this discrepancy, Wever proposed the "volley" concept of neural firing. Subsequently, FFR has been observed at higher levels of the auditory pathway (*2*).

Considerable controversy has existed over whether this response reflects neural activity, or some nonneural mechanism such as cross talk in the recording system, remote pickup of the cochlear microphonic (CM), or some artifact generated by such physical factors as acoustic vibration at the interface of brain and electrode. Evidence supporting a neural interpretation of FFR has been reported by a number of authors (*3*). The principal observations are: (i) FFR has latency appropriate to the neural level from which it is recorded; (ii) in contrast to the graded onset of the CM, FFR has an abrupt onset and a discrete threshold; (iii) FFR is so narrowly localized within the auditory pathway that it is not recorded at points a millimeter away; and (iv) under anoxia the disappearance of FFR is concurrent with that of known neural activity (for example, evoked potentials) whereas CM persists. Since the possible importance of FFR for processing of neural information and for theories of hearing depends upon an unequivocal demonstration of its neural basis, two experiments were undertaken to resolve this issue.

In the first experiment (Fig. 1), section of the eighth nerve abolished FFR at the cochlear nucleus, as well as the evoked potential, without affecting the CM recorded at the round window.

Since the functional integrity of the recording electrode in the cochlear nucleus is not demonstrable after transection of the eighth nerve, a second experiment was performed. Reversible blocking was produced by inserting a cryoprobe to cool the left cochlear nucleus. Both the evoked potential and