An Attempt to Produce Mutations by Sound

Since the pioneer work of MULLER[1], it has been known that X-rays and other radiations can produce mutations in the reproductive cells of animals. That high intensity sounds might be mutagenic has been suspected, but definite proof is lacking. HERSH *et al.*[2], studying visible mutations, obtained negative results when adult *Drosophila* were treated in a container immersed in an underwater ultrasonic field. FRITZ-NIGGLI *et al.*[3], likewise using ultrasonic frequencies but treating eggs, larvae, and pupae of *Drosophila* immersed in a gelled salt solution, found damage to the insects but no lethal mutations, as tested by the standard *ClB* procedure. On the other hand, WALLACE *et al.*[4] reported the production of lethal and visible mutations through treatment of adult *Drosophila* by ultrasound, but they gave no details of method. They later[5] reported nuclear destruction and chromosome fragmentation in plant materials treated with ultrasound. All these experiments utilized ultrasonic frequencies with various methods of treatment of the subjects, and the discrepancies in results could have been due to the differences in techniques.

As part of a general research program at this college on the biological effects of high intensity air-borne sound at high sonic and near-ultrasonic frequencies, it seemed advisible to study the possible production of mutations by high intensity sound at lower frequencies than those tried previously. Popular fear in some quarters of possible production of mutations in man by high intensity air-borne sound gave the study added interest.

The organism used for the work was *Drosophila melanogaster*, males of which were exposed to a high intensity sound field, at 6 Kc frequency, produced by the siren built by ALLEN and RUDNICK[6]. The induction of lethal mutations was tested by the standard *ClB* method. For the treatment, flies were mounted by fixing the wings to a small triangle of wax-paper attached to a glass rod[7]. Thus mounted they could be suspended at any desired place in the sound field. The insects were treated at two points in the sound beam, where the sound pressures were 154 and 163 db respectively (relative intensity 10^{-16} watts/cm^2). Untreated control flies were similarly mounted and suspended for corresponding times. After treatment, the flies were removed by cutting off the wings and were mated to *ClB* females.

The first experiments were made to determine the length of time of exposure to the sound a male *Drosophila* could tolerate. This was found to be about 45 min at 163 db, and two treatment times were therefore selected for use at this intensity: 40 min and 20 min. The treatment time at 154 db was 40 min. It was hoped that, if mutations appeared, any dependence upon time of treatment or intensity of treatment could thus be found.

The results of the experiments are given in the table. The "Number of treated X-chromosomes tested" refers to the number of F_1 *ClB* females which were bred to white eyed males to give the F_2. As this clearly shows, no lethal mutations were produced.

Treatment	No. of Treated Males	No. of treated X-chromosomes tested	No. of lethals
Controls	9	287	0
154 db, 40 min	11	317	0
163 db, 20 min	13	379	0
163 db, 40 min	19	478	0

This study obviously does not check that of WALLACE *et al.*[1], but the difference is probably due to differences in the methods of treatment. It is certain that in our experiments a large part of the sound energy was reflected from the body surface and was thus unavailable for effective action on the testes of the flies. This interpretation is supported by the fact that the flies were killed only after being in the sound field for 45 min. Roaches, for instance, which do absorb the sound and die as a result of internal heating, perish within a few minutes[2].

In mammals, the harmful effects of exposure to high intensity air-borne sound are almost entirely due to heating of the fur through absorption of sound[3]. In a relatively hairless mammal, such as man, the body surface reflects most of the incident sound, and protection is thus afforded. The results of the present experiment suggest that, in such a case, the possibility of harmful genetic effects through exposure to high intensity sound fields is remote[4].

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Zusammenfassung

Es wurde versucht, bei *Drosophila melanogaster* durch Einwirkung sehr intensiven Schalls (154 und 163 db; bei 6 kHz) eine Letalmutation zu erzielen. Die Experimente blieben jedoch ohne Erfolg.

[1] H. J. MULLER, Science *66*, 84 (1927).

[2] A. H. HERSH, E. KARRER, and A. L. LOOMIS, Amer. Natural. *64*, 552 (1930).

[3] H. FRITZ-NIGGLI and A. BÖNI, Science *112*, 120 (1950).

[4] R. H. WALLACE, R. J. BUSHNELL, and E. H. NEWCOMER, Science *107*, 577 (1948).

[5] E. H. NEWCOMER and R. H. WALLACE, Amer. J. Bot. *36*, 230 (1949).

[6] C. H. ALLEN and I. RUDNICK, J. Acoust. Soc. Amer. *19*, 857 (1947).

[7] H. FRINGS, Turtox News *24*, 150 (1946).

[1] R. H. WALLACE, R. J. BUSHNELL, and E. H. NEWCOMER, Science *107*, 577 (1948).

[2] C. H. ALLEN, H. FRINGS, and I. RUDNICK, J. Acoust. Soc. Amer. *20*, 62 (1948).

[3] H. FRINGS, C. H. ALLEN, and I. RUDNICK, J. Cell. Comp. Physiol. *31*, 339 (1948).

[4] Paper No. 1639 in the Journal Series of the Pennsylvania Agricultural Experiment Station. The work was sponsored in part by the U. S. Air Force, Air Materiel Command, Wright-Patterson Air Force Base, Dayton, Ohio, under Contract AF—33 (038) —786.

Sweet Taste in the Cat and the Taste-Spectrum

ZOTTERMAN has recently called attention to his inability to record action potentials from the taste fibres of the cat when sucrose solution was applied to the tongue[1]. These results, he noted, were supported by PFAFFMANN's[2] earlier study, although PFAFFMANN did not deny the existence of receptors for sweet substances in the cat. ZOTTERMAN explained his results by the statement that "the cat as opposed to the dog has no liking for sugar or sweet tasting food in general".

I have previously reported[3] that every animal which I had tested accepted sucrose solutions eagerly when

[1] Y. ZOTTERMAN, Exper. *6*, 57 (1950); Skand. Arch. Physiol. *72*, 73 (1935); Acta Physiol. Scand. *18*, 181 (1949).—B. ANDERSSON *et al.*, Acta Physiol. Scand. *21*, 105 (1950).

[2] C. PFAFFMANN, J. Cell. Comp. Physiol. *17*, 243 (1941).

[3] H. FRINGS, Turtox News *24*, 133 (1946).

hungry. This included even such unlikely subjects as spiders, rabbits, mantids, snails, and quail. With this in mind, BEVERLY COX, a student at this college, and I tested cats for acceptance of, or better, preference for sweet solutions and found that cats accept sucrose as a food when offered in diluted milk, easily distinguishing diluted milk with sugar from the same without sugar.

Ten cats (5 adults, 5 kittens) were housed in small animal cages and were given water *ad lib.*, but were deprived of food for various intervals of time before testing. A period of 24 hours of inanition proved to be quite satisfactory. Then each cat was offered two similar dishes containing solutions—one contained milk diluted with four times its volume of water, the other contained milk similarly diluted but with sucrose added to make it 0·5 M[1] with respect to sucrose. Diluted milk was selected as the medium for the sucrose after it was found that consumption of water or water with sugar was too little to give reliable results. Whole milk was unsuitable, because this was taken avidly by hungry cats with or without sucrose added. Diluted milk, on the other hand, was either refused or taken in very small amounts by the cats after they sampled it, while the same diluted milk to which sugar had been added was taken eagerly by all of the animals. By randomizing the positions of the containers in the cages in replicated tests, it was easy to determine that the cats could distinguish between the two solutions, and that, once both had been sampled, they would take only the one containing sucrose. This, of course, suggests the possibility of self-regulatory selection of diet in cats, as is claimed for rats[2]. Since the purpose of our experiments was not to study this, no discussion of the subject is offered here. Suffice it to say that cats can distinguish "sweet" things from "non-sweet", and that they do "like" the "sweet", if conditions are right.

A possible explanation for the lack of success in finding nerve potentials on stimulation with sucrose has already been offered[3]. On the basis of comparative studies of taste thresholds with six species of animals using many compounds, I have formulated a "taste-spectrum hypothesis" which discards the primary taste qualities (salt, sweet, sour, and bitter) and assumes that the taste quality is dependent upon the stimulative power of the sapid substance. Stimulation seems to involve penetration or a surface effect, such as adsorption. Those substances which are least stimulating are sweet, those which are next most stimulating are salty (or better, salty-like, since only NaCl is "pure" salty), those next most stimulating are bitter (a wide and poorly defined range), and those most stimulating are sour. Since it would be unlikely that different qualities could be aroused by different levels of stimulation of the same end-organs, it seems reasonable to assume a population of receptors on the tongue differently susceptible to penetration or surface action. Stimulation of the most easily stimulated cells would thus be interpreted as sweet; stimulation of these plus others would be interpreted as salty; stimulation of still more would be interpreted as bitter; and stimulation of all or nearly all as sour. There would be no breaks in this continuous series, thus accounting for intermediate tastes and for the loose specificity of taste of individual compounds. On this basis, the so-called primary taste qualities are merely points of familiarity on a stimulus-continuum and are no more primary than are those falling intermediate in the scale. Using this concept, it is possible to predict the thresholds and taste qualities for inorganic compounds for which these data are unknown. The idea explains further the shift in taste quality (sweet to salty with NaCl, for instance) with increasing concentration found with many compounds.

On the basis of this theory, the reason for the lack of success in finding sweet taste fibers would be that the necessary end-organs, being only the most sensitive, are present in the smallest numbers. With whole nerve preparations, the potentials might thus be too small or might be masked. With single fiber preparations, the chances that any specific fiber would be from one of these organs would be small.

The taste-spectrum theory has recently been criticized for underrating the importance of possible specific receptors[1]. Actually, it does deny the existence of qualitatively different receptors, but it does not exclude the possibility of regional localization of end-organs of quantitatively different susceptibility. Thus, the most sensitive cells might be located on the tongue in the region commonly designated as most sensitive to sweet, and so with the other end-organs. Regional stimulation by a specific substance would occur not because the end-organs were specific for that substance, but because the stimulative power (rate of penetration or adsorption) of the substance was such as to stimulate the end-organs in that region. Taste quality would be determined actually from the distributional pattern of stimulation of quantitatively different receptors.

It is true that the taste-spectrum theory is best supported by studies of comparative thresholds[2], and leaves combination tastes (i. e., sweet-sour) difficult to explain. These may possibly be explained, however, if penetration or adsorption is involved in stimulation, by the existence of two series of stimulating substances—inorganic (polar) and organic (non-polar). Thus, simultaneous stimulation by two different substances from the two series would be possible. This idea is supported by PFAFFMANN's finding salt-acid and quinine-acid fibers, and also by the well-known effect of Gymnema extract in abolishing first the sweet taste (receptors most susceptible), then the bitter, leaving acid and salt unaffected. Further studies on mixed sapid solutions, in which the presence of one substance may influence the stimulative power of the other, would illuminate this problem.

The hypothesis of four primary taste qualities certainly needs careful reexamination and reconsideration, since its acceptance has sometimes had a stultifying effect. As a result of it, for instance, many students of taste have restricted their studies to relatively few substances (NaCl, quinine, HCl, acetic acid, and sucrose) without making fruitful comparative studies with a wide variety of compounds. The taste-spectrum hypothesis has been put forward not so much with the belief that it offers an explanation for all the complexities of taste, but with the hope that it will encourage a re-

[1] A note should be added here about the prevalent use of percentage designations for concentrations of solutions in taste studies. Comparisons based on percentages are actually based on weight only. For studies of the chemical senses, it is certainly more reasonable to compare substances on a molecular or ionic basis, and molar or normal units should therefore be used.

[2] C. RICHTER, Harvey Lecture Series *38*, 63 (1942); J. Comp. Physiol. Psychol. *40*, 129 (1947).

[3] H. FRINGS, Biol. Bull. *88*, 37 (1945); J. Exp. Zool. *102*, 23 (1946); J. Comp. Physiol. Psychol. *41*, 25 (1948).—H. FRINGS and B. R. O'NEAL, J. Exp. Zool. *103*, 61 (1946).

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[2] L. CHADWICK and V. DETHIER, J. Gen. Physiol. *30*, 247, 255 (1947); *32*, 139 (1948); *32*, 445 (1949); *33*, 589 (1950).—V. DETHIER, Amer. J. Physiol. *165*, 247 (1951).

examination of older ideas and a gathering of data from which more exact theories may be inductively derived.

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Zusammenfassung

Es konnte festgestellt werden, daß Katzen die Geschmacksempfindung «süß» nicht besitzen. Sie können aber «süß» von «nicht süß» unterscheiden. Elektrophysiologisch läßt sich dieser Befund nicht belegen. Zur Erklärung wird auf die Geschmacksspektrum-Theorie verwiesen.

Manifestations cycliques indiquant la présence de terminaisons nerveuses libres et de synapses dans la couche moléculaire à la surface du cervelet chez le Rat

La surface des lamelles cérébelleuses est recouverte par une couche moléculaire dans laquelle, indépendamment de quelques cellules nerveuses ou névrogliques, se rencontrent sous une forme prodigieusement fine un grand nombre de dendrites, particulièrement ceux des éléments de Purkinje ou des cellules à corbeilles et de neurites, fibres grimpantes ou prolongements innombrables des grains du cervelet. Les schémas de cette zone mince et compliquée restent encore dans les livres classiques ceux observés jadis avec la méthode de Golgi. L'emploi des techniques modernes qui permettent l'imprégnation argentique de tous les filaments nerveux de cette portion des centres, donne des images si embrouillées que, quelle que soit la théorie à laquelle on se rattache, neuroniste ou antineuroniste, il est impossible de démontrer soit des extrémités libres, soit un réseau comme ceux de HELD[1] ou de BAUER[2].

Dans ces dernières années, WEBER[3] a montré que les véritables extrémités libres des fibres nerveuses, les appareils métaterminaux, présentent des modifications cycliques qui amènent leur destruction, puis leur régénération. La dégénérescence de ces filaments extraordinairement fins se traduit le plus souvent par le gonflement du granule qui se trouve à leur extrémité et qui s'aplatit ensuite pour donner naissance à un petit anneau à bords épais ou rondelle. Pendant ce temps, un nouveau filament métaterminal se reconstitue. La désintégration de ces fines structures laisse souvent des traces granuleuses sur leur parcours. D'autre part lorsque l'extrémité d'un filament métaterminal appartenant à un neurite s'altère et se gonfle, il est assez fréquent de voir ce même processus d'imbibition se produire sur le dendrite qui réalise un contact, au niveau de cette synapse axo-dendritique. Entre les deux formations globuleuses en question, une mince fente, visible avec une bonne optique, délimite le territoire des deux neurones en présence. Tous ces faits constatés par WEBER aussi bien à la périphérie que dans les centres sont visibles dans cette zone difficilement analysable au microscope, même avec d'excellentes imprégnations, et indiquent d'une façon indiscutable la présence de terminaisons nerveuses libres.

C'est principalement dans la région la plus profonde de la couche moléculaire que sont visibles les aspects que je vais décrire; on les trouve beaucoup plus rarement au voisinage de la pie-mère. Ainsi, non loin des cellules de Purkinje, l'attention est attirée par des groupements de rondelles qui semblent occuper de petites plages dont le diamètre oscille entre 35 et 40 μ et qu'on retrouve sur la succession des coupes, dans une épaisseur de 20 à 30 μ environ. Quelques-unes de ces formations annulaires sont encore attachées à la fibre qui leur a donné naissance, par épaississement de l'appareil métaterminal (fig. 1).

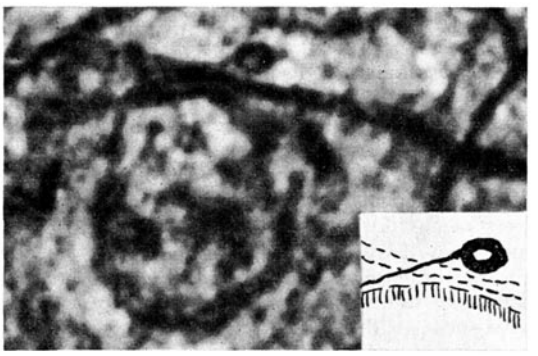

Fig. 1. – Cervelet de Rat. Fixation SW 24. Imprégnation argentique. Couche moléculaire. Microphoto, grossissement 3500:1. Au-dessus d'un noyau névroglique, fibre fine terminée par une rondelle.

Mais bientôt le filament en question se désintègre et se fragmente (fig. 2), laissant la rondelle libre et comme

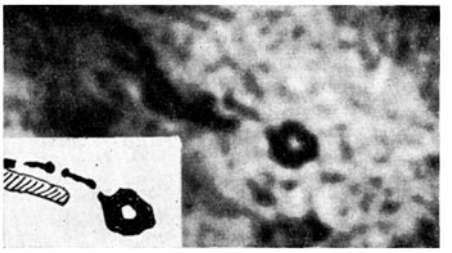

Fig. 2. – Même région, même grossissement. La fibre aboutissant à la rondelle se fragmente.

autre trace, une rangée de granulations. Grâce aux répétitions cycliques de ce phénomène, une même fibre peut ainsi donner naissance à des rondelles qui se placent les unes à côté des autres, à la suite de rangées de granulations presque parallèles (fig. 3). En d'autres points,

Fig. 3. – Même région. Grossissement 3000:1. Dessin à la chambre claire. Appareil métaterminal en voie de reconstruction après avoir donné naissance à deux rondelles isolées et à des granules de dégénérescence.

il est possible de constater que l'épaississement du granule situé à l'extrémité de l'appareil métaterminal, a déterminé dans la terminaison nerveuse qui forme synapse avec lui, un gonflement qui met bien en évidence le contact et non la continuité des deux fibres en présence.

En résumé, dans la couche moléculaire profondément compliquée qui recouvre la substance corticale du cervelet, chez le Rat, se retrouvent comme partout dans les

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[2] K. BAUER, Z. Zellforsch. mikr. Anat. *30*, 751 (1940).

[3] A. WEBER, Exper. *4*, 394 (1948); *5*, 461 (1949); Bull. Histol. appl. *27*, 73, 163 (1950).