The Effect of Exposure to Loud Tones upon Animal and Human Responses to Acoustic Clicks[1]

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Auditory fatigue can be defined broadly as a more or less temporary loss of auditory sensitivity due to previous auditory stimulation. There seem to be several types of fatigue, characterized by different time courses, degrees of reversibility, and frequency patterns of impaired response, but the great variability in reported data precludes a precise definition of these types. The advent of electrophysiological recording from the ear suggested that measurement of the electrical output of the ear might yield an objective index of fatigue. The results obtained in previous studies of cochlear potentials, however, were largely disappointing. A depression of the electrical response was usually obtained only when the stimulation was intense enough to cause damage to the responding mechanism.

During World War II more data on the effects of exposure to loud sounds in animal and man become available (*1, 3*). But renewed efforts to correlate temporary deafness in man with changes in the "electrical audiogram" (*2*) in animals again met with failure.

The electric potentials recorded from electrodes in contact with the cochlea are made up of at least two components: (1) the so-called cochlear microphonic, which reproduces fairly accurately the waveform of the acoustic stimulus and (2) the action potentials, presumably derived from neural structures in the lower auditory pathways. In general, when the electrical response of the ear to pure tones is recorded (and this was the case in the studies referred to above) the microphonic potentials swamp the neural components. On the other hand, in the responses to impulsive stimuli, such as sharp clicks at low and moderate intensities, the microphonic and the neural components may appear separated in time.

A typical response to a click recorded by means of a wire electrode placed near a cat's round window is shown in Fig. 1 (upper right-hand corner). The acoustic click is produced by feeding an electric pulse of 0.1 msec duration to a Permoflux (PDR-10) earphone.

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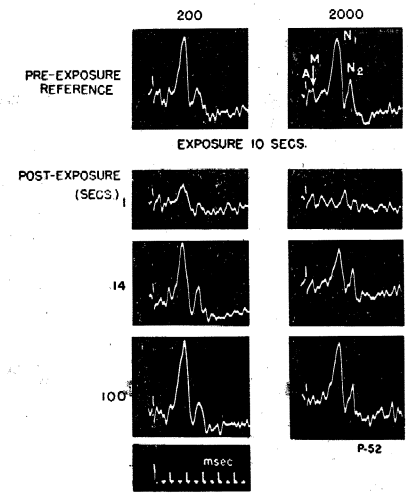

FIG. 1. Effect of exposure to loud tones (200 and 2000 cycles per sec) upon the response to clicks as recorded from the round window. The top line shows typical responses to weak clicks (about 15 db above human threshold). The earliest regular upward deflection on all records (*A*) is an artifact of 0.1-msec duration caused by the electric pulse delivered to the earphone. *M* is the earliest and in most cases most prominent microphonic component. N_1 and N_2 are the so-called first and second neural components. Typical post-exposure pictures, taken after exposures to tones of 200 and 2000 c for 40 sec at a sound pressure level of about 105 db, are presented. Note: (a) both neural components are reduced in approximately the same proportions; (b) recovery is quicker after exposure to the 200-c tone and the first neural component becomes "supernormal."

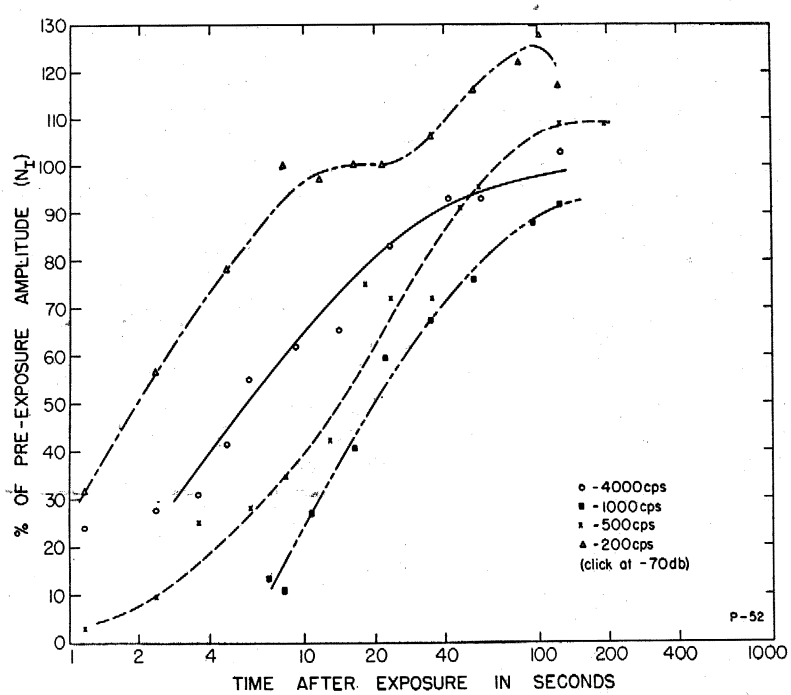

FIG. 2. Effect of exposure to various frequencies. All exposures were for 10 sec at a sound pressure level of about 105 db. The course of recovery of the first neural component is traced by comparing its amplitude at any given time after exposure with the standard or pre-exposure amplitude. Note the "supernormality" after recovery from exposure to tones of 200 and 500 c.

If the cat's ear is exposed to loud tones having a sound pressure level (SPL) of about 105 db and the responses to clicks before and after exposure are compared, significant changes are evident (see Fig. 1).[2] A sizable temporary reduction in the amplitude of the neural components follows even a relatively short exposure to moderately intense tones. Furthermore, the neural response to weak clicks is abolished for a time. Thus the exposure not only reduces the neural response to clicks; it also raises the threshold of the neural components. The course of recovery depends upon the frequency of the exposure tone (see Fig. 2) and it depends also, of course, upon the intensity and the duration of the exposure. For example, after one fairly severe exposure (2000 cycles per sec for 15 min at 125 db SPL) 10,000 sec elapsed before both components returned to about 85% of their pre-exposure amplitude. During the same period, however, the amplitude of the most prominent microphonic component showed only minor fluctuations.

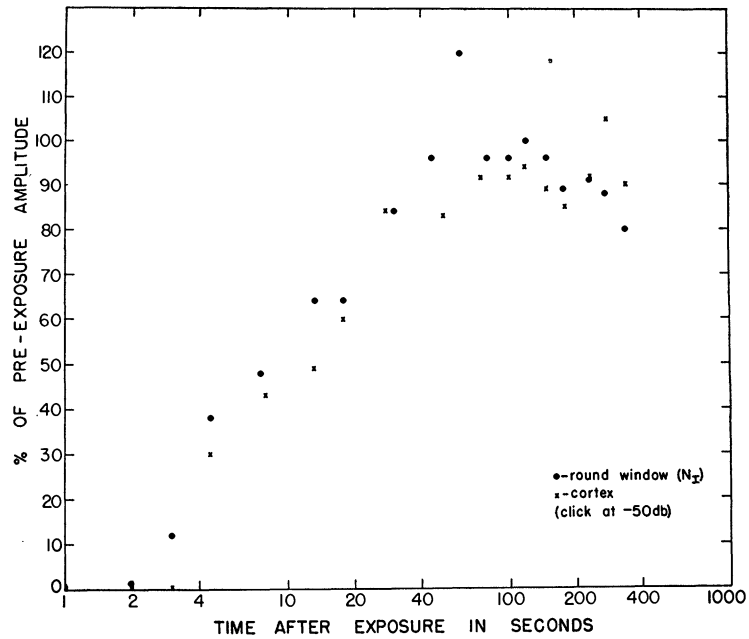

FIG. 3. Comparison between recovery at round window and recovery at the auditory cortex. The exposure stimulus was a 500-c tone presented for 60 sec at a sound pressure level of about 115 db.

At the auditory cortex the size of the electrical response to weak and moderately loud clicks is related monotonically to the acoustic input. It seemed worth while, therefore, to check the effect of an exposure to loud tones upon this cortical click response. Fig. 3 shows that the recovery curves for the so-called first neural component at the round window and for the cortical click response are hardly distinguishable.

Our data on the effect of exposure upon the size of the neural component show that the effect is greatest for the weak clicks and decreases as the intensity of the click increases. This may be related to the findings of Davis and his collaborators (*1*), who have shown that, whereas exposure to loud tones may elevate the threshold, the apparent loudness of sounds 80 to 100 db above threshold remains unchanged.

So far, we have been concerned with changes in the magnitude of a neural response to a given supraliminal stimulus. Auditory fatigue in man, on the other hand, is usually defined in terms of a change in threshold to a stimulus after exposure. Although these two manifestations of auditory sensitivity may represent different phenomena, there is good reason to believe that there are common physiological factors at work.

It is of interest, therefore, to inquire what happens to the human threshold for clicks after exposure to loud tones. Since no data were available on this point, we undertook the following experiment. After exposing an observer to pure tones of different frequencies, intensities, and durations, we presented a group of four clicks every 3 sec. The four clicks within each group were at different intensities separated by steps of 4 db. The number of clicks the observer reports permits an estimate of his threshold. We find that the threshold for clicks is raised by about 10 db after exposure to certain tones (e.g., 250 and 500 c for 2 or 3 min at 130 db SPL). The ear recovers rapidly and is normal after about 40 sec, a time not unlike that required for the cat's neural response to return to normal (cf. Fig. 2). After this recovery, however, the threshold goes up again by slightly less than 10 db, and there follows a long recovery period whose duration depends upon the frequency and duration of the exposure tone.

To summarize: Exposure to loud tones modifies the response to clicks as recorded from the round window of cats. The microphonic component shows little or no change, either to clicks or to the exposure tone itself. The neural components of the response to clicks are reduced in size immediately after exposure, and the course of their recovery depends upon the intensity, frequency, and duration of the exposure tone. There is a striking parallel between the recovery of the first neural component recorded at the round window and the recovery of the neural response recorded at the auditory cortex. Although it is hazardous to compare human thresholds with magnitudes of neural responses in animals, our preliminary findings suggest the possibility of a relation between these two phenomena.

Observations similar to some of those reported here have been made independently, and approximately during the same period, by Hawkins and Kniazuk at the Merck Institute for Therapeutic Research. Both groups re-

[2] Preliminary observations on guinea pigs confirm the data obtained from cats. We are indebted to Drs. H. Davis and C. Fernandez for their assistance in making these observations on guinea pigs.

ported their findings at the November 1949 (St. Louis) meeting of the Acoustical Society of America.

References

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Identification of Estrogens by Paper Chromatography

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In animals, estrogenic steroids occur almost invariably in mixture, but in spite of the great differences in their biological activity, chemical and biological assays are usually reported in terms of a single estrogen, owing to the technical difficulties in their separation and identification. Partition chromatography on paper can be used for separating closely related compounds, provided they show a difference in distribution coefficients and can be detected in small amounts. This technique has been applied successfully to the identification of ketosteroids by Zaffaroni *et al.* (*3*). A simple and rapid procedure for the identification of minute amounts of estrone, estradiol, and estriol by paper chromatography is presented here, which may be applicable to differential determination of estrogen content of biological specimens.

Colored derivatives of the estrogens are prepared by coupling with diazotized *p*-nitrobenzeneazodimethoxyaniline (Fast Black Salt K[1]). This reagent, proposed by Marx and Sobotka (*1*) for the determination of equilenin and dihydroequilenin, was reported as not coupling readily with estrone, estradiol, or estriol. However, K salt can be made to react with these steroids in a strongly alkaline medium, either by heating the mixture or by allowing it to stand overnight. A saturated aqueous solution of K salt is prepared freshly and filtered.[2] The estrogens are dissolved in absolute ethanol and 0.1 ml of each solution, containing a minimum of 3 μg of any steroid, is placed in a test tube, and a tube containing 0.1 ml alcohol is used as a blank. Then 0.2 ml of the diazo reagent and 0.1 ml of 20% Na_2CO_3 solution are added and the contents of the tubes are mixed and heated in a boiling water bath for 10 min and later cooled in water. To check evaporation, a glass bulb is placed in the mouth of each tube. Coupling also takes place if the mixture is allowed to stand overnight. The color deepens on standing and, once developed, it is stable for weeks. To extract the dyes, 0.2 ml benzene is added, and the tubes are shaken vigorously. The contents of each tube are drawn up into a medicine dropper with a fine tip, allowed to separate, the aqueous phase is expelled, and the entire benzene solution is applied to the filter paper.

[1] Emil Greiner Co., New York City.

[2] The solution keeps at least a day at room temperature and several days at 5° C, but it should be filtered before use to minimize the blank.

TABLE 1

COLOR AND LOCATION OF SPOTS IN THE PAPER CHROMATOGRAM OF ESTROGENS AND RELATED COMPOUNDS

Compounds	Color of spots	R_f values
Estrone*	purple	0.95 ± .03
Estradiol*	"	0.81 ± .06
Estriol*	"	0.07 ± .03
Estrone and estradiol†	"	0.96 and 0.85
Estrone and estriol†	"	0.96 and 0.09
Estradiol and estriol†	"	0.85 and 0.09
Estrone, estradiol, and estriol†	"	0.96, 0.85 and 0.09
Estrone sulfate‡	"	0.95
Equilin§	"	0.96
Phenol	red	0.50
Catechol	yellow	0.00
Diethylstilbesterol‖	none	...
1-Methylestradiol diacetate§	purple	0.81 and 0.96
Dehydrocorticosterone acetate‖	yellow	0.95
Progesterone*	"	0.74
Pregnanediol¶	none	...
Dehydroisoandrosterone acetate*	"	...
cis-Testosterone**	yellow	0.21
Isoandrosterone††	none	...
Δ^4-Androstene-3,17-dione††	yellow	0.60 and 0.97
Δ^1-Androstene-3,17-dione§	none	...
$\Delta^{1,4}$-Androstadiene-3,17-dione§	"	...

* Courtesy of Schering Co.
† Equal parts.
‡ Courtesy of Ayerst, McKenna, and Harrison, Ltd.
§ Courtesy of Dr. Martin I. Rubin, Georgetown University.
‖ Merck and Co., Inc.
¶ Bios Laboratories, Inc.
** Courtesy of Ciba Pharmaceutical Products, Inc.
†† Courtesy of Organon, Inc.

The solvent mixture found most suitable for the separation of the three dyes is prepared by mixing 200 ml toluene, 100 ml petroleum ether (boiling range 35°–60° C), and 30 ml ethanol with 70 ml water in a separatory funnel. After overnight equilibration, the water layer is drawn off into an open vessel and used in keeping the atmosphere of the chromatographic chamber saturated with respect to this phase. Equilibration and chromatography are carried out in an air-conditioned laboratory kept at 75° ± 3° F. The ascending method of chromatography (*2*) is used. The solvent mixture is placed directly on the bottom of a cylindrical glass jar, 12 in. high and 8 in. inside diam. The vessel, a so-called museum jar, has a flat glass cover with a rubber sealing ring and can be made airtight by means of a screw clamp. The bottom is raised in the center, which results in a saving of solvent. Sheets of Whatman filter paper No. 1, 18½ × 22½ in., are cut in half, and subsequently either cut into strips, which are suspended from hooks inside the lid of the jar, or, for up to 15 chromatograms, stapled together as cylinders 11¼ in. high and 18½ in. in circumference.

The paper is held over a hot plate and the benzene solution of the dye is placed on a pencil line, 1½ in. from the bottom edge. By repeatedly delivering small amounts of solution from the medicine dropper the entire benzene solution can be evaporated without making the spot un-