Induction of Convulsive Seizures in Sound Sensitive Albino Mice: Response to Various Signal Frequencies[1] (36659)

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Inbred laboratory mice, O'Grady strain, convulse when exposed to sound produced by a variety of means, including rattling keys, telephone rings and a door bell (1–3). However, no spontaneous seizures have been observed in these mice even when they have been kept in noisy surroundings (2). It appeared likely that only signals of certain specific frequencies could induce seizures.

We have investigated, therefore, the effect of sound signals of varying frequencies and intensities on seizure induction.

Materials and Methods. Audiosensitive albino mice were purchased from Flora O'Grady, Bronx, N.Y. (O'Grady strain). These mice were derived from the Swiss strain and bred for sensitivity to sound over 30–32 generations. Approximately 600 males were used in this study. Nonaudiosensitive Swiss mice obtained from the same source served as controls. All animals were received at weaning (*i.e.*, 20–22 days of age), and were tested over a period of several weeks. One day after arrival the mice were separated into groups matched according to weight and susceptibility to sound. Control Swiss mice were matched by weight only.

Sound signals of defined, sinusoidal waves were produced by a RCA signal generator, Model No. WA 44C, an amplifier (either General Radio Model 1206B-1203B or Lafayette Model 765), and a speaker (first an "extended range" speaker, then a University Sphericon Tweeter) mounted on a roof of the test chamber. The chamber, 18 in. × 10½ in. × 6 in., was made of clear polycarbonate with rounded edges and corners. Overall signal intensity was measured with a sound-level meter, Bruel and Kjaer Model 2203, weighted according to the USASI standard A network. Frequencies between 5–35 KHz at sound pressure of 50–100 dBA over 2×10^{-4} microbar (20 $\mu N/m^2$) were employed. Convulsive dose of sound (pressure expressed in dBA) required for 50% seizure response (CD_{50}) at various frequencies was determined by the method of Doren *et al.* (4), using mouse population ranging from 10 to 30 animals per group.

In addition, individual seizure responses were graded on a scale of 0–10 points: strong irritation, total immobility or occasional body tremors rated 2.5; uncontrollable running, characteristic for sound-sensitive mice, 5.0; mild clonic convulsions, 7.5; tonic-clonic convulsions with extension of the extremities, 10.0. Score for a group of mice was then expressed as percentage of maximum possible score:

$$\frac{\Sigma \text{ Individual responses}}{\text{Number of mice} \times 10} \times 100.$$

No group was smaller than ten mice. Time lag from onset of sound to seizure response was also recorded.

To the extent that sound level meters measuring total sound pressures and employing USASI weighting curves compensate for human ear characteristics, they tend to overemphasize the contribution of frequencies which are inaudible to the mouse. We have, therefore, analyzed the composition of background noise and signals by determining sound pressure levels within various frequency ranges with the aid of an octave band filter set,

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Bruel and Kjaer Model 1613, equipped with either 1 or .5 in. diameter condenser microphones, Type 4131 and 4133, which permitted screening out of most frequencies not applicable to the mouse. The contribution of our signals to the sound pressures within the mouse frequency range was evaluated (5, 6).

Results. Seizures induced by sound stimulation began within 2–8 sec with the typical wild running characteristic of audiosensitive mice, which changed within 4–15 sec into tonic seizures with flexion and extension of extremities. The animals recovered within 30–50 sec and showed no visible aftereffects. All survived. When the signal was kept deliberately mild either in intensity or in frequency, the mice ran for 10–15 sec, but did not have tonic-clonic seizures. Signals of overall pressure of 74 dBA (*i.e.*, 20 dB higher than the laboratory background noise) produced 100% seizure response at all frequencies lying between 10–25 kHz (Fig. 1). Lower intensity signals (66 dBA) produced the strongest reaction between 11–15 kHz.

The maximum effect with the minimum sound pressure was achieved at 13 kHz. This frequency was optimal for seizure induction. This fact was confirmed when the average time lag from the start of the signal to the onset of seizures was found to be shortest at 13 kHz. When the dominant frequency was changed from 13 to 22 kHz an increase in overall intensity from 59 to 68 dBA was necessary to elicit 100% seizure response in 21 day old mice. Although less efficient, the latter frequency was found useful because it was inaudible and thus nonirritating to humans. Signals below 5 kHz or over 35 kHz, regardless of intensity, did not lead to tonic seizures. Although room background noise level ranged from 52–55 dBA, no spontaneous seizures were observed in our colony. Our control Swiss mice did not convulse upon exposure to sound, even at 100 dBA.

Analysis of frequency distribution of background noise in our laboratory with octave band filters proved that the noise is composed mostly of sounds inaudible to the mouse. Measured with the sound level meter calibrated with the USASI A scale, it registered 54 dBA, but it contained only 13 dB re 2×10^{-4} μbar of noise registering with the band centered on the frequency of 16 kHz and covering the spectrum from 10.7–21.3 kHz, extending over the main mouse hearing range when assayed with the octave band filter (Fig. 2). A few examples should suffice to demonstrate the differences between the overall signals as they are heard by man and recorded by sound level meters adjusted for

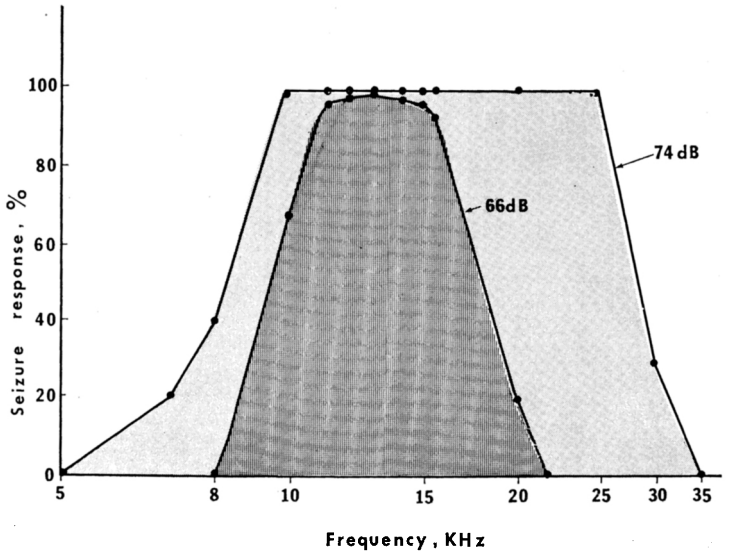

FIG. 1. Tonic-clonic seizures in O'Grady mice in response to stimuli produced by sound pressures of 66 and 74 dBA varying in frequency from 5 to 35 kHz.

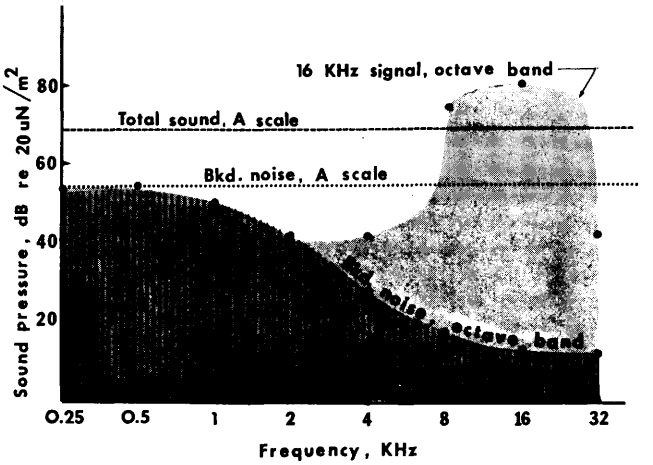

FIG. 2. Octave band analysis of background noise and the contribution of a 16 kHz signal. Background noise rated 54 dBA and signal plus background totaled 69 dBA.

human hearing and as they must appear to the laboratory mouse against this very quiet laboratory background level. The weakest signal produced by a 5-in. bell in a metal drum registered a total sound pressure corrected for the A curve of 70 dBA re 2×10^{-4} μbar, 16 dBA above background. Measured with the 16 kHz octave band filter the sound pressure in the 10.7–21.3 kHz range proved to be 89 dB re 2×10^{-4} μbar. Thus the bell "added" a striking 76 dB above background. A 16 kHz signal produced by our signal generator with the power input adjusted so that the total sound pressure on the A scale was 69 dBA, 15 dBA over background (Fig. 2), proved to be 80 dB, or 67 dB over the 13 dB background when assayed within the range of 10.7–21.3 kHz. A weak 16 kHz signal, which raised total pressure by only 2 dBA over background, probably did not appear weak to the mouse, for it contributed 35 dB above background noise, for an overall total of 48 dB within the mouse hearing range.

The sound intensities required to produce

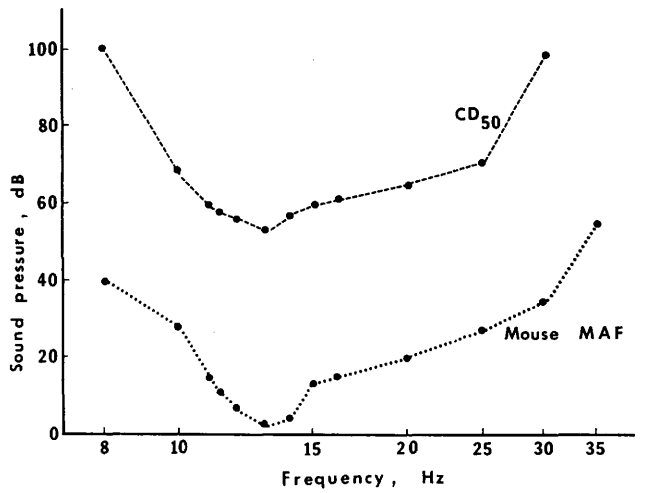

FIG. 3. Sound intensity required for 50% convulsive seizure induction (CD_{50}) recorded in dB re 2×10^{-4} μbar with an octave band filter for the main mouse hearing range (10.7–21.3 kHz), compared to the mouse minimum audible field (6). Stimulus frequencies ranged from 8–35 kHz.

a 50% convulsive response at frequencies between 8–35 kHz range were reinvestigated with the aid of an octave band filter set for the 10.7–21.3 kHz range. The signal intensity required for a CD_{50} response proved almost parallel to the mouse minimum hearing acuity as reported in literature (6) for frequencies lying between 10 and 25 kHz, but higher at either end of the spectrum (Fig. 3).

Discussion. The intensity of sound required to produce seizures in the O'Grady strain was lowest at 13 kHz. At frequencies below 8 and above 30 kHz signals of very high intensity were necessary. At intermediate frequencies, our frequency/intensity curve (Fig. 3) paralleled the published hearing acuity curves obtained with the conditioned galvanic skin response as an index of hearing or directly with microelectrodes implanted in auditory units (6). Thus, the hearing range of audiosensitive mice did not appear to differ significantly from the range of control mice, whose hearing acuity was found to lie between 10–20 kHz, with peak frequency response closer to 10 kHz. All signals over the main mouse hearing range were convulsive. The degree of their seizure effectiveness depended largely on the animal's hearing acuity at any given frequency. For CD_{50}, pressures of 46.1 $\pm$ 3.1 dB above the minimum audible field values were required to induce seizures between 10–25 kHz. Only marginal frequencies (8 and 30 kHz), were less effective, requiring over 60 dB of sound pressure. Thus, no particular specific irritating frequency emerged as being responsible for seizure induction, but a broad band of frequencies proved convulsive. The location on the band of the frequency scale appared to depend solely on hearing acuity of the mice.

Reflex epilepsy depends on sensory perception. More intense stimuli generally produce a greater incidence of seizures in susceptible subjects. Intensity of the stimulus, however, must be defined in terms of the subject. Particular care must be exercised in sound definitions where the units themselves are anthropocentric. By definition, the level of 0 dB re 2×10^{-4} μbar is based on minimal *human* hearing acuity (5). Sound level meters have built-in calibration curves which compensate for human hearing contours and drop off sharply at higher frequencies. Other equipment for sound measurement or reproduction, including microphones, speakers, *etc.*, suffers from the same defect. When it comes to calibrating intensity of sound reaching the CNS of an experimental animal, specific adjustments are required. Noise intense to humans need not appear that way to a mouse. Fortunately, in our case, the octave band centered on 16 kHz $\pm$ 30% covered the main mouse hearing range and a filter for that band was used for a rough approximation of the intensity of sound audible to the mouse.

The difference between the data that we have obtained with a sound level meter corrected for human total hearing acuity (USASI A curve) and with the octave band filter within the mouse hearing range explains the otherwise puzzling fact that spontaneous seizures have never been observed in our mice in spite of the high and variable background noise level. It also explains why the seemingly small increments of total noise produced by our signal generator as determined with the sound level meter were sufficient to induce seizures with reliability and reproducibility. Sound pressures with optima within human range proved misleading when applied to the mouse. The increments due to our signal were not small for the animals; our generator raised the overall sound pressure audible to the mouse by 35–80 dB re 2×10^{-4} μbar. It is likely that two separate, though perhaps related, mechanisms underlie induction of audiogenic seizures. Both a specific epileptogenic focus and a general metabolic instability distinguish O'Grady mice from matched Swiss controls: the general susceptibility to seizures does not disappear upon maturation (Alexander and Gray, unpublished data) but the sensitivity of the specific sound-seizure mechanism decreases with age.

General metabolic instability predisposing our animals to convulsive seizures can involve many biochemical systems. To mention only two examples, abnormal myelin has been observed in aberrant mice strains (6) and high cholesterol diets have been useful in treatment of epilepsies of diverse etiologies

including audiogenic seizures (7). Abnormal phenylalanine metabolism and neurotransmitter turnover rates have been observed in audiosensitive animals (8, 9). No detailed review of pertinent literature can be presented here.

The specific causative factor, unique to the audiogenic-seizure animals, involves a heightened sensitivity to auditory stimuli: either an increased auditory acuity or an unusal seizure response at normal auditory thresholds. Proper hearing is essential: O'Grady mice ceased convulsing when their hearing was impaired either physically or chemically (6, 10). However, we have found in this study that the hearing range of the inbred mice did not differ significantly from that of control mice. Inasmuch as control mice did not convulse even when exposed to extremely strong signals, it is unlikely that mere differences in auditory acuity could account for susceptibility to audiogenic seizures in inbred strains. The specific system responsible for induction of audiogenic seizures in O'Grady mice appears to be, at least in part, serotonin dependent and can be blocked rapidly by the inhibitor of serotonin biosynthesis, *p*-chlorophenylalanine (11) although the same drug enhances other forms of seizures. The mechanism for this effect is unknown, but the phenomemon underscores again the specificity and uniqueness of the process of induction of audiogenic seizures.

Few known counterparts to sound-induced seizures exist in human experience except for the so-called "musicogenic seizures". Most reflex epilepsy in humans is induced by visual stimuli (12). Specific sounds which do not add to overall noise and therefore escape detection but which significantly alter the properties of the noise could account for some "spontaneous" attacks of otherwise unknown etiology. However, our data on the absence of specific wavelengths responsible for induction of seizures in mice and on the nearly equal ability of all audible sounds to act as inducers, cast considerable doubt on the possibility that sound frequency changes underlie any human idiopathic epileptic seizures.

Summary. Convulsive seizures were induced in audiosensitive seizure-prone mice, O'Grady strain, with defined sound signals. Optimum frequency for seizure induction was 13 kHz, but a signal of 22 kHz, inaudible to human observers, was also satisfactory. Measurements with an octave band filter covering the main mouse hearing range established that no one wavelength was specifically responsible for seizure induction: all frequencies between 8–30 kHz were convulsive, depending only on the mouse hearing acuity.

1. Swinyard, E. A., *in* "Psychophysiologie, Neuropharmacologie et Biochimie de la Crise Audiogène" p. 405. Centre National de la Recherche Scientifique, Paris (1963).

2. Alexander, G. J., and Gray, R., Fed. Proc. **29**, 779 (1970).

3. Fink, G. B., and Iturrian, W. B., *in* "Physiological Effects of Noise" (B. L. Welch and A. S. Welch, eds.), p. 211. Plenum Press, New York (1970).

4. Doren, E., Miller, M., and Taber, R. I., Fed. Proc. **29**, 779 (1970).

5. Petersen, A. P. G., and Gross, E. E., Jr., "Handbook of Noise Measurement," 6th ed. General Radio Co., West Concord (1967).

6. Fuller, J. L., and Wimer, R. E., *in* "Biology of the Laboratory Mouse" (E. L. Green, ed.), 2nd ed., p. 606. McGraw–Hill, New York (1966).

7. Alexander, G. J., and Kopeloff, L. M., Exp. Neurol. **32**, 134 (1971).

8. Schlesinger, K., *in* "Mind as a Tissue" (C. Rupp, ed.), p. 271. Harper and Row, New York (1968).

9. Busnel, R. G., and Lehmann, A., J. Physiol. (Paris) **53**, 285 (1961).

10. Geller, L. M., Cowan, D., and Wolf, A., Exp. Neurol. **14**, 86 (1966).

11. Alexander, G. J., Kopeloff, L. M., and Alexander, R. B., Life Sci. **10**, 877 (1971).

12. Forster, F. M., *in* "Physiological Effects of Noise" (B. L. Welch and A. S. Welch, eds.), p. 151. Plenum Press, New York (1970).

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