($p < 0.05$). Interestingly, animals maintained on a 2L:22D photoperiod also show this seasonal difference ($p < 0.01$), suggesting that it is not dependent on length of daily photoperiod. The work of Wirz-Justice[9], also using an in vitro uptake model, implies support for our results by showing maximum monoamine uptake in brain slices during October and minimum uptake during June. The fact that these seasonal differences persist in the laboratory environment as well as in field conditions suggests either an endogenous generator mechanism or perhaps a subtle geophysical mechanism. One current molecular theory attributes rhythms to membrane changes which, via an ion transport system, could influence the conformational state of the membrane[11]. This may have important implications for changes in monoamine reuptake sites, synaptic vesicles, and control factors in neuronal activity.

Yet, environmental lighting is the major and most consistent external variable and Zeitgeber to affect both the suprachiasmatic nucleus[12] and the pineal[13] via direct neuronal links with the retina. Furthermore pinealectomy, which removes the hormonal output of this gland, has been shown to induce cytochemical changes in certain cells within the suprachiasmatic nucleus suggesting modulation of suprachiasmatic neuronal or secretory activity[14]. Reiter has demonstrated that the pineal is critical for the annual reproductive capability of the golden hamster[15], and others have suggested that the pineal may function as an integrating device between the primary synchronizer of environmental photoperiod and various regional brain indole rhythms. The roles of the various serotonergic, catecholaminergic and other chemical mediators in the physiology of the suprachiasmatic nucleus are not completely understood, but it does appear very likely now that the importance of this nucleus in mediating light information for central nervous system periodicity is widespread among mammals[12]. The importance of monoamine periodicity in the suprachiasmatic nucleus, and of related circannual endocrine rhythms, may stem, at least in part, from adaptations to appropriately timed reproductive trophic and steroid hormones for optimal reproductive success and thus, species survival.

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Experimentally induced otitis and audiogenic seizure in the mouse

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Summary. Audiogenic seizures can be induced in genetically non-susceptible 17-day-old mice (Rb/3 strain) with various results. Priming only induces 9% of seizures, auditory insulation 3,8%, while experimental otitis leads to 79%. The hypothesis concerning disuse supersensitivity subsequent to acoustic deprivation was not confirmed by the experiment. However, modification of acoustic transmission at middle ear level induced by otitis or ear physical damage during the maturation period, exposes the upper nervous centers to intense stimulation to which the reaction is a recruiting response.

Recent studies have demonstrated that audiogenic seizures (A.S.) could be induced in genetically non-susceptible mice following acoustic priming[1,2], tympanic membrane perforation[3], or plugging of the external meatus[4], during a critical period of postnatal development (17–25 days). It was hypothesized that the acoustic deprivation resulting from such modifications produces a subsequent hyperreactivity (disuse supersensitivity) of the CNS auditory centers. However, on one hand, the effects of acoustic priming on A.S. susceptibility are different in 2 genetically resistant strains of mice (17% A.S. in the C57 BL Charles River and only 9% A.S. in the Rb/3) while the auditory impairment measured by cochlear potential shifts are identical[5]. (Rb: Swiss albinos has 2 substrains: Rb/1 genetically susceptible to A.S. Rb/3 genetically resistant [Mouse News Lett. *1959*, Companion issue No. 21, p. 42].) Therefore, mechanisms other than that of disuse supersensitivity must be involved in the induction of seizure susceptibility.

On the other hand, spontaneous middle ear infections enhance A.S. sensitization following acoustic priming by an electric bell in genetically non-susceptible mice[6]. Another priming (10 kHz, 120 dB) gave a 62% seizure rate in the Rb/3 resistant strain, but 23% of these animals had spontaneous middle ear infection. 1 year later, however, identical experiments produced significantly different results: 23% A.S. and 5% otitis (Niaussat, unpublished data). Considering these findings, a study was undertaken to define the exact influence of otitis on A.S. sensitization, in genetically non-susceptible mice. Irritative otitis was chemically induced in such mice and an investigation was conducted to determine the possible relationship between varying degrees of ear damage and the nature of the resulting seizures.

Materials and methods. The otitis induced was to be irritative but non-infectious to avoid introduction of a pathological reaction of the organism to infection. After preliminary trials with various chemicals: e.g., silver nitrate, trichloracetic acid, formol, B-methyl-metacrylate, the latter was found to be most irritative to the external and middle ear skin and mucosa. B-methyl-metacrylate is a dental cement catalyzer which was discovered to be irritative to mouse skin when used in excess during implantation of chronic electrodes (personal observation). 17-day-old mice from the non-susceptible Rb strain were

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used. The 216 animals selected were divided into 5 groups to differentiate the influence of the chemical from those of the various manipulations involved in the procedure. Group 1: 62 mice received several drops of B-methylmetacrylate in each ear. To insure a close contact between the chemical and the skin of the ear canal, a cotton bolus was introduced into the meatus and was held in place by folding the pinna over itself and clamping it shut with a 7.5 mm surgical wound clip. This treatment not only irritated the external ear with the corrosive chemical but also partially insulated the mouse from ambiant noise. Group 2: 51 mice received only sterile cotton in the ear canal held in place as described above. Group 3: 33 mice had only a slight acoustic insulation by occlusion of the pinna as in groups 1 and 2. Group 4: 22 mice were acoustically deprived by being kept in an individual anechoïc chamber (background noise = 31 dB, linear measure). Cotton wool replaced the normal litter to absorb the noise of the animal's own movements. Group 5: 44 control mice were given equal handling but underwent no experimental manipulations.

After a 5-day-interval, the pinna and the meatus (groups 1–3) were cleared and all animals were tested with an electric bell (broadband 10–40 kHz, 110 dB SPL) for 1 min, or until convulsive seizure occurred: either wild running (R), clonic (Cl), or tonic seizure (T). After testing the mice were killed and dissections of the peripheral auditory system were performed to study and classify the incidence and nature of ear damage.

Results. Results for the 5 experimental groups are given in the table. No otitis and no audiogenic seizures were observed in the control group. However, acoustic deprivation in the anechoïc chamber induced a slight occurence of A.S. (3,8%) without otitis. Although the irritative chemical induced a high proportion of A.S. and ear damage (79% and 86%) in the other 3 groups, the sterile cotton and even the clamp alone brought about a relatively high, but non significant, incidence of A.S. and otitis. On the other hand, these are global results which do not differentiate between the 3 types of convulsive seizures (wild running, clonic and tonic seizures) and the various degrees of ear damage.

To determine the relationship between these various reactions, the 142 cases of ear damage observed out of the 216 ear autopsies performed were re-examined and classed into 3 categories: 1. Mild irritation of the ear canal and of the pinna due to the wound caused by the clamp or to a skin reaction to the cotton bolus. 2. Otitis externa with congestion of the ear canal, hypersecretion of the ceruminous glands and occasional vasodilation of the ear drum. 3. Otitis media with or without perforation of the tympanic membrane. The irritant chemical induced either otitis externa or media.

– Of the 104 mice exhibiting non convulsive seizures, a 40% otitis rate was nevertheless observed.

– Of the 111 mice exhibiting A.S., an average rate of 90% ear damage, principally otitis media and externa, was observed.

The difference between these 2 percentages is highly significant (Student's t-test, t: 7.5 $p < 0.001$).

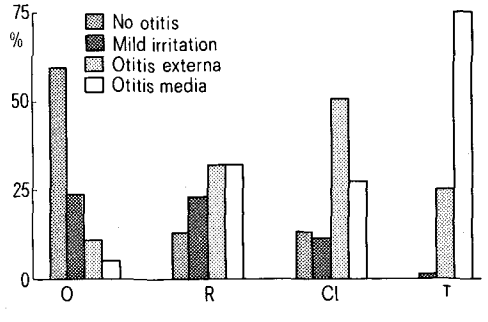
The percentages of various degrees of ear damage for each type of convulsive reaction to acoustic stimulation are shown in the figure. 75% of the mice exhibiting tonic seizure, 28% of those exhibiting clonic seizures, and 31% of those exhibiting wild running were afflicted with otitis media. The results indicate that there is a direct relationship between the proportion of otitis media observed and the severity of the convulsive reaction. Otitis externa is observed most often in mice exhibiting clonic seizures (52%).

Discussion. A positive correlation exists between the presence of otitis and the onset of A.S. after intense acoustic stimulation. This relationship has been established in the rat[7]. Specific pathogenic-free animals have a lower seizure susceptibility than conventional animals[8]. We propose the following hypothesis: the presence of a congestive state or a middle ear effusion modifies acoustic transmission along the ossicular chain by impairing contractions of the middle ear muscles, and as a result, the auditory system is no longer protected from acoustic overloading. This can be related to the recruiting phenomenon already observed in genetically susceptible mice[9]. The hypothesis concerning auditory hypersensitivity due to sensory deprivation[1–4] was not confirmed in this experiment as only 3,8% of the acoustically deprived mice reacted with A.S. The acoustic deprivation obtained by plugging the ear canal was effective only when accompanied by congestion of the external or middle ear.

Percentage of A.S. and ear damage in control and experimental groups and their statistical values (t-test)

Treatment at 17 days	Numbers of mice	Per cent of A.S. at 22 days	Per cent ear damage
Control	44	0	0
Liquid, cotton wool and clamp	62	79[a]	86[d]
Cotton and clamp	51	52[b]	45[e]
Clamp only	33	24[c]	27[f]
Acoustic deprivation	22	3.8	0

[a,b] $p < 0.0001$ (HS); [b,c] $p < 0.05$ (NS); [d,e] $p < 0.00001$ (HS); [e,f] $p < 0.13$ (NS).



Relation between each type of convulsive reaction and various degrees of ear damage. *O* No seizure; *R* running only; *Cl* clonic seizure; *T* tonic seizure.

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