

Changes in temporal acuity with age and with hearing impairment in the mouse: A study of the acoustic startle reflex and its inhibition by brief decrements in noise level

James R. Ison, Punit Agrawal, James Pak, and William J. Vaughn

Department of Brain and Cognitive Sciences, Meliora Hall, University of Rochester, Rochester, New York 14627

(Received 8 August 1997; accepted for publication 28 April 1998)

Temporal acuity for brief gaps in noise was studied in mice of different ages (1–36 months) from strains with differing susceptibility to age-related hearing loss, using reflex modification audiometry. Prepulse inhibition of the acoustic startle reflex (ASR) increased with gap depth (GD: 10–40 dB in 70 dB SPL noise) and lead time (LT: 1–15 ms). The increase in inhibition with LT followed an exponential function in which the two parameters, asymptotic inhibition (AINH) and the time constant (τ), were both affected by GD. AINH rapidly declined from 1 to 6 and then to 18 months of age in C57BL/6J mice with progressively severe hearing loss, but first increased with maturation and then gradually declined beyond 6–12 months of age in CBA/CaJ and CBA×C57BL F1-hybrid mice, which show no apparent change in sensory function at these ages. In contrast, τ was unaffected by hearing loss or by age, this suggesting that age-related changes in this form of temporal acuity occur because of a reduction in the efficiency with which gaps are centrally processed, not from any reduced ability to follow their rapid shift in noise level. © 1998 Acoustical Society of America. [S0001-4966(98)03608-X]

PACS numbers: 43.80.Lb, 43.66.Sr, 43.66.Mk [FD]

INTRODUCTION

The objective of this research was to describe developmental changes in temporal acuity in auditory processing in the mouse over the life span, from early maturation to late senescence. Temporal acuity was measured by a variant of the “gap detection” paradigm, which is assumed to measure the sensory persistence of a stimulus after its physical offset (Plomp, 1964). The behavioral method of reflex modification audiometry (Young and Fechter, 1983) was used in the present work to exploit the observation that the offset of a background noise inhibits the acoustic startle reflex (ASR) if it is presented immediately before the noise burst that normally elicits the reflex (Stitt *et al.*, 1973). One strain of mice, the C57BL/6J, was chosen for its well-characterized age-related hearing loss, and two others, the CBA/CaJ and the F1 hybrid of a C57 and the CBA, because of their minimal expression of hearing loss with age (see Henry and Chole, 1980; Li and Borg, 1993; Mikaelian, 1979; and especially Willott, 1996).

Gap detection thresholds improve with age in children (for example, Davis and McCroskey, 1980; Irwin *et al.*, 1985; and others) and rat pups (Kellogg *et al.*, 1983; Dean *et al.*, 1990), and are then raised in aged compared to young adult humans (McCroskey and Davis, 1976, cited in McCroskey and Kasten, 1982; Schneider *et al.*, 1994; Snell, 1997), and also in aged mice compared to young mice (Ison *et al.*, 1993). Threshold changes are seen especially in aged listeners with sensorineural hearing loss (for example, Lutman, 1990; Moore *et al.*, 1992), which also increases gap detection thresholds in young humans (for example, Fitzgibbons and Wightman, 1982; Buss and Florentine, 1985; and

others), and young chinchillas (Giraudi-Perry *et al.*, 1982; Salvi and Arehole, 1985).

Gap detection, which is accepted as a simple and convenient measure of temporal acuity, has been shown to correlate with measures of speech perception in humans (Tyler *et al.*, 1982), and it is therefore encouraging that the alteration of gap thresholds with age and hearing loss seen in humans, described above, can be captured in animal models that afford the possibility of studying its physiological bases (Walton *et al.*, 1997). In this latter pursuit, it is of considerable interest to determine if these observed changes in gap detection thresholds are necessarily best understood as an effect of age and/or hearing loss on an intrinsic temporal characteristic of auditory function, or whether, instead, the effects might be better interpreted as a change in the overall “efficiency” of a central detection mechanism. This theoretical distinction was introduced by Patterson *et al.* (1982) in an abstract model intended to account for the effects of age on the critical ratio and masking in human listeners, and it was subsequently extended to gap detection by Plack and Moore (1990). Hall and Grose (1994) found that the improvement in temporal acuity seen in children (not using gap detection, but temporal modulation transfer functions to measure temporal acuity) resulted from an increase in the efficiency of a central processor in detecting a shift in the level of its afferent input, rather than to a change in ability to follow and encode the temporal course of the amplitude modulated stimulus. Given these data we were then concerned to see if a similar account might be appropriate for gap detection obtained under various conditions in the mouse.

In our previous work we have shown that noise offset is in fact inhibitory, that is, it reduces the ASR below that

obtained when the reflex is elicited in the absence of the background noise (Ison, 1982). Further, for gaps of the same sized decrement but beginning at different background levels, neither the rate of development of inhibition nor its asymptote varies with noise level, but larger decrements result in different asymptotic levels of inhibition; further, explicit variation in decay time at the beginning of the gap alters the rate of development of inhibition (Ison *et al.*, 1996): the implications of these findings are that in the present experiment reflex inhibition should be little affected by at least moderate shifts in the audibility of the noise carrier (as is true also for psychophysical experiments with human listeners: Buss and Florentine, 1985); that a change in the “internal” signal-to-noise ratio of the neural representation of gaps, which would be expected from a change in processing efficiency, should be evident in a change in the asymptotic level of inhibition; and a change in its “internal” decay time indicative of a change in an intrinsic temporal process should be apparent in the time course of inhibition rather than in its asymptotic level.

I. METHOD

A. Subjects

The subjects were 156 mice (92 male and 64 female), 24 acquired as breeding stock from the Jackson Laboratories and Harlan Sprague Dawley, and 132 born and raised in the vivarium at the University of Rochester. The strains and nominal ages at testing were as follows: the C57BL/6J at 1 month of age ($n=6$), 3 months ($n=6$), 6 months ($n=15$), 1 year ($n=10$), and 18 months ($n=9$); the CBA/CaJ at 1 month of age ($n=7$), 3 months ($n=6$), 6 months ($n=6$), 1 year ($n=10$), 18 months ($n=12$), and 2 years ($n=9$); and the F1-hybrid cross of a CBA male and a C57BL female at 1 month of age ($n=8$), 2 months ($n=9$), 3 months ($n=13$), 6 months ($n=8$), 1 year ($n=6$, all of which had been tested at 6 months of age), 2 years ($n=19$, three previously tested at 6 months of age), and 3 years ($n=10$, four of which had been tested at 2 years of age). The actual ages varied slightly from those listed above because of limitations in the availability of subjects of specified ages and because the testing time varied from 7 days to about 14 days across different cohorts. The actual mean ages for the groups are given in the results section. The mice were maintained generally in group cages, rarely in single cages because of fighting, under conditions of constant temperature and humidity, with a 12-12 h light-dark cycle (lights on at 6 a.m.). Food and water were available at all times. Tests were run from about 9 a.m. to 5 p.m.

B. Apparatus

The subjects were confined for testing in a cage made of acrylic plastic (7 cm long, 5 cm wide, and 4 cm high) with slotted sides and roof for free sound penetration, which could be mounted on a suspended acrylic platform directly over an attached accelerometer (model SA-2-300, Statham Laboratories, Beverly Hills, CA). The platform was placed in an anechoic chamber (inside dimensions $84 \times 84 \times 84$ cm, manufactured by the Eckel Corp., Cambridge MA). The acceler-

ometer was sensitive to the vertical force exerted in the startle reflex, and its output was amplified and then integrated over a 100-ms interval beginning with the startle stimulus. The startle stimulus was a noise burst provided by a wideband noise generator that was gated through an electronic switch, amplified, then delivered through a high-frequency speaker. It was presented at 115 dB SPL, and was 20 ms in duration with near instantaneous rise and fall times. The subjects were run in a noise background at 70 dB SPL, provided by the same noise generator but delivered through a Panasonic high-frequency leaf tweeter. The level of the background noise was controlled by programmable attenuators with near instantaneous response times. The spectrum of the startle stimulus varied by no more than ± 2 dB over a range of octave bands centered at 1–32 kHz, while the noise background varied by no more than ± 6 dB over a range of 2–100 kHz. (These were measured with a 1/4-in. Bruel & Kjaer microphone, model 4135, connecting to a measuring amplifier, Bruel & Kjaer, model 2610.) Stimulus presentation and response recording were under computer control.

C. Procedure

The subjects received trials on which the startle stimulus was delivered alone in the noise background and trials on which the startle stimulus was preceded by the partial offset of the noise, to noise floors of 30, 40, 50, or 60 dB (gap decrements (GD) of 40, 30, 20, and 10). These decrements led the onset of the startle stimulus by 1, 2, 4, 6, 8, 10, or 15 ms. The experiment was continued over 4 test days, each 2–4 days apart. On any one test day only one level within the gap was used but all of the gap intervals were presented in random order within blocks of trials. Each block consisted of three control trials (with startle stimuli not preceded by a gap, in order to provide the baseline startle response), and one each of the gap conditions, for a total of ten trials within each block. Eleven blocks were presented on each day with a mean interval between trials of 20 s. The order of testing for the different floor levels was random.

D. Data analysis

Two indices of response strength were used for the primary statistical analyses. For the first, mean integrated response amplitudes (in units that are linearly related to volts) were calculated within subjects for each stimulus condition. This measure for the baseline control response was useful for assessing the effects of age and strain on the average force exerted in the startle reflex, that is, for showing the effect of maturation, senescence, and hearing loss on the baseline acoustic reflex response. The second measure provided the fundamental index of reflex inhibition, and was based on the ratio formed by dividing the inhibited response amplitude score for each condition for each mouse by the baseline control response for that subject in that particular floor condition (that is, $1 - \text{mean prestimulus condition}/\text{mean control}$). This relative inhibition measure provided the metric on which the temporal course of inhibition (τ values) and the asymptotic levels of inhibition (AINH values) were determined from the formula

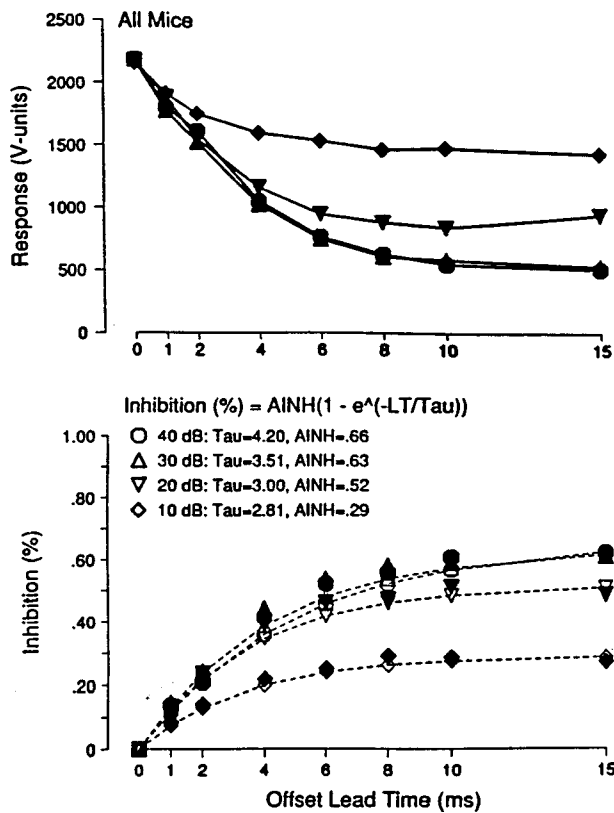


FIG. 1. (Top) Mean amplitudes of the acoustic startle reflex on control trials (at 0 ms) and on trials when the startle stimulus was preceded by a decrement in a 70-dB background noise of 10–40 dB, at lead intervals of 1–15 ms, for all subjects. (Bottom) Mean relative inhibition of the startle response for these same stimulus conditions, obtained data in the solid symbols, and calculated values derived from an exponential growth function in open symbols connected by broken lines. The inset gives the parameter values for each noise decrement, as the time constant (τ) and the asymptotic value for inhibition (AINH).

$$\text{Relative inhibition} = AINH(1 - e^{-LT/\tau})$$

with LT as the lead time of noise offset. Both response measures were analyzed by mixed design analyses of variance (ANOVA) in which strain and age were the between-*S* variables and gap duration and floor level were the within-*S* variables. Degrees of freedom for repeated measures were adjusted for nonhomogeneity of between cell correlations by the Hunyh–Feldt procedure.

II. RESULTS

A. Overall analysis of LT and GD at noise offset

Figure 1 presents the mean values for the two major response indices, ASR amplitude in the upper portion and relative inhibition in the lower, as they varied with the lead time and the depth of the noise decrement, this taken across all groups. Inhibition increased with LT towards an asymptotic limit determined by GD, but it may be noted that even the smallest decrement (10 dB) at the smallest LT (1 ms) significantly reduced the ASR, $F(1/150) = 29.90$, $p < 0.01$, and there was also a small but significant difference between the noise floors at this interval, $F(3/450) = 4.63$, $p < 0.01$. The solid symbols in the lower portion of Fig. 1 give the observed data points for relative inhibition across the

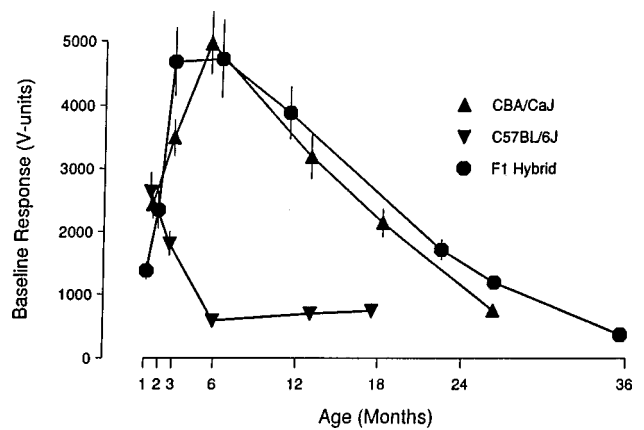


FIG. 2. Mean amplitudes for the baseline control reflex (± 1 SEM) for each of three strains of mice at each age.

entire set of subjects, and the open symbols are the predicted values provided by the best fitting exponential function, these values being highly correlated, $r = +0.994$. The two parameters of this function for each of the noise floors are given in the upper left corner in this figure. The size of the noise decrement affected both parameters, a greater decrement resulting in a slower growth rate of inhibition but also a greater asymptotic level of inhibition. The inferential analysis of these parameters was complicated because while grouped data were stable and suitable for curve fitting, the data provided by individual subjects often had random irregularities, particularly for the lower GD, which could not be reasonably fit with an exponential function. For this reason the analysis of the differences in AINH described in Fig. 1 used the obtained values for relative inhibition for each subject at the 10- and 15-ms intervals as the surrogates for the predicted values. This analysis of variance showed an overall significant effect for GD, $F(3/504) = 96.86$, $p < 0.01$, large and significant differences between the adjacent 10- vs 20-dB and the 20- vs 30-dB decrements ($p < 0.01$), and a small but significant difference between the 30- vs 40-dB decrements ($p < 0.05$). A second surrogate measure was devised to analyze the growth rates, in which the values of inhibition for each subject became proportions of maximum inhibition in that particular condition (for example, the data for a subject with 30% inhibition at 2 ms and 60% at 15 ms would be converted to scores of 50% at 2 ms and 100% at 15 ms). These relative growth values were analyzed by a nonparametric test of proportions, with the values for each adjacent noise floor subtracted from each other for each lead time. This analysis showed that the relative growth of inhibition was greater for the 10-dB vs the 20-dB decrement at 1 ms ($p = 0.056$); for the 20-dB vs the 30-dB decrement at 2, 4, 6, and 10 ms; and for the 30-dB vs 40-dB decrement at 2 ms (all $p < 0.05$), indicating that the inhibitory effect of the smaller noise decrement was approaching its (smaller) asymptotic level at a faster relative rate than the adjacent greater decrement.

B. Startle response baseline

Figure 2 presents the mean ASR control values for each age and strain. The age-related patterns in ASR amplitudes

for the CBA and the F1 mice were similar, with an increase from 1 to 3 or to 6 months of age (maturation) followed by a steady decline in response amplitudes (senescence). In contrast, the C57 mouse responded most vigorously at 1 month of age, with substantial decrements to 3 and then to 6 months, and a constant but low level of responding apparent from 6 to 18 months of age. The overall analyses of the effects of age within each strain were all significant ($p < 0.01$). Within the C57BL strain, analyses of adjacent ages showed that the drop from 1 to 3 months was significant, $F(1/10) = 5.29$, $p < 0.05$, as was that from 3 to 6 months, $F(1/19) = 88.56$, $p < 0.01$, while ASR values at 6, 12, and 18 months were not different ($p > 0.20$). Within the CBA strain ASR increases from 1 to 3 months and from 3 to 6 months were both significant, $F(1/11) = 8.87$, $F(1/10) = 7.09$, $p < 0.05$, as were the subsequent declines from 6 to 12 months of age, from 12 to 18 months of age, and from 18 to 26 months of age: $F(1/14) = 9.52$, $p < 0.01$; $F(1/20) = 6.97$, $p < 0.05$; $F(1/19) = 29.31$, $p < 0.01$. Within the F1-hybrid strain the increases from 1 to 2 months of age and from 2 to 3 months of age were both significant, $F(1/15) = 11.51$, $F(1/20) = 16.50$, $p < 0.01$; as were the decrements in response from 12 to 22 months, $F(1/17) = 42.04$, $p < 0.01$, from 22 to 26 months, $F(1/17) = 6.96$, $p < 0.05$, and from 26 to 36 months, $F(1/22) = 234.39$, $p < 0.01$. Comparing across the three strains, for young mice averaging 6 weeks of age there was no difference in the ASR, $p > 0.05$, while at 3 months of age the C57BL strain responded less than the CBA and F1-hybrid strains, $F(2/30) = 13.41$, $p < 0.01$. ASR amplitudes for the CBA and the hybrid F1 strains were significantly different only at 26 months of age, $F(1/13) = 40.86$, $p < 0.01$. The 3-year-old F1 mice responded less vigorously (mean ASR=355) than the 2-year-old CBA mice (ASR=737) and the 18-month-old C57BL mice (ASR=730), $F(2/25) = 10.28$, $p < 0.01$, which were not different from each other, $p > 0.2$. The mean ASR of each of these groups save the 3-year-old F1 hybrids was significantly higher than would be expected on the basis of spontaneous activity in the absence of a startle stimulus, which in other experiments typically has had a median value of 200–250 V units.

C. Effects of age and hearing loss on the parameter values for relative inhibition

Eight τ values are missing because the obtained group means for those conditions could not be fitted successfully with the exponential formula, six of them because the level of inhibition was zero at 10 and 15 ms. For these six scores the AINH value was taken as 0.

1. Asymptotic levels of inhibition (AINH)

Figure 3 presents the calculated AINF values at each GD for each age and each strain, the C57BL at the top, the CBA in the center, and the F1-hybrid mouse at the bottom. The effect of hearing loss, which must be entirely responsible for the early changes seen in the C57BL data, but was confounded with senescence in the oldest group of this strain, was to substantially reduce AINH, especially for the higher GD. This change was apparent at 3 months, and while noise

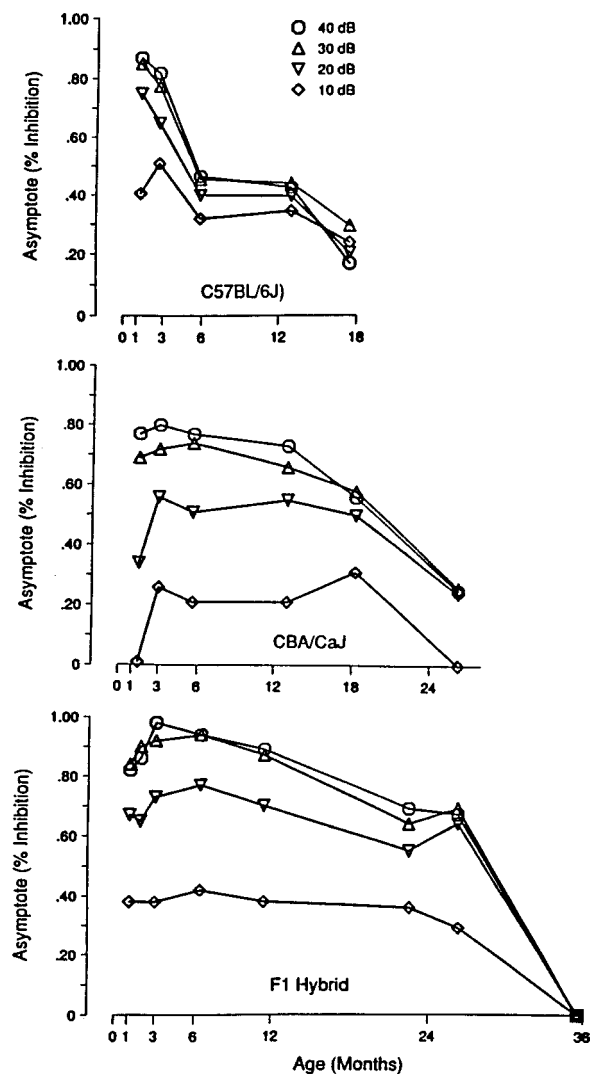


FIG. 3. Calculated values for asymptotic levels of inhibition, provided for each group on the basis of the data included in Fig. 1 (bottom), for each of the three strains and the four stimulus conditions, at each age.

decrements still provided significant inhibition at 18 months ($p < 0.01$), there was no differential effect of GD at this age. There were two effects of age in the CBA and F1-hybrid strains: a modest maturational effect of increasing inhibition that lasted up to 3 or 6 months of age, followed by a senescence effect beginning at 6 or 12 months of age, possibly confounded with an increasing degree of hearing loss beyond 18 months of age. The analysis of variance of all three strains through 12 months of age (in which the two earliest F1 groups were combined, their mean age approximating that of the CBA and C57BL strains, at 1.5 months) provided significant effects for strain, $F(2/98) = 24.07$, $p < 0.01$; for age, $F(3/98) = 7.96$, $p < 0.01$; and for their interaction, $F(6/98) = 9.72$, $p < 0.01$. All of these effects interacted with GD (beyond $p < 0.01$), which attests to the reliability of the data showing that the decremental effects of hearing loss and senescence were both more pronounced when there was a greater noise decrement. In the young mice below 3 months of age, the C57BL mouse and the F1-hybrid mice did not differ from each other, but both showed greater inhibition than the comparable CBA mouse. In contrast, at 6 and 12

months of age the C57BL mice showed less inhibition than the CBA, a difference to be understood as some consequence of hearing loss in the C57BL strain, while the difference favoring the F1-hybrid over the CBA strain remained. An analysis of the data at 1.5 vs 6 months in the hybrid and the CBA mice showed that the overall increase in inhibition between these ages was significant, $F(1/36)$, $p < 0.05$, supporting the reliability of the graphic indication that the processes responsible for the detection of the noise decrements or for their inhibitory effects matured with age. In contrast, the C57BL mouse showed a significant overall decline in inhibition over this same period, $F(1/19) = 29.66$, $p < 0.01$, which can be again attributed to its early onset hearing loss. In fact, in the C57BL mouse the decrement in inhibition was significant for the greater noise decrements in the comparison of the 1 vs 3 month data, $F(1/10) = 5.90$, $p < 0.05$. Across all three strains combined, there were no significant age differences in inhibition between the ages of 6 and 12 months. However, it is interesting that even with its evident substantial hearing loss at 6 and 12 months of age the inhibitory effect of the 10-dB decrement was greater in the C57BL than in the CBA, $F(1/37) = 4.11$, $p < 0.05$, and equal in strength to that of its F1-hybrid offspring.

The inhibitory effect of the noise decrements diminished beyond 12 months of age in each strain. For the C57BL there was a significant and general decrement in inhibition at 18 months compared to the apparent plateau of 6 and 12 months, $F(1/32) = 7.65$, $p < 0.01$, even in the absence of changes in the control ASR baseline. For the CBA mouse at 18 compared to 12 months there was a selective loss of inhibition for the higher noise decrements, this was apparent in an interaction between age and GD, $F(3/60) = 4.36$, $p < 0.05$; and at 26 compared to 18 months of age there was a general loss of inhibition across all of the stimulus conditions including the smallest decrements, $F(1/19) = 73.19$, $p < 0.01$. In the F1 mouse at 22 compared to 12 months of age there was a significant loss of inhibition with age, $F(1/17) = 8.00$, $p < 0.01$, and a marginal age \times GD interaction, $F(1/17) = 3.46$, $p = 0.08$, resulting because there was no change with age with the 10-dB decrement, $F < 1$, but a loss for the larger decrements, $F(1/17) = 8.84$, $p < 0.01$. The 22 month group did not differ from the 26-month-old mice, but from 26 to 36 months there was a total loss of inhibition. However, the interpretation of this observation is confounded by the particularly low level of the startle baseline obtained in the most aged mice.

2. The time constant for the development of inhibition (τ)

Figure 4 presents the τ values for each of the groups. The median τ value was 3.37 ms, the interquartile range 2.66–4.09 ms. The τ values showed the same modest effect of GD seen in the overall analysis, above, being in general greater for large compared to small decrements in noise (thus, 14 of the 18 groups had lower τ values for their lowest compared to their highest decrement, $p < 0.05$). No systematic effect of age or hearing loss on the growth rates for inhibition is evident in Fig. 4: thus for the C57BL, going across all stimulus conditions from one age to the next there

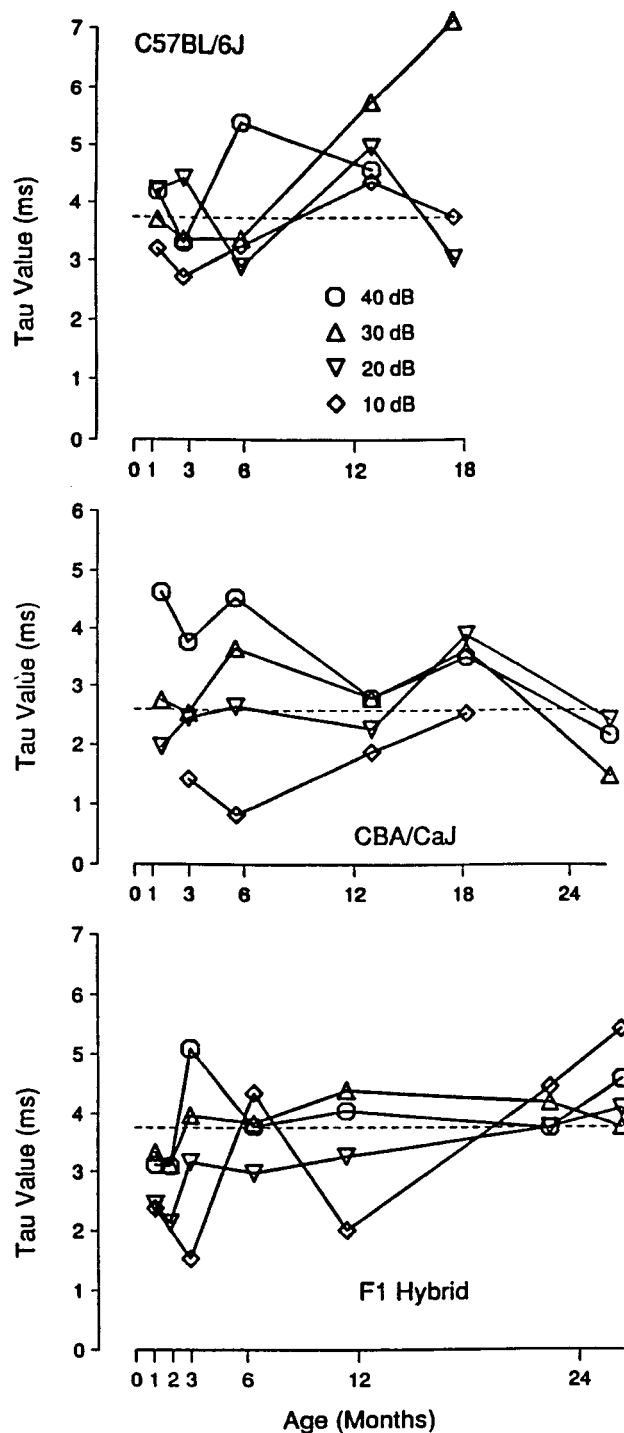


FIG. 4. Calculated values for the time constant τ , provided for each group on the basis of the data included in Fig. 1 (bottom), for each of the three strains and the four stimulus conditions, at each age. Eight data points are not included, six because the stimulus did not inhibit the response, and two because the obtained functions were too irregular.

were eight increments in τ and seven decrements; for the CBA, nine increments and nine decrements; and for the F1-hybrid, 12 increments and nine decrements; and across all three strains, looking just at changes in τ beyond 12 months of age, there was a total of ten increments and eight decrements. The null hypothesis that the time course for the development of inhibition does not change with age or with hearing impairment seems to be strongly suggested in these

data, especially in comparison to their large significant systematic consequences for asymptotic levels of inhibition.

III. DISCUSSION

A. Changes in ASR amplitudes with age and hearing impairment

The ASR increases in the first month of life in C57BL mice (Shnerson and Willott, 1980), in parallel with the development of auditory function seen in compound action potentials (Shnerson and Pujol, 1981). Parham and Willott (1988) reported that ASR levels progressively decline in the middle aged C57BL mice, 6 and 10 months of age, while older CBA mice show relatively small but progressive decrements at 12 and then 18 months. Our findings generally agree with this report, save that we found that CBA mice, like the F1 hybrid offspring, were most reactive in middle age, with smaller reactions at and beyond 12 months of age and below 2 to 3 months of age. A similar nonmonotone age effect is apparent in humans. Ornitz *et al.* (1986) showed an ASR increase in children at least up to 8 years of age, and Ford *et al.* (1995) showed a ASR decrement in aged compared to young adults (mean ages 69 vs 22 years). While the early ASR decrement in the C57BL must be a consequence, either primary or secondary, of its rapidly developing hearing loss, in general ASR age effects seem not best attributed to changes in auditory function, at least as auditory function is captured in detection thresholds. Profound changes in muscle mass and muscle composition are apparent during early development (Wirtz *et al.*, 1983) and in late senescence (Einseidel and Luff, 1992), and these must account for some of the age-related changes in the ASR. But Krauter *et al.* (1981) showed that the startlelike reflex elicited by a cutaneous stimulus was affected by age much less than was the ASR, suggesting that some change in the auditory system is important for the age effect on the ASR. Willott and Bross (1990) found age-related changes in the numbers, density, and dendritic arborization of octopus cells of the ventral cochlear nucleus, particularly in the first year of life in the C57BL and in the second year in the CBA. These periods coincide with the substantial loss in the vigor of the ASR even in mice that typically have no evident signs of hair cell pathology. Although it is not certain that octopus cells contribute to the startle reflex arc, they are large cells with a fast onset response across wide frequency bands, characteristics that would be favorable for the ASR. It may be that in early maturation and later senescence changes in large onset cells in the cochlear nucleus contribute to the age-related modification of the ASR that has been noted in both humans and in rodents.

B. The physiology of acoustic startle, and a model for its inhibition by noise offset

The ASR pathway begins with the haircells, then the auditory nerve and cells in the ventral cochlear nucleus (or possibly the cochlear root nucleus, Lee *et al.*, 1996); thence passing into the caudal pontine nucleus at the head of the reticulospinal tract (Wu *et al.*, 1988; Lingenhohl and Friauf, 1992); and terminating in the spinal motor neurons that in-

nervate the flexor and extensor muscles. Consistent with this depiction of perhaps no more than three central synapses, EMG measures of the ASR appear with a latency as rapid as 5 ms and a duration of only about 10 to 20 ms in rodents (Ison *et al.*, 1973; Wu, 1987). The physiological basis of ASR inhibition by noise offset is uncertain, but because of its temporal characteristics some reasonable hypotheses can be considered. First, because background noise may facilitate the startle reflex (in the rat: Davis, 1974; Hoffman and Searle, 1965; Ison and Hammond, 1971; and in the mouse, Ison *et al.*, 1996), it might be that depression at noise offset results simply because a stimulus condition that is favorable for ASR expression has been removed. But noise produced facilitation of the ASR depends on the integrity of forebrain mechanisms (Davis and Gendleman, 1977). It seems impossible that a signal for noise offset could reach the forebrain, turn off the arousal mechanism, and then have its effect register back in the brainstem, when the delay between noise offset and startle stimulus onset is just 1 ms, and when even the delay between offset and EMG activity is only 6 or 7 ms. Further problems with the "defacilitation" hypothesis arise first, because the optimal noise level depends on the level of the startle stimulus, and there is little if any facilitation apparent with a 115-dB startle stimulus in the presence of a 70-dB background (Ison *et al.*, 1996: the optimal noise level would be 50 dB); and second, because noise decrements depress the ASR considerably below the level obtained in the long-term absence of background noise (Ison, 1982). This latter observation means that noise offsets provide active inhibition of the ASR rather than passive "defacilitation."

A second possible explanation is that noise offset inhibition is a consequence of auditory adaptation resulting from the prior noise exposure. Thus classic reports (Kiang *et al.*, 1965; Young and Sachs, 1973a) show that if an auditory-nerve fiber is driven by a prolonged stimulus there is an initial fast rate of firing at stimulus onset and a subsequent decline to a steady state, followed, as in the present data, by a transient depression at noise offset below the spontaneous rate. Similar effects are apparent in psychophysical judgments (Young and Sachs, 1973b). Clearly, the ASR does not agree with the time course of these latter measures of either adaptation or of detection thresholds, both of which are most depressed immediately at stimulus offset and then recover in strength with an increasing delay between noise offset and the startle stimulus. The data contradict the hypothesis that sensory adaptation is critical for ASR inhibition.

A third possibility is that inhibition at noise offset results from a relatively complex form of forward masking. The typical forward masking effect, like the "neural adaptation" effects studied by Young and Sachs, is a simple continuation of simultaneous masking, and declines in strength with a delay between the masker and the test stimulus. But while the physiology of the auditory nerve may not allow for increasing inhibitory or masking effects over a delay period, masking studies in the cochlear nucleus do yield such functions (Kaltenbach *et al.*, 1993). For some cells in the dorsal cochlear nucleus forward masking is not immediately apparent at noise offset but increases in strength as a probe tone is separated from the masking tone, over a range of some 5–10

ms or even more. The relevant observation of Kaltenbach *et al.* is that forward masking occurs only to the extent that there is a distinguishable gap between the signal and the masker: we find this critically interesting, because it mirrors the effect seen in our behavioral data. Given this observation and the inadequacies of other hypotheses, we suggest that ASR inhibition at noise offset is a form of behavioral “gap detection” that depends on active inhibitory circuits present in the lower brainstem.

C. The inhibitory effect of noise offset

1. Asymptotic levels of inhibition

The asymptotic inhibitory effect of a noise decrement was (a) greater the larger the decrement; (b) progressively reduced by hearing loss in early middle aged C57BL mice; and (c) first increased with maturation and then declined in middle age in CBA and F1-hybrid mice with minimal hearing loss. Where direct comparisons are available, these results agree with those for psychophysical measures of temporal acuity in humans and with other behavioral measures of gap detection in animals: thus, human gap detection (a) is related to the depth of the gap (e.g., Forrest and Green, 1987); (b) is reduced with hearing impairment (e.g., Fitzgibbons and Wightman, 1982); and (c) is increased with maturation in childhood and then degraded with advancing age (e.g., Davis and McCroskey, 1980; Snell, 1997). These similarities support the assumption that inhibition of the ASR shares with these other measures of temporal acuity a common dependence on underlying sensory and integrative neural mechanisms.

One difference between the present data and those obtained in the more usual study of gap thresholds is that here a continuous behavioral function is obtained across the range of gap durations, including evident suprathreshold gap values. The most evident special outcome in the present data is that almost all of the effects are captured as differences in the mean level of reflex inhibition at asymptote, and thus can be attributed to differences in the inhibitory strength of “forward-masking-type” neural circuits presumed to be activated at the offset of the noise. How might activity in such hypothetical inhibitory circuits be modulated by these variables? One way is to change the level of their input, so that, as was seen, the greater the noise decrement then the greater the level of inhibition. A similar effect might be responsible for changes in inhibition in the middle aged C57BL mouse with sensorineural hearing loss, because as the higher frequency components of the noise background are progressively lost, they obviously could not contribute to the inhibitory effect at noise offset. In fact, providing a background masker of variable bandwidth to eliminate the contribution of particular frequencies to noise offset does reduce its inhibitory effect, and thus appears to simulate the effect of high-frequency hearing loss in the C57BL mouse (work in preparation). Other work has shown that reductions of 10 dB off noise levels ranging from 40 to 80 dB yields constant levels of ASR inhibition, and these data suggest that a hearing loss for particular components of the background noise would reduce the levels of ASR inhibition only if the loss

was substantial, i.e., beyond 40 dB. The hearing loss of the 6-month-old C57BL mouse might approach this value for frequencies above about 20 kHz, and the 18-month-old C57BL mouse would have a loss approaching this magnitude across the frequency spectrum (Li and Borg, 1993). That in these mice the strength of inhibition was no different for decrements of 10–40 dB suggests that noise levels of less than 60 dB (that is, floor levels that were more than 10 dB below the 70-dB carrier level) may have all been below their hearing threshold, and so could not differentially affect behavior. Perhaps the most surprising effects seen in these old C57BL mice is that they did provide both reliable ASR values on the control trials and then reliable inhibition with a small decrement in the background level.

It is unlikely that either the CBA or the F1-hybrid mice had a peripheral hearing loss so serious as to render significant components of the background noise inaudible, and for these mice it is more reasonable to suggest that central changes in auditory function were responsible for the steady losses in asymptotic inhibition that are apparent in their data beyond 6 or 12 months of age. For most groups the age-related loss of inhibition was apparent primarily for the greater noise decrements, suggesting that the fundamental problem was that of not being able to generate a substantial degree of inhibition (only two groups, the youngest and the oldest of the CBA mice, were particularly insensitive to small decrements while being responsive to larger decrements). It is then interesting that Raza *et al.* (1994) and Milbrandt and Caspary (1995) report age-related declines in the numbers of glycine receptors and in the production of GABA in the brainstem of the rat. Either of these effects, if present in aged mice, could account for the obtained age-related changes in sensitivity and asymptotic levels of behavioral inhibition.

2. The time constant for inhibition

The relative growth of inhibition was most rapid with the smallest decrement of 10 dB, and overall was negatively related to the asymptotic level of inhibition provided by each offset condition (though it was not at all related to age-related differences in asymptotic inhibition). It is possible that this change in the time constant for the development of inhibition resulted either in the “afferent” task of detecting the change in stimulus input or in the “efferent” task of generating its inhibitory consequence. This could happen on the afferent side because the decay in neural excitation per unit time immediately after the noise decrement is to some extent independent of the size of the decrement, or on the efferent side because the generation of inhibition proceeds with the same inhibitory output per unit time more or less independently of the asymptotic level of inhibition generated in that particular stimulus condition. Thus the afferent processing necessary for a small decrement in neural excitation would be completed more swiftly as would the task of generating a small amount of neural inhibition: and in either case, the time course of development for the inhibitory effect of a small decrement in noise level must necessarily have a smaller time constant. But in the present experiment the most interesting outcome is that the rate of development of inhi-

bition was not systematically different across groups varying in their age or in their degree of hearing loss. The primary effect of each of these variables was to change the asymptotic level of inhibition, but not to change its temporal rate of development. This outcome suggests that increasing age or increasing hearing loss must reduce the amount of inhibition that can be generated per unit time just as it reduces the asymptotic level of potential inhibition: that is, there may be no age-related change in the relative growth rate of inhibition, but there must be an age-related change in the absolute growth rate of inhibition. This suggestion is entirely consistent with the hypothesis that the underlying neural effect underlying the developmental and age-related effects seen in the present experiment is a change in the amount of neurotransmitter release at noise offset, and/or a decrease in the number of receptors sensitive to that release, because both of these effects would change the absolute growth of inhibition per unit time in tandem with the asymptotic level of inhibition.

D. Concluding comments

The present experiment provided parallels to outcomes apparent in studies of gap detection in human listeners in showing that compared to young adult mice, very young and very old mice and hearing impaired middle aged mice are all deficient in responding to gaps in noise. These deficits in responsiveness to brief gaps in noise are particularly interesting in that they are attributable entirely to changes in asymptotic levels of inhibition, and not in the relative rate of development of inhibition. In light of the model for gap detection presented by Plack and Moore (1990), it could be argued, therefore, that the deficits in temporal acuity are not due to the inability of these mice to follow the temporal envelope of the noise decrement, but to a loss in the efficiency of more central parts of the auditory system that are responsible for responding to rapid shifts in afferent input and then moderating the level of the startle reflex. It is of great interest that Hall and Grose (1994) found in their study of children that age effects on temporal acuity were also restricted entirely to differences in sensitivity at asymptote for high levels of modulation depth, with no effects apparent in the time constants of the modulation contrast functions. The similarity of the findings, even to the point that the time constants presented by Hall and Grose approximate those found in the present work, suggests that this conclusion has a considerable degree of generality.

ACKNOWLEDGMENTS

This research was supported by USPHS Research Grant No. AG09524, a Center Support Grant to the Center for Visual Science, EY01319, and by the Rochester International Center for Hearing and Speech Research.

Buus, S., and Florentine, M. (1985). "Gap detection in normal and impaired listeners: The effect of level and frequency," *Time Resolution in Auditory Systems*, edited by A. Michelsen (Springer-Verlag, Berlin), pp. 159–179.
 Davis, M. (1974). "Signal-to-noise ratio as a predictor of startle amplitude and habituation in the rat," *J. Comp. Physiol. Psychol.* **86**, 812–825.

Davis, M., and Gendleman, P. M. (1977). "Plasticity of the acoustic startle reflex in acutely decerebrate rats," *J. Comp. Physiol. Psychol.* **91**, 549–563.
 Davis, S. M., and McCroskey, R. L. (1980). "Auditory fusion in children," *Child Dev.* **51**, 75–80.
 Dean, K. F., Sheets, L. P., Crofton, K. M., and Reiter, L. W. (1990). "The effect of age and experience on inhibition of the acoustic startle response by gaps in background noise," *Psychobiology* **18**, 89–95.
 Einsiedel, L. J., and Luff, A. R. (1992). "Alterations in the contractile properties of motor units within the ageing rat medial gastrocnemius," *J. Neurol. Sci.* **112**, 170–177.
 Fitzgibbons, P. J., and Wightman, F. L. (1982). "Gap detection in normal and hearing-impaired listeners," *J. Acoust. Soc. Am.* **72**, 761–765.
 Ford, J. M., Roth, W. T., Isaacks, B. G., White, P. M., Hood, S. H., and Pfefferbaum, A. (1995). "Elderly men and women are less responsive to startling noises: N1, P3 and blink evidence," *Biol. Psychol.* **39**, 57–80.
 Forrest, T. G., and Green, D. M. (1987). "Detection of partially filled gaps in noise and the temporal modulation transfer function," *J. Acoust. Soc. Am.* **82**, 1933–1943.
 Giraudi-Perry, D. M., Salvi, R. J., and Henderson, D. (1982). "Gap detection in hearing-impaired chinchillas," *J. Acoust. Soc. Am.* **72**, 1387–1393.
 Hall, J. W., and Grose, J. H. (1994). "Development of temporal resolution in children as measured by the temporal modulation transfer function," *J. Acoust. Soc. Am.* **96**, 150–154.
 Henry, K. R., and Chole, R. A. (1980). "Genotypic differences in behavioral, physiological, and anatomical expressions of age-related hearing loss in the laboratory mouse," *Audiology* **19**, 369–383.
 Hoffman, H. S., and Searle, J. L. (1965). "Acoustic variables in the modification of the startle reaction in the rat," *J. Comp. Physiol. Psychol.* **60**, 53–58.
 Irwin, R. J., Ball, A., Kay, N., Stillman, J., and Rosser, J. (1985). "The development of auditory temporal acuity in children," *Child Dev.* **56**, 614–620.
 Ison, J. R. (1982). "Temporal acuity in auditory function in the rat: Reflex inhibition by brief gaps in noise," *J. Comp. Physiol. Psychol.* **96**, 945–954.
 Ison, J. R., Bowen, G. P., Barlow, J. A., Taylor, M., and Walton, J. P. (1993). "Diminished response to acoustic transients in aged rodents," *J. Acoust. Soc. Am.* **93**, 2409.
 Ison, J. R., Gutierrez, E., Agawal, P., Pak, J., and Vaughn, W. (1996). "Reflex inhibition by partially filled gaps in developing and aged mice: Ontogenetic changes in processing efficiency not temporal ability of audibility," *Soc. Neurosci. Abstr.* **22**, 1822.
 Ison, J. R., and Hammond, G. R. (1971). "Modification of the startle reflex in the rat by changes in the auditory and visual environments," *J. Comp. Physiol. Psychol.* **75**, 435–452.
 Ison, J. R., McAdam, D. W., and Hammond, G. R. (1973). "Latency and amplitude changes in the acoustic startle reflex of the rat produced by variations in auditory prestimulation," *Physiol. Beh.* **10**, 1035–1039.
 Kaltenbach, J. A., Meleca, R. J., Falzarano, P. R., Myers, S. F., and Simpson, T. H. (1993). "Forward masking properties of neurons in the dorsal cochlear nucleus: possible role in the process of echo suppression," *Hearing Res.* **67**, 35–44.
 Kellogg, C., Ison, J. R., and Miller, R. K. (1983). "Prenatal diazepam exposure: Effects on auditory temporal resolution in rats," *Psychopharmacol.* **79**, 332–337.
 Kiang, N. Y. S., Watanabe, T., Thomas, A. C., and Clark, L. F. (1965). *Discharge Patterns of Single Fibers in the Cat's Auditory Nerve* (MIT, Cambridge, MA).
 Krauter, E. E., Wallace, J. E., and Campbell, B. A. (1981). "Sensory-motor function in the aging rat," *Beh. & Neural Biol.* **31**, 367–392.
 Lee, Y., Lopez, D. E., Meloni, E. G., and Davis, M. (1996). "A primary acoustic startle pathway: Obligatory role of cochlear root neurons and the nucleus reticularis pontis caudalis," *J. Neurosci.* **16**, 3775–3789.
 Li, H.-S., and Borg, E. (1993). "Auditory degeneration after acoustic trauma in two genotypes of mice," *Hearing Res.* **68**, 19–27.
 Lingenhohl, K., and Friauf, E. (1992). "Giant neurons in the caudal pontine reticular formation receive short latency acoustic input: an intracellular recording and HRP-study in the rat," *J. Comp. Neurol.* **325**, 473–492.
 Lutman, M. E. (1990). "Degradations in frequency and temporal resolution with age and their impact on speech identification," *Acta Oto-Laryngol. Suppl.* **476**, 120–125.
 McCroskey, R. L., and Davis, S. M. (1976). "Auditory function: develop-

- mental trends," Convention of American Speech and Hearing Association, Houston, Texas (unpublished).
- McCroskey, R. L., and Kasten, R. N. (1982). "Temporal factors and the aging auditory system," *Ear Hear.* **3**, 124–127.
- Mikaelian, D. O. (1979). "Development and degeneration of hearing in the C57/b16 mouse: relation of electrophysiologic responses from the round window and cochlear nucleus to cochlear anatomy and behavioral responses," *Laryngoscope* **89**, 1–15.
- Milbrandt, J. C., and Caspary, D. M. (1995). "Age-related reduction of [3H]strychnine binding sites in the cochlear nucleus of the Fischer 344 rat," *Neuroscience* **67**, 713–719.
- Moore, B. C., Peters, R. W., and Glasberg, B. R. (1992). "Detection of temporal gaps in sinusoids by elderly subjects with and without hearing loss," *J. Acoust. Soc. Am.* **92**, 1923–1932.
- Ornitz, E. M., Guthrie, D., Kaplan, A. R., Lane, S. J., and Norman, R. J. (1986). "Maturation of startle modulation," *Psychophysiology* **23**, 624–634.
- Parham, K., and Willott, J. F. (1988). "Acoustic startle response in young and aging C57BL/6J and CBA/J mice," *Behav. Neurosci.* **102**, 881–886.
- Patterson, R. D., Nimmo-Smith, I., Weber, D. L., and Milroy, R. (1982). "The deterioration of hearing with age: frequency selectivity, the critical ratio, the audiogram, and speech threshold," *J. Acoust. Soc. Am.* **72**, 1788–1803.
- Plack, C. J., and Moore, B. C. J. (1990). "Temporal window shape as a function of frequency and level," *J. Acoust. Soc. Am.* **87**, 2178–2187.
- Plomp, R. (1964). "Rate of decay of auditory sensation," *J. Acoust. Soc. Am.* **36**, 277–282.
- Raza, A., Milbrandt, J. C., Arneric, S. P., and Caspary, D. M. (1994). "Age-related changes in brainstem auditory neurotransmitters: measures of GABA and acetylcholine function," *Hearing Res.* **77**, 221–230.
- Salvi, R. J., and Arehole, S. (1985). "Gap detection in chinchillas with temporary high-frequency hearing loss," *J. Acoust. Soc. Am.* **77**, 1173–1177.
- Schneider, B. A., Pichora-Fuller, M. K., Kowalchuk, D., and Lamb, M. (1994). "Gap detection and the precedence effect in young and old adults," *J. Acoust. Soc. Am.* **95**, 980–991.
- Shnerson, A., and Pujol, R. (1981). "Age-related changes in the C57BL/6J mouse cochlea. I. Physiological findings," *Brain Res.* **254**, 65–75.
- Shnerson, A., and Willott, J. F. (1980). "Ontogeny of the acoustic startle response in C57BL/6J mouse pups," *J. Comp. Physiol. Psychol.* **94**, 36–40.
- Snell, K. B. (1997). "Age-related changes in temporal gap detection," *J. Acoust. Soc. Am.* **101**, 2214–2220.
- Stitt, C. L., Hoffman, H. S., and Marsh, R. (1973). "Modification of the rat's startle reaction by termination of antecedent acoustic signals," *J. Comp. Physiol. Psychol.* **84**, 207–215.
- Tyler, R. S., Summerfield, Q., Wood, E., and Fernandes, M. (1982). "Psychoacoustic and phonetic temporal processing in normal and hearing impaired listeners," *J. Acoust. Soc. Am.* **72**, 740–752.
- Walton, J. P., Frisina, R. D., Ison, J. R., and O'Neill, W. E. (1997). "Neural correlates of behavioral gap detection in the inferior colliculus of the young CBA mouse," *J. Comp. Physiol. A* **181**, 161–176.
- Willott, J. F. (1996). "Anatomic and physiologic aging: a behavioral neuroscience perspective," *J. Am. Acad. Audiol.* **7**, 141–151.
- Willott, J. F., and Bross, L. S. (1990). "Morphology of the octopus cell area of the cochlear nucleus in young and aging C57BL/6J and CBA/J mice," *J. Comp. Neurol.* **300**, 61–81.
- Wirtz, P., Loermans, H. M., Peer, P. G., and Reintjes, A. G. (1983). "Post-natal growth and differentiation of muscle fibres in the mouse. II. A histochemical and morphometrical investigation of dystrophic muscle," *J. Anat.* **137**, 127–142.
- Wu, M.-F. (1987). "Effect of prestimuli, background noise, and noise-offset on the acoustic pinna reflex: the relationships between emg and multiple-unit responses in the inferior colliculus of spinally transected rats," Doctoral dissertation, Department of Psychology, University of Rochester, pp. 1–159.
- Wu, M. F., Suzuki, S. S., and Siegel, J. M. (1988). "Anatomical distribution and response patterns of reticular neurons active in relation to acoustic startle," *Brain Res.* **457**, 399–406.
- Young, E., and Sachs, M. B. (1973a). "Recovery from sound exposure in auditory-nerve fibers," *J. Acoust. Soc. Am.* **54**, 1535–1543.
- Young, E., and Sachs, M. B. (1973b). "Recovery of detection probability following sound exposure: Comparison of physiology and psychophysics," *J. Acoust. Soc. Am.* **54**, 1544–1553.
- Young, J., and Fechter, L. D. (1983). "Reflex inhibition procedures for animal audiometry: A technique for assessing ototoxicity," *J. Acoust. Soc. Am.* **73**, 1686–1693.