

Handbook of **Mouse Auditory Research**

From Behavior
to Molecular Biology

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5 The Acoustic Startle Response: Reflex Elicitation and Reflex Modification by Preliminary Stimuli

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INTRODUCTION

The study of the behavioral effects of apparently irrelevant sensory and motoric events on reflex expression in the alert and behaving animal began toward the end of the nineteenth century, when it was becoming understood that reflex activities are not driven by stimuli acting through fixed and isolated sensory-neural pathways, but are very sensitive to subtle events of psychological significance and, thus, by complex neural activities in the brain. These early experiments on reflex modification (described in Ison and Hoffman, 1983) studied the basic phenomena of reflex modification and also its application, and demonstrated the strength and ubiquity of prestimulus effects in species as disparate as frogs (Yerkes, 1905) and humans (Lombard, 1887; Bowditch and Warren, 1890). These workers showed that both facilitation of reflex expression and its inhibition can be produced by near-threshold stimuli and by the initiation of voluntary movements, the outcome depending on small differences in the timing between their onset and subsequent reflex elicitation. Some variables that affect the strength of these reflex phenomena are important in other research domains, notably sensation and perception (L.H. Cohen et al., 1933), emotion (J.S. Brown et al., 1951), and aspects of psychopathology in humans (Braff et al., 1978). The comparability of reflex findings in rats and humans has supported the development of animal models in these areas that prove useful in understanding both normal function and disease, and this has extended the scope and the importance of research on reflex behavior. Recent advances in molecular biology have made inbred, transgenic, and knock-out mice the most powerful animal models for analyzing the functional effects of gene expression. The purpose of this chapter is to review the research on reflex elicitation and modification in mice, in order to extend these models for the more fruitful neurobehavioral analysis of genetic manipulation.

Much of the recent development in this field began with the large and systematic body of empirical work in rats and in humans published over the years by Howard Hoffman and his colleagues and students, beginning with their rediscovery of prepulse inhibition and prepulse facilitation in the acoustic startle reflex of the rat (Hoffman and Fleshler, 1963). These researchers provided an extensive series of integrated parametric experiments on the various phenomena of reflex inhibition and reflex facilitation in rats, pigeons, frogs, and humans. Among them, for example, Cohen et al. (1983) demonstrated the inhibitory effects of knowledge of stimulus presentation on the startle reflex in humans; DeIpezzo and Hoffman (1980) described the increased inhibitory effect of prestimuli to which humans attend; Hoffman and Searle (1965) analyzed the time course of prepulse inhibition of acoustic startle in the rat; Leitner et al. (1981) discovered a role for the reticular formation in prepulse inhibition; Marsh et al. (1973) showed the effects of

binaural interactions on reflex inhibition in humans; and Stitt et al. (1976) described startle inhibition of a light-induced reflex in pigeons by noise prepulses. Other work included the application of reflex augmentation in newborn children to the study of audition and sensorimotor control in the neonatal clinic (e.g., Anday et al., 1991).

Hoffman studied reflex elicitation and reflex modification variously in human subjects, rats, pigeons, and frogs, but never in mice. Given that the basic principles of reflex modification have been demonstrated in these several and diverse species, it seems very likely that research on the mouse should now yield a similar set of findings. But rather than assume their similarity, it may be instructive to catalogue different reflex phenomena in mice in order to describe their normative reflex behavior and its similarities and differences with other species, as well as begin to compare that behavior across inbred strains. This investigation of the conditions important for reflex elicitation and modification in mice should help us to better use these animal models, and in addition, further study of the basic phenomena in these mice may help solve the intrinsic problem of how neural processes of excitation and inhibition are organized in sensorimotor control.

THE STARTLE REFLEX

The study of reflex modification depends first on the reliable presence of a behavioral response, which can then be modified. Thus, it is not surprising that a program of research intended to study reflex modification should begin with an investigation of the important conditions for reflex elicitation. One of the first projects in Hoffman's laboratory was taken on by Morton Fleshler (1965) in a doctoral dissertation intended to determine "the adequate acoustic stimulus for [the] startle reaction in the rat." In a series of three experiments, Fleshler determined the threshold of the response with variation in the age of the rat (40, 130, and 270 days); in the rise time of his startle tones (2.5, 10, 25, and 50 ms); in their spectral frequency (720, 6380, and 13250 Hz); and in their duration (6, 12, 24, and 48 ms). His overall empirical findings were that threshold sensitivity first slightly increased, then more substantially declined with age; that reflex expression was high and about equal at rise times of 2.5 and 10 ms, and then low and about equal at rise times of 25 and 50 ms; that reflex sensitivity increased markedly with the frequency increase from 720 to 6380 Hz, and then modestly with the further increase to 13250 Hz; and that response thresholds did not vary with duration. Overall, the lowest threshold recorded for the rat was about 95 dB SPL, obtained with a 13,250-Hz stimulus presented at a rise time of 2.5 ms.

There is no single study that examines all of these variables in mice, but several experiments jointly suggest the importance of the same variables. In the first experiment concerned with the parametric control of startle thresholds in the mouse, Shneron and Willott (1980) studied startle responding in three age groups of preweanling inbred C57BL/6J mice, 12 to 13 days old, 13 to 14 days, and 15 to 17 days, for a total of 150 trials in which the startle tones varied in their spectral frequency (5, 7, 10, 15, and 20 kHz) and their level (80, 90, and 100 dB SPL). Startle incidence increased with age and with stimulus level, and non-monotonically with stimulus frequency, being more likely for mid-frequencies. It is interesting that the threshold for startle defined as 50% incidence was less than 80 dB for the mid-frequency stimuli. This is much less than the 95 dB reported by Fleshler, but his transducer may have filtered out slow reactions that would count as startle responses in the mouse study (see below). One very informative subtlety in the developmental data presented by Shneron and Willott was that the age-related increase in response incidence was seen at its earliest at low frequencies and lagged behind for 15-kHz and especially 20-kHz stimuli. This reveals the presence of a maturational delay in sensitivity for the higher compared to the lower frequencies, which, as the authors note, had been previously seen in other sorts of experiments by Hack (1968) and by Ehret (1976a). That age-related differences in a "startle response audiogram" confirmed these earlier observations is testimony to the ease with which objective differences in startle reflex elicitation can be used to assess certain aspects of sensory function in mice.

The C57BL/6J mouse is a frequent participant in auditory research because it has a slowly developing, progressive age-related hearing loss that appears in the animal around 2 months of age and then continues for perhaps another 12 months or so. The initial work demonstrating this sensory loss in behavioral experiments and then beginning to trace out its electrophysiological and anatomical correlates was reported by Mikaelian (1979). In interesting contrast to the C57BL/6J, the DBA/2J mouse shows a remarkably rapid degeneration in the ear that becomes evident as early as 2 to 3 weeks of age. To contrast changes in sensorimotor function in C57BL/6J and DBA/2J mice, Willott, Kulig, and Satterfield (1984) provided a near-replication of the experiment of Shnerson and Willott (1980) but tested the mice at the slightly older ages of 22 and 29 days (which is an important window on the auditory system of the DBA/2J but not the C57BL/6J mouse). Response amplitude rather than incidence was the dependent measure in this study. Both strains showed the expected increasing response amplitude with an increase in stimulus level over a range of 80 to 100 dB and, in the young mice, peak responding occurred at 12 kHz. The response then fell off from 12 kHz to 8 kHz and 4 kHz on one side, and 16 kHz and 24 kHz on the other. An interesting and not unexpected age by strain interaction resulted as response amplitudes tended to increase with age in C57BL/6J mice but showed a pronounced decrease in DBA/2J mice, consistent with their more rapid peripheral degeneration.

A later study reported by Parham and Willott (1988) focused on the development of the less rapid age-related degeneration in the C57BL/6J mouse. They contrasted the effect of age on startle response amplitudes in the C57BL/6J with those of the CBA/J mouse, which maintains normal hearing until well into its second year of life. To demonstrate hearing loss in the C57BL/6J mouse, they extended the age range beyond those used earlier and compared groups tested at 1, 6, and 10 months with CBA/J mice tested at 1, 12, and 24 months. Again, the response in the young mice was most sensitive to the mid-frequency of 12 kHz, with thresholds as low as 70 to 75 dB SPL. These response thresholds increased with age in both mouse strains, thus echoing the age-related loss of reflex sensitivity found in the rat by Fleshler; but, as expected, the decline in startle expression was most pronounced for the high-frequency stimuli in the C57BL/6J strain. These findings are exactly those anticipated on the basis of their deteriorating sensory thresholds at 6 months and especially at 10 months of age. However, more recent data (Ison et al., 2000; and below) reveal that under some conditions sensory loss can yield a transient increase in acoustic reflex sensitivity in the middle-aged C57BL/6J mouse. This finding, combined with data from other research paradigms, provides an interesting description of a shifting balance of excitatory and inhibitory processes that may accompany the withdrawal of afferent sensory input from central auditory structures.

Even in the Parham and Willott experiment, as the authors note, while the progressive change in the response to high-frequency stimulation in C57BL/6J mice is entirely compatible with their hearing loss, it is curious that there were similar if milder changes for low-frequency stimuli as well as high frequencies in the middle-aged mice, low frequencies where the ABR shows no threshold change. There are perhaps two quite different reasons for this broad behavioral effect of an apparent narrow-band hearing loss, which are not mutually exclusive. One is based on the fact that a startle tone presented at the high intensities and brief rise times characteristic of startle experiments produces a transient click at tone onset. The low spectral frequencies present within the main body of the startle tone are thus surrounded by the broad-band frequencies of the click at tone onset, and these surrounding features could contribute to startle expression even if they do not provide much of the energy in the long-duration stimulus. Any high-frequency transients occurring at onset would be less audible for mice with a high-frequency hearing loss, and thus reflex expression could be reduced in these mice even for startle stimuli with mostly a low-frequency content. While this is a plausible and testable hypothesis, an alternative is much more interesting. It may be that central changes in suprathreshold levels of excitation and inhibition develop in the hearing impaired mouse in partial compensation for peripheral sensory loss, and thus the startle response may be revealing a central effect of peripheral hearing loss that is not apparent at the

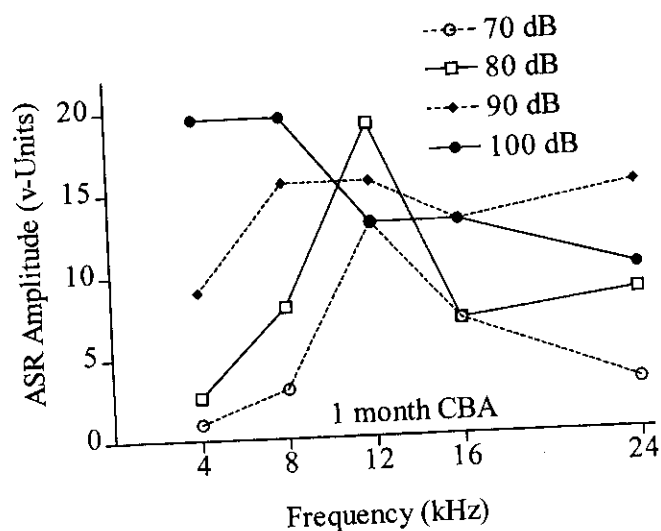


FIGURE 5.1 Mean amplitudes of the acoustic startle reflex in a group of young CBA mice as a function of the spectral frequency of tone pips and their level. (Adapted from K. Parham and J. F. Willott, 1988, *Behav. Neurosci.*, 102, 881-886. With permission.) Copyright 1988 by the American Psychological Association.

sensory threshold. This hypothesis is consistent with the observed changes in the low-frequency receptive fields in many central auditory structures following peripheral loss, beginning with Willott et al. (1982), and is interesting in its showing again that changes in reflex elicitation can generate interesting hypotheses for subsequent research.

Fleshler had concluded that the differences obtained in startle thresholds across frequency are most easily accounted for by the "sensitivity of the ear" rather than by "some special startle stimulus-sensing mechanism which has a unique sensitivity function." The data obtained by Willott and colleagues reveal a similar process working in the mouse, or at least working to affect startle expression in the young mouse with normal hearing. Their data in fact do raise questions about the extent to which sensory thresholds as defined by the ABR audiogram are predictive of startle thresholds and suprathreshold behavioral measures in middle-aged C57BL/6J mice provided in a later discussion. However, there are some other indications in the data provided by Parham and Willott that reflex expression for stimuli at levels over the threshold for reflex elicitation may follow quite a different path from those seen in sensory thresholds even in young mice with normal hearing, and these effects are quite important in understanding the ways in which the nervous system deals with high levels of sensory input. The basic observation is that in some conditions, startle in the mouse first increases with an increase in stimulus level above its threshold, but a further increase results in a terminal loss of response strength. This effect is seen in Figure 5.1. These authors did not take any special notice of this aspect of their data, perhaps because non-monotonic rate-level functions are common in their studies of neural activity in the central auditory system; but, in contrast, they are at least unusual and, prior to this report, perhaps even unknown in the startle literature.

For example, the rat's startle reaction has been shown to be monotonically related to startle level up to 140 dB (Hoffman and Searle, 1968; and see below); but for Parham and Willott, the youngest group of CBA/J mice responded most vigorously for an 80-dB stimulus at their optimal frequency of 12 kHz, but then in an abrupt reversal the response dropped at 90 dB and again at 100 dB. Their data for the youngest C57BL/6J mice was not quite as unusual as this, but were strange enough — when presented at the best frequency of 12 kHz, startle-tone pips ranging in level from 70 dB to 100 dB were all equally effective in eliciting the response. Most certainly, this

effect would never be seen in either rats or in human subjects. In fact, in these mouse studies, the prototypical monotonic increasing functions were evident only in two conditions: in the responses of older mice overall and in those of younger mice specifically for low-frequency tone pips away from the most sensitive hearing region.

It might be that not too much should be made of an isolated finding in one experiment, but to the reader conditioned to expect a monotonic increasing function between stimulus level and response amplitude, this observation was very surprising. For this reason, we ran a small experiment on this behavioral "rate/level" function to see for ourselves what might happen in a direct comparison of rats vs. mice. A total of just 9 animals were run, 5 naïve Long Evans hooded rats (3 months old), and 4 naïve C57BL/6J mice (10 weeks old), all female. The apparatus and the procedures were the same for the two species, save that the test cage for the mouse was much smaller than that of the rat, and weighed about 100 compared to about 600 g. The eliciting stimuli were different from those of Parham and Willott, but stimuli with which we were more familiar, namely, white noise bursts, 25-ms duration with 5-ms rise and decay times. They were presented at seven levels (76, 85, 94, 103, 112, 121, and 130 dB SPL), randomized in order within each of 11 blocks of trials that included a no-stimulus trial. The details of the standard procedures used are given, for example, in Ison et al. (1998a). The experiment was run under computer control, with trials scheduled every 20 s on average. The test cage is in a sound-attenuating room, and is placed on a flexible shelf over an accelerometer that records the force of a flinch response to the sound burst. The response is integrated for 100 ms after stimulus onset, and the means of the integrated responses calculated for each subject for each condition.

The results of this experiment are shown for each of the nine subjects in Figure 5.2, with the individual means for the rats above, the mice below. The two groups were similar in showing some slight increase in activity to 76- and 85-dB noise bursts, but then a sharp increase in responding at 94 dB (except for one mouse that had a shallow response function). Examining the tracings of the small responses obtained at 76 and 85 dB revealed that their fundamental frequency was lower than that provided by the larger responses to higher levels of stimulation. A careful reading of the description of the response sensor built by Hoffman and Fleshler (1965, especially p. 308) suggests that it would be insensitive to slow-wave input from non-impulsive responses to low-level stimuli. This may provide an explanation of the threshold differences described above in rats and mice, because consistent with this interpretation, it is observed that both species respond with small responses at 76 dB, about the level reported for mice, and with large responses at 94 dB, about the level reported by Fleshler in rats.

The similarity of the response amplitudes within the group of rats is striking, especially in contrast to the much greater variation between the four mice, which may be an indication that their age-related hearing loss had already had some effect. These mice were older than those in the earlier reports, and we have found that the age of onset of hearing loss can be quite variable even in this inbred strain. It is also interesting that two of the mice were jumping as vigorously as the rats for the lower stimulus levels, and it should be noted that the gain on the recording amplifier was the same for mice and rats. The rats outweighed the mice by about 10 to 1, and thus must have exerted a substantially greater force on the accelerometer. However, both the mouse and the mouse cage weigh much less than the rat and the rat cage, and therefore the point is really that the startle reflexes in the small mouse and the large rat are about equally able to quickly dislodge the animal from its normal posture, and thus provide our measuring instrument with about the same impulsive accelerative response. All of these effects are of some interest, but in context, the most important observation in this experiment is that while the rats showed a steady increase in response amplitude over this range of stimulus levels (with but two small reversals across all of them combined), for every mouse the reflex amplitudes first increased with the initial increase in stimulus level and then exhibited a terminal decline. This non-monotone effect of startle stimulus level on reflex expression is one consistent difference between the startle reactions of mouse and rat, and the terminal flattening or reversal of the reflex in mice appears again in this report.

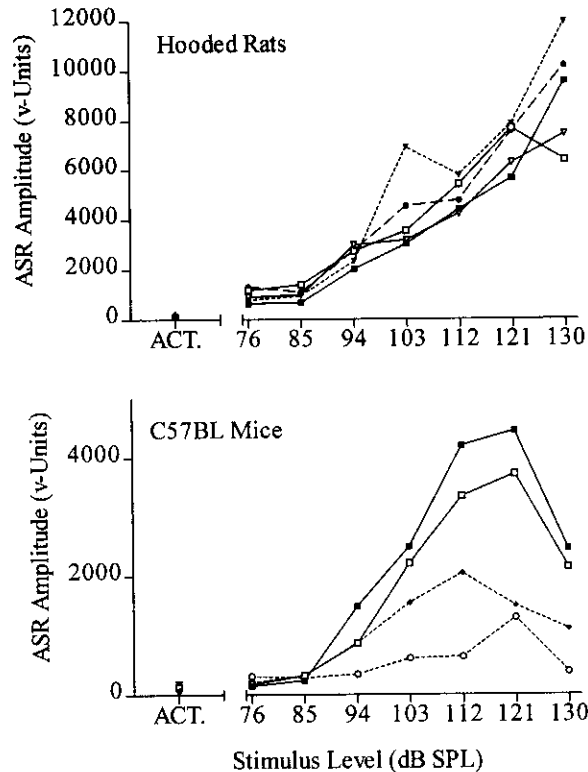


FIGURE 5.2 A comparison of startle amplitudes in rats ($n = 5$; above) and mice ($n = 4$; below) elicited by noise bursts of different levels (76 to 130 dB SPL).

The rise time of the eliciting stimulus appears to be a variable that is likely to be important for startle elicitation in the mouse, especially given that the “suddenness” of the stimulus is traditionally thought of as being with “unexpectedness” one of its two defining attributes (Landis and Hunt, 1939). Fleshler reported for the rat that while the threshold expression of the startle reaction was related to the rise time of the eliciting 4350-Hz tone burst, the actual function was essentially two-valued, with the thresholds for the two lower values of 2.5 and 10 ms being about indistinguishable, and much different from the two higher values of 25 and 50 ms. Fleshler concluded that the critical variable was not rise time as such; instead, “the stimulus must reach some critical intensity within the first 12 or so ms, but the manner in which it achieves this criterion probably has no substantial effect” (1965, p. 204). No similar parametric experiment has been published for mice, although Willott et al. (1979) showed in a single comparison that having a brief rise time is important for startle in the young C57BL/6J mouse. They reported that if an 80-dB, 10-kHz tone had a 5-ms rise time, then the mice responded over 90% of the trials and the responses had an overall mean of about 16 mV; but if the rise time was 20 ms, then the mice responded 52% of the time with a mean response of 2 mV. To determine whether a more detailed rise time function for the mouse would show further similarities with the rat data of Fleshler, we ran an experiment with 8-week-old male CBA/CaJ mice ($n = 8$), using 110-dB SPL noise bursts as the eliciting stimuli and jointly varying their (linear) rise and decay times (RDT) and total stimulus duration (SD) so as to roughly match the energy in each of six types of noise burst: 0 ms RDT and 30 ms SD; 2 ms RDT and 32 ms SD; 5 ms RDT and 35 ms SD; 10 ms RDT and 40 ms SD; 20 ms RDT and 50 ms SD; and 30 ms RDT and 60 ms SD. A total of 60 trials was given in 10 blocks. The data are presented in Figure 5.3, the group means (of individual medians) in the top graph and each trial in each condition for one mouse in the lower graph. The ASR data agree largely with Fleshler in

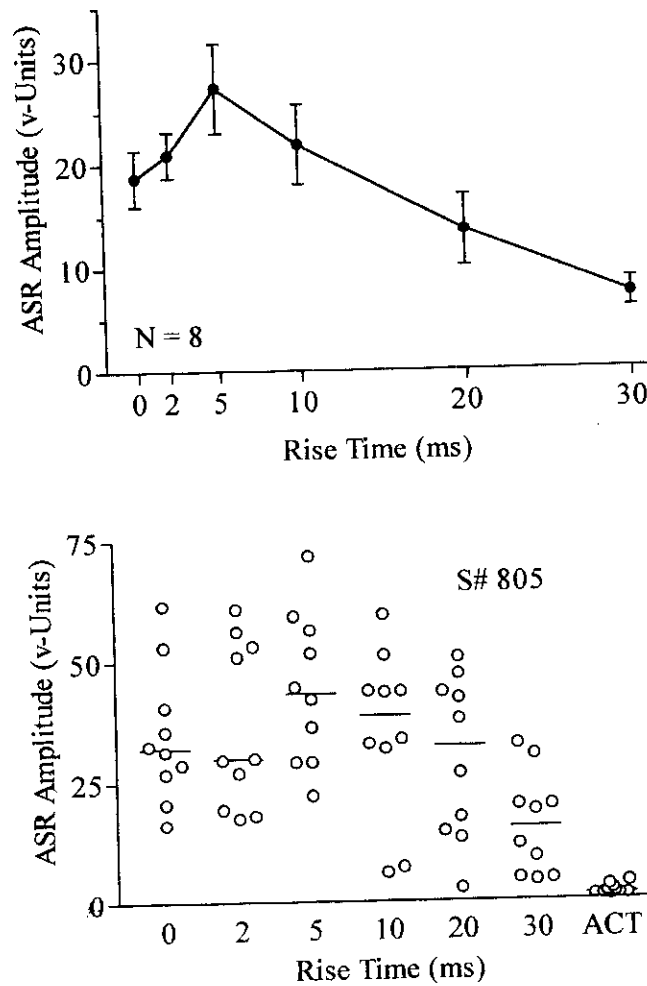


FIGURE 5.3 Group mean startle response amplitudes (\pm SEM) in mice to 110 dB SPL noise stimuli varying in rise time (above), and the amplitudes of all trials in each rise time condition for one mouse, showing the condition means.

finding major differences between short rise times vs. long rise times, but there is an important difference in detail arising probably because of procedural differences, namely that Fleshler used tonal stimuli that “click” at short rise times, while our noise bursts provide a click-like stimulus at all rise times. Our data show that there is a peak ASR within the short rise times that occur at 5 ms, $t = 3.75$, $df = 7$, $p < 0.01$, rather than a flat function from 0 to 10 ms. Thus, ASR amplitude in mice seems determined largely by the intensity reached within about 10 ms of onset, but there is an advantage to small, compared to 0 ms, rise times.

Fleshler concluded that the rat ASR was unaffected by stimulus duration, but his minimal duration was 6 ms. A larger study by Marsh et al. (1973) found ASR integration for brief durations. There is one experiment that studied this variable in mice (Hogan and Ison, 2000). In one of several conditions run in this experiment, young normal-hearing F1 hybrid mice (male, 6 weeks of age, $n = 8$) from a CBA X C57BL mating, received 144 noise bursts, presented for durations of 1, 2, 4, 8, or 16 ms, and levels of 105, 115, and 125 dB. Each stimulus condition was given in random order within blocks of trials that included a “no-stimulus” activity condition. The mouse data from Hogan and Ison is presented in the lower graph of Figure 5.4, and for comparison the rat data from

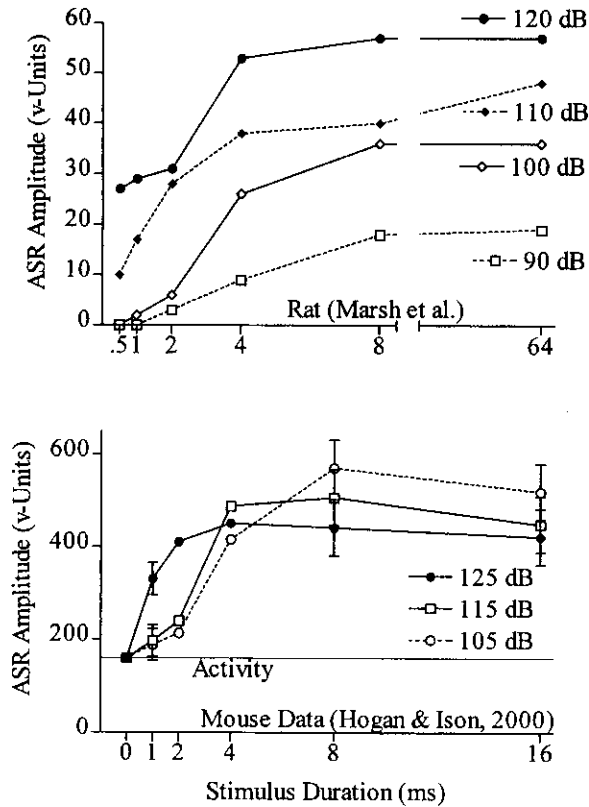


FIGURE 5.4 Mean startle responses in the rat to stimuli varying in level (90 to 120 dB SPL) and duration (0.5 to 64 ms), above; and in mice (\pm SEM), with levels of 105 to 125 dB SPL, and durations of 1 to 16 ms. (Adapted from R. Marsh, H.S. Hoffman, and C.L. Stitt, 1973, *J. Comp. Physiol. Psych.*, 82, 507-511; and from Hogan and Ison, 2000. With permission.)

Marsh et al. are presented at the top. The two sets of data agree in showing a rapid increase in amplitude with an increase in stimulus duration up to about 4 ms at all stimulus levels. In addition, for the lowest stimulus level of 105 dB in our data, and for the two lower levels of 90 and 100 dB in the data of Marsh et al., there is a further increase in response amplitude with an increase in duration to 8 ms. Regardless of stimulus level, there was no further increase beyond about 8 ms in either the mouse or rat data. In most respects, the findings for the mouse are very similar to those obtained in the rat, despite notable differences in the experimental procedures, such as, for example, our use of a noise stimulus in contrast to the tone burst in Marsh et al. The one difference is again an observation of a reversal in the effect of stimulus level on response output in the mouse. In contrast, the rat provides the very simple and readily explicable monotone relationship between startle reflex amplitude and stimulus level at all durations. This function is entirely consistent with the notion that the excitatory drive for the startle reflex is directly related to the greater amount of excitatory neural activity engendered by more intense acoustic stimuli. In this simple view of stimulus-driven neural activity in the reflex pathway, the behavior of the young mouse is an almost completely anomalous finding — although as mentioned previously, it is not an uncommon attribute of input/output functions in the central auditory system. It is interesting that a significant positive relationship between startle amplitude and stimulus level can be seen in the young mouse with good hearing, but one that is entirely confined to the very brief stimulus durations of 1 and 2 ms. At a 4-ms duration, stimulus level had no effect (as it had no effect in the young C57BL/6J mice in the experiment of Parham and Willott, described above). At the longer durations of 8 and 16 ms,

the functions reversed so that the more intense the stimulus, the lower the response amplitude (just as it reversed at the best-frequency of young CBA/J mice in Parham and Willott).

Overall, the data on temporal integration of the startle reflex in the mouse suggest a complex dynamic balancing of positive and negative interactive effects of stimulus levels and durations, which occur over such a small period of time that they are likely to be intrinsic components of the startle mechanism itself. Thus far, this type of response/level reversal has appeared in three experiments, counting Parham and Willott, our pilot experiment, and Hogan and Ison. While the formation of a testable hypothesis to explain this effect remains elusive, it does invite speculation that perhaps in the mouse, but not so apparent in the rat or human, the massive acoustic input of a startle-eliciting stimulus engages both immediate excitation and then a briefly delayed inhibition that limits reflex expression.

In most respects, startle elicitation in the mouse is similar to that seen in the rat and the human in its substantial dependence on the intrinsic characteristics of the eliciting stimulus. But the nicety of these functional relationships is always conditioned on our aggregating groups of trials within similar conditions, a necessary stratagem that was immediately apparent to Lombard when he began his research in 1887. In fact, the expression of startle reflex behavior in both rats and mice, as well as humans and frogs, is affected by other less evident variables. As much as we all may have tried to present clean and well-controlled stimuli in a constant environment, response amplitudes always vary from one moment to the next, and the range of variation can be considerable. There is no doubt that some of this variation is produced by differences in what the animal happens to be doing at the time of the stimulus, just as the knee-jerk is affected by the Jendrassik maneuver in the early work of Lombard and Bowditch and Warren. A similar mixed excitatory/inhibitory effect of extraneous movement has been reported for the various components of the cutaneous eyeblink EMG in humans (Sanes, 1984), where there is also a pronounced mixed effect of voluntary stimulus presentation that is very precisely timed (Ison et al., 1990). Consistent with these findings, the startle reflex in the rat is substantially depressed if the rat is active at about the time of stimulus (Wecker and Ison, 1986a), and it is also reasonable to think that these movements would facilitate the startle reflex if they could be sufficiently well-synchronized with the response (Ison and Krauter, 1975). Thus, it may be suggested that one appropriate way to reduce trial-to-trial variability in the response would be to closely observe the subject and refrain from presenting stimuli while the animal is moving or fidgeting in the test cage. This was common practice in the days before computer control; but to prevent any chance of experimenter bias, it is a procedure that at best requires the continuous attention of two experimenters, one to set up the stimulus condition unbeknown to the other, who was then responsible for delivering the trial. Currently, we usually leave control over the procedure to the completely unbiased computer, but it probably leaves us more vulnerable to unwanted levels of variability. Variability might be reduced if the computer detected the presence of extraneous movement and delayed stimulus presentation until the baseline returned to normal, but we have not yet put this hypothesis to the test.

REFLEX MODIFICATION

The attempt to understand and control the sources of trial-to-trial variability leads us to the study of reflex modification, in the search for extrinsic stimulus conditions and internal state-like conditions that can alter the function of the reflex pathways, without themselves being part of the intrinsic reflex arc. The first contemporary study of startle modification by preliminary stimuli was that of Hoffman and Fleshler (1963). Figure 5.5 is adapted from their data showing the amplitude of the startle reaction to the first 60 presentations of a brief but very loud click, given in blocks of 10 trials (10 s inter-trial interval), each block in either ambient background noise (an average of 58 dB SPL, but of unknown spectral content); a steady wide-band noise (85 dB SPL); or in pulsed wide-band noise, cycled every 0.5 s. In ambient noise, the response was moderately large overall but variable from trial to trial; it was completely eliminated on almost all trials in the pulsed noise condition,

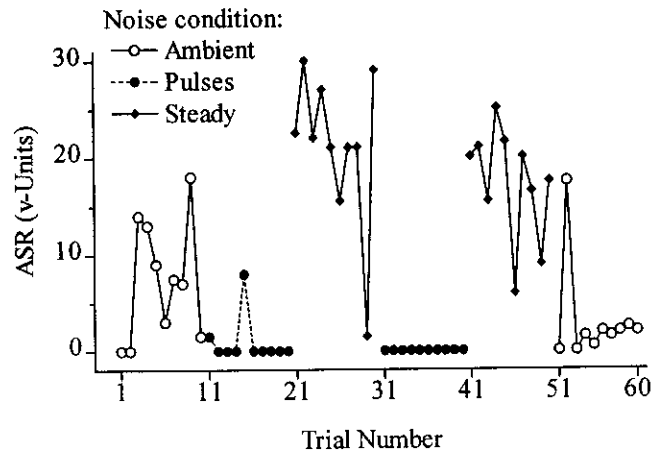


FIGURE 5.5 Startle response amplitudes for 60 trials for one rat in different blocked background noise conditions. (Adapted from H.S. Hoffman and M. Flesher, 1963, *Science*, 141, 928-930. With permission.)

but was substantially exaggerated in the steady background noise condition. There are some incidental findings that are also interesting, one being the robust appearance of habituation but only in the aggregate; note that the first two trials for this rat had zero amplitude. Another was a brief description of an experiment done with a steady vs. pulsating light that had no effect on startle, but this conclusion was later overturned by further developments.

The next series of experiments from Hoffman's laboratory provided ever more careful control over the specification of the important stimulus conditions for these facilitatory and inhibitory effects. Most notably, they included a rediscovery of the critical importance of the temporal relationships between the stimuli to the strength of inhibition (Hoffman and Searle, 1965). Much of the early development of this work and its theoretical significance was reviewed by Hoffman and Ison (1980), but here our specific concern is how these phenomena of reflex modification might appear in the behavior of the mouse. As can be clearly seen in Figure 5.5, the presence of a steady background noise has a very powerful facilitative effect on the behavior of the rat: does this variable affect startle in the mouse? The effect in the rat came to be seen as being more complicated than Figure 5.5 reveals. Later parametric variation in background level showed that facilitation increases up to some optimal level of wide-band noise and then is reduced in strength (Ison and Hammond, 1971). Further, the optimal noise level depends in part on the relative spectral compositions of the noise and the eliciting stimuli (Gerrard and Ison, 1990), and noise with a low-frequency composition tends to facilitate startle, but with a relatively high-frequency composition tends to suppress the response. Davis (1974) also demonstrated that the optimal level of background noise varied with the intensity of the eliciting stimulus and, further, on the degree to which the response has been habituated to that stimulus. These complicated positive and negative effects of noise interacting with past experience were shown in rats, and now the available evidence, although not as rich in parametric detail, indicates that they are also found in startle reactions in mice.

In one experiment, we examined startle in a group of seven young CBA/CaJ mice (10 weeks old, all male) with the reflex elicited by wide-band noise bursts, 25 ms in duration (including 5 ms RDT) presented at levels of 94, 103, 112, 121, 130, and 139 dB SPL. These were presented in a quiet background or in a 70-dB wide-band noise (which had a broad peak at 8 kHz to 16 kHz and varied by ± 6 dB over a range of 2 to 100 kHz). Trials were given in 11 blocks, which included each condition presented in random order. Figure 5.6 gives the means across all conditions; and in accord with the findings of Davis, it is clear that the effect of the noise depended on the intensity of the startle stimulus: the noise partially suppressed the response elicited by the weaker stimuli of 94 and 103 dB, had no effect at 112 dB, but enhanced the reflex elicited by stimuli of 121, 130,

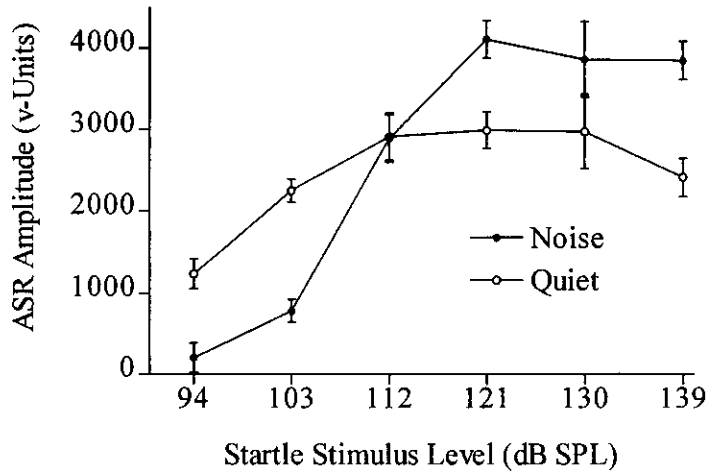


FIGURE 5.6 Startle response means (\pm SEM) for a group of mice to noise bursts ranging in level from 94 to 139 dB, in quiet or in the presence of a 70-dB noise.

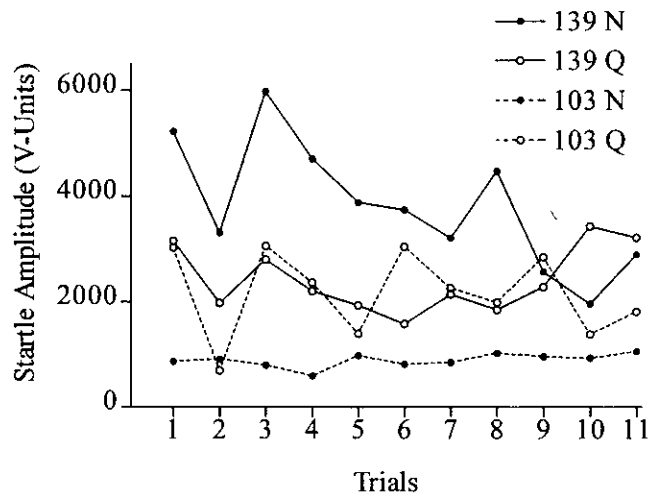


FIGURE 5.7 Startle response means on the 11 trials of a day for a group of mice to noise bursts of 103 and 139 dB, in quiet and in the presence of a 70-dB noise.

and 139 dB. An additional confirmation of the similarity between mouse and rat is given in the changing effects of the noise across the test session. Figure 5.7 gives the group means across each trial block, comparing quiet vs. the noise conditions for just the 103-dB and 139-dB stimuli. As in Figure 5.8, the 103-dB stimulus was more effective when it was presented in quiet, and this was a stable effect across the experiment. In contrast, the facilitative effect of noise for the response elicited by the 139-dB stimulus was most evident at the beginning, and was completely lost in the three final blocks when habituation was most advanced. One simple interpretation of these data is that it is noise facilitation of responses that habituates, not the responses themselves; however, the hypothesis favored by Davis on the basis of his data is that habituated reflexes are less susceptible to noise potentiation.

Another point of similarity between startle in the mouse and the rat is presented in the research of Carlson and Willott, in their examination of the effects of jointly varying the spectral composition of the background and the startle stimulus in the C57BL/6J mouse at different ages (Carlson and

Willott, Chapter 6 in this volume). Their findings are consistent with those obtained by Gerrard and Ison (1990) in the rat, showing that responses are augmented when the background is lower in frequency than the startle stimulus and suppressed in the reverse condition, with both effects exaggerated with an increase in the background stimulus. Clearly, background noise can have two effects on the acoustic startle reaction in both rat and mouse, providing in both species a positive exaggeration of the response and its opposite, a negative effect in which it appears to function as a masking stimulus. The data are also convincing in showing that facilitation is most apparent (a) in the naïve animal, (b) when the animal is presented with relatively intense eliciting stimuli in which high frequencies dominate, and (c) when the animal is in a background in which low frequencies dominate. However, Carlson and Willott caution that neither the positive arousal-like effect of noise nor its masking-like effect are well understood. They do conclude that the effects of background noise on startle are rich in their theoretical implications, and they also described their practical implications that should influence our choice of experimental procedures. They suggest that its experimental analysis may yield ideas about how the auditory system assembles a shifting balance of excitatory and inhibitory mechanisms to deal with different sources of moderate to high levels of both chronic and acoustic input. It may be that our understanding of this complexity will benefit from finding a different balance of excitation and inhibition in different strains of inbred mice and different types of mutant knock-out mice.

The other effect reported by Hoffman and Fleshler (1963) and captured in Figure 5.5 was that a pulsing noise inhibited the startle reflex, and (they mentioned in passing) a flashing light did not have any such effect. Do these observations also hold for the mouse? The first experiment described here shows that contrary to their finding in the rat, in mice the acoustic startle reflex is inhibited by a flash of light. The reasons for reporting this experiment here may appear somewhat obscure, given that the text is "mouse auditory research" and that Hoffman and Fleshler reported a negative finding in the rat for light flash inhibition. However, later research showed that rat startle is inhibited by both sound pulses and light flashes (Buckland et al., 1969; Ison and Hammond, 1971; Schwartz et al., 1976), but that flashes have a shorter temporal window in which they are effective. The idea is that the light flashes used by Hoffman and Fleshler were ineffective because they were not synchronized with the startle, and thus did not fall within the necessary time period. Additionally, the question of whether mice show visual inhibition of an auditory reflex is a question about plasticity of auditory function as well as a question about vision. This question would become especially vexing, for example, if light flashes did not affect the startle reflex in mice known to possess adequate visual abilities.

In this experiment, we examined the effects of brief light flashes on the startle reflex in young C57BL/6J mice (7 female, 5 male, 10 weeks old). A 20-ms flash of light (6 ft-c) at various lead times, 10 to 220 ms, before a 115-dB wide-band noise eliciting stimulus, the usual 25-ms long noise burst including 5-ms rise and decay times. The data from this experiment are presented in Figure 5.8. Clearly, the startle reaction in these mice was inhibited by the light flash. At the lead time of 40 ms, all 12 mice showed inhibition, at least in the sense of all having a response value relative to the baseline control of less than 1.0. At 40 ms, the range of relative scores was from 48 to 97% (i.e., a range of relative inhibition scores of 52 to 3%). It is probably important that the optimal lead time for inhibition was at 40 ms in only four of these mice, the others showing their maximal scores at 50 ms ($n = 3$), 70 ms ($n = 2$), and 110 ms ($n = 3$). The peak of inhibition in young rats is less variable, and in rats major shifts in the function toward longer lead times are associated with retinal degeneration (Wecker and Ison, 1986b; Ison et al., 1992; and see below). It will be interesting to follow these mice as they grow older, to find out if as a group they will exhibit the pattern of delayed inhibition across lead times and then the final loss of inhibition that is characteristic of age-related retinal dysfunction in the rat.

Given this reliable inhibitory effect of pulses of light on startle reflexes in the mouse, it would be very surprising if pulses of noise did not have the same effect. In this experiment, we examined the temporal development in inhibition over various lead times, from 5 to 640 ms. Standard wide-band

The Acoustic Startle Response: Reflex Elicitation/Reflex Modification by Preliminary Stimulus

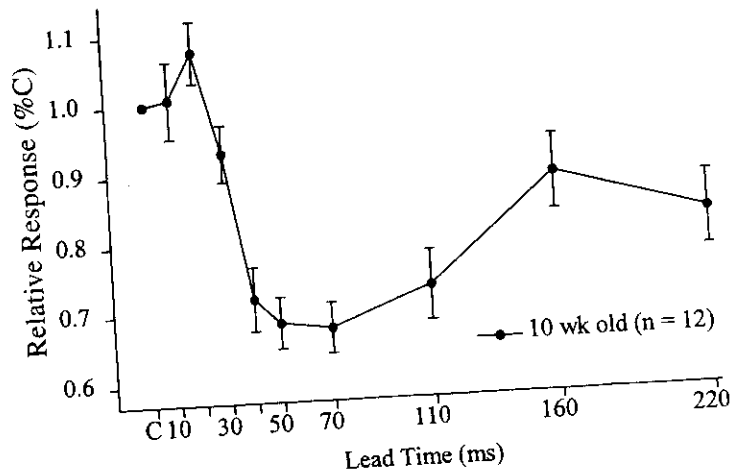


FIGURE 5.8 Mean relative response means (\pm SEM) for a group of mice when the startle stimulus was preceded by light flash, at intervals of 10 to 220 ms.

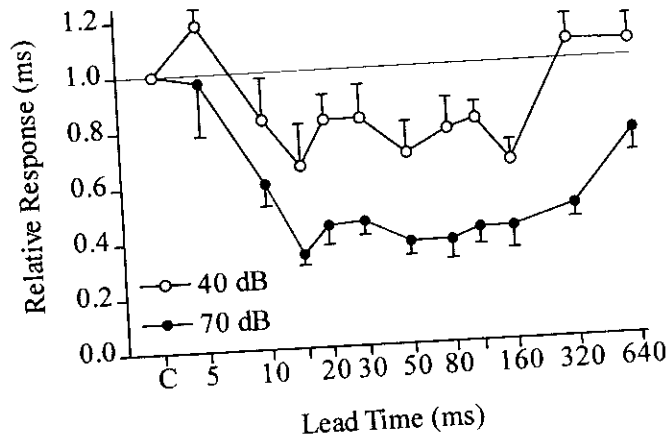


FIGURE 5.9 Mean relative response means (\pm SEM) for a group of mice when the startle stimulus was preceded by a noise burst of 40 dB or 70 dB, at intervals of 5 to 640 ms.

noise pulses were given at levels of 40 and 70 dB (on separate days), at 11 different lead times prior to the presentation of a 25-ms noise pulse at 115 dB. A total of 143 trials were given on each of two test days, 11 blocks of 13 trials each. Each block included one trial for each lead time plus two startle-alone control trials. The subjects were five female CBA/CaJ mice, about 2 months of age. The resulting data are presented in Figure 5.9, the amplitude measures again being converted to relative values. The data are similar to that obtained in similar conditions for the rat reported by Hoffman et al. (1980), except that inhibition developed more rapidly in the mouse. Three major effects are apparent. First, the peak strength of inhibition was greater for the more intense prepulse. Second, inhibition developed rapidly in both stimulus conditions, reaching its peak value within 15 ms. Third, inhibition persisted at that plateau for about 150 ms for the weaker stimulus and much longer for the more intense stimulus.

A more subtle effect is the slight increase in the response when the 40-dB stimulus was presented at a 5-ms lead time, which was close to significance even in this small group of subjects ($t = 2.67$, $p = 0.056$). Recent experiments have shown that early facilitation is a common finding in rats,

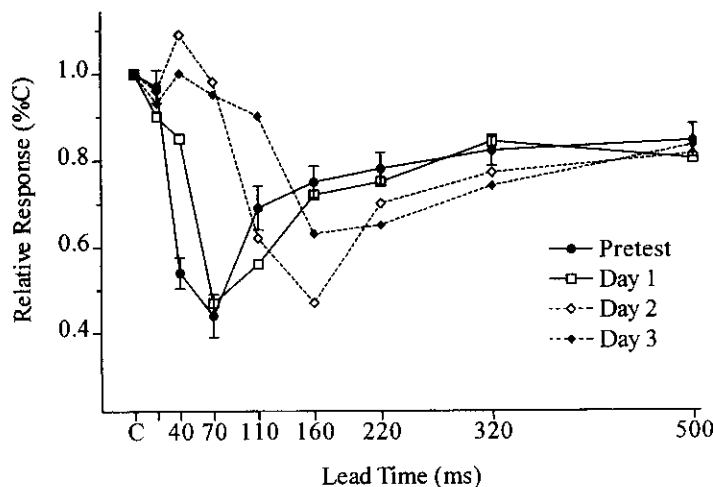


FIGURE 5.12 Mean relative startle amplitudes in groups of albino rats to a 120-dB tone startle stimulus preceded by a light flash at lead times of from 20 to 500 ms, in a pretest (mean \pm 95% confidence intervals), and groups exposed to light for 1, 2, or 3 days.

reflex. The paradox, however, is that while for inhibition the “more effective prestimulus” is the more intense prestimulus, for augmentation the “more effective prestimulus” is the less intense.

For this phenomenon of reflex modification, the data reveal that augmentation is strongest with small increments in a background noise (Ison et al., 1997, and above in Figure 5.10), perhaps because in this condition, delaying inhibition onset unmasks facilitation. Are there data that might support this hypothesis that sensory loss yields a delay in the development of inhibition? There are, in the changes in prepulse inhibition observed in the rat during the early stages of retinal blinding produced by light exposure. In one of our research projects, we collected baseline data on a total of 155 Fischer 344 rats at 5 to 6 weeks of age, measuring the inhibitory effects of a light flash on the acoustic startle reflex. The general procedures were exactly those we used in the experiment reported above for mice, that led to the data presented in Figure 5.8. Some of these rats ($n = 18$) were then exposed to fluorescent light for 1 month at 24 h/day. This procedure produces complete retinal degeneration in the rat, and, by the end of the month, results in a complete loss of light-produced reflex inhibition (accounts of this experiment are presented in del Cerro et al., 1991). We tested subgroups of these rats after 24 h of exposure ($n = 5$), 48 h ($n = 6$), and 72 h ($n = 7$), with the results shown in Figure 5.12, showing the data for relative responses at each lead time in the entire group of 155 rats for the pretest, and then the three subgroups with their different levels of exposure. The normal function has a sharp drop in the relative response at 40 ms, which deepens to 70 ms, and then begins a more or less exponential return toward the baseline control level. In contrast, the exposed groups show a progressive loss in inhibition, not first in the strength of its peak effect, but rather in the loss of inhibition for the brief lead times, and thus a progressive slowing of the time at which peak inhibition appeared: from peaks of 40 and 70 ms in normal rats, to 70 ms and 110 ms after 24-h exposure, 160 ms after 48 h exposure, and 160 ms and 220 ms after 72 h exposure. These changes in the kinetics of light inhibition in the rat with a degenerating retina capture the central outcome seen in Figure 5.10 for the middle-aged C57BL/6J mouse with a degenerating basilar membrane (i.e., a severe slowing of inhibition without change in peak level). This effect in the light-blinded rat cannot be simulated by manipulating the stimulus conditions, as diminished prepulse duration or power shifts both the time and the strength of peak, while light adaptation maintains the time of the peak, but reduces its strength and its persistence (Ison et al., 1992).

measure of activity in the (minimally two) neural mechanisms that control reflex strength must necessarily reflect their combined action, but we cannot determine *a priori* how their separate activities vary with time, nor can we work out how they are separately affected by variables such as stimulus level. We had hoped to do a pharmacological dissection using GABA agonists to block excitation and thus isolate the effects of inhibition; but unlike background noise or fear potentiation of startle (Kellogg et al., 1991; Davis, 1979), diazepam does not affect noise increment facilitation (Ison et al., 1997b). Thus, when we find that a 6-dB stimulus facilitates startle at 10 ms but inhibits at 60 ms, we cannot tell if this results because the additional 50 ms has allowed more inhibition to accumulate, or allowed facilitation to dissipate.

There are serious implications for the practical use of reflex modification in these findings. When we use reflex inhibition as objective evidence that a prestimulus has been detected or as an index of stimulus salience (e.g., Young and Fechter, 1983), we are assuming that the stimulus is entirely inhibitory in its effect. But if stimuli simultaneously engage multiple neural mechanisms that follow independent time courses, and if one mechanism augments while another depresses reflex expression, there will be lead times at which both are active. To some extent, their joint effort will result in mutual cancellation. When there is a significant effect, whether it be facilitation or inhibition, we are assured that the stimulus was presented above the threshold: the problem is that the absence of an effect does not mean the stimulus was below threshold, because we cannot distinguish between this circumstance and that of mutual cancellation, unless our experimental designs are very clever.

An interesting example of this phenomenon and the interpretive problems to which it may lead is seen in studies of reflex inhibition by tone pips in the C57BL/6J mouse, in comparing young mice against older mice with high-frequency hearing loss. It is now well-known that receptive fields in the older mouse are reorganized following the loss of afferent input from high-frequency regions of the cochlea. The central targets of the missing hair cells are not thereafter "quiet," but instead shift in their best-frequency to become very sensitive to low-frequency input (e.g., Willott, 1984; 1986; Willott et al., 1991; 1993). Given that older mice have a larger number of low-frequency neurons, what are they able to do with low-frequency input that is beyond the ability of the young? Willott et al. (1994) used a prepulse inhibition procedure in their approach to this question, with a very positive outcome. (It is worth noting that this interesting question is also being asked of humans with high-frequency hearing loss, with mixed success: compare, for example, McDermott et al., 1998, and Buss et al., 1998.) The startle stimulus was a noise pip at 100 dB SPL, the prestimuli tones pips, variously 50 to 80 dB in level, at frequencies of 4, 8, 12, 16, and 24 kHz. The interval between S1 and S2 was a constant 100 ms. The data showed first that the inhibitory effect of high-frequency tone pips was very much reduced in 12-month-old C57BL/6J mice, and slightly reduced in 5-month-old mice, as might be expected; but in great contrast, the inhibitory effect of low-frequency tone pips was greatest in the 12-month-old mice and next in the 5-month-olds. And moreover, for the mid-frequencies, the 5-month-old mice showed the most inhibition. Similar effects were found in DBA/2J mice as well, but at an earlier age, as is consistent with their relatively rapid peripheral degeneration.

The data demonstrate a strikingly simple (but deceptive!) correspondence between the behavioral salience of low-frequency tonal input and the relative numbers of neurons in the auditory system that come to be activated by such stimuli following central reorganization. One implication of this result is that if the mechanisms of neural plasticity are similar in mice and humans, then a human listener with high-frequency hearing loss may be able to compensate by making more effective use of low-frequency input. However, the next paper published in this series showed that this behavioral effect of high-frequency loss is more complicated than a simple enhancement of reflex inhibition would suggest. In Willott et al. (1994), the experiment varied prepulse frequency and level, but maintained the lead time of the prepulse at 100 ms. Willott and Carlson (1995) used the same general procedures as Willott et al. (1994a), including their varying prepulse frequency (again 4 to 24 kHz), but now they fixed the level of the prepulse at 70 dB SPL while varying their

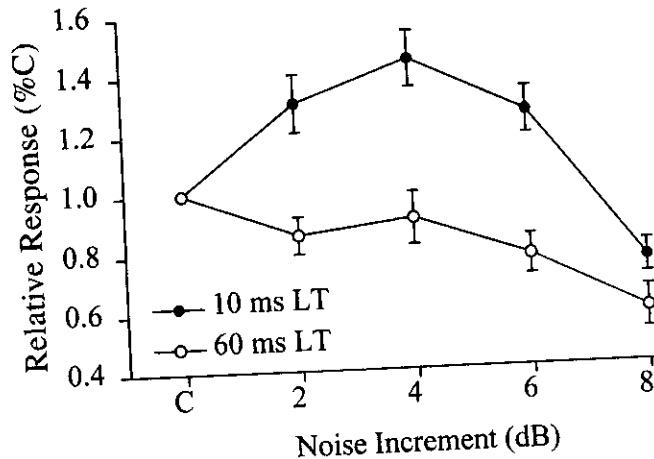


FIGURE 5.10 Mean relative response amplitudes (\pm SEM) for a group of mice when the startle stimuli were preceded by noise increments of 2, 4, 6, and 8 dB, at 10 ms prior to the startle stimulus, or 60 ms.

obtained usually under stimulus conditions that yield small or delayed inhibitory effects. The most powerful excitatory effects of a prepulse are seen in rats when the preliminary stimuli are small increments in a constant background noise (Ison et al., 1997). For the rat, noise increments of 3 to 6 dB facilitate startle with increasing strength as lead time increases up to about 20 to 30 ms. They may double the size of the response at the peak of effectiveness, but brief increments beyond about 10 dB tend to inhibit rather than facilitate reflex expression at this lead time. These experiments in the rat reveal that the acoustic startle reflex is governed by a dynamic balancing of excitation and inhibition in which the kinetics of excitation and inhibition are determined in large part by the intensity of the prestimulus.

We were interested in how these complex intensity- and time-dependent functions might appear in the startle reaction of the mouse under similar stimulus conditions. In the next experiment, the subjects were 10 CBA/CAJ mice, 5 to 8 months of age, 3 male, 7 female. They were run in a 58-dB background noise with 20-ms long 115-dB wide-band startle stimuli, and 0-ms rise times. Increments in the background of 2, 4, 6, and 8 dB (0-ms rise time) were presented with lead times of 10 or 60 ms, using a 10-ms duration for 10-dB lead times, and 20 ms for 60-ms lead times. The data (Figure 5.10) were very similar to those of the rat, with the exception that inhibition seems stronger in the mouse and appears earlier, although the differences are small enough that a firm conclusion should await a direct comparison of the two species. The most obvious effect is that at the 10-ms lead time, increments of 2, 4, and 6 dB were facilitatory (all $p < 0.01$), while at this same interval, the 8-dB increment was inhibitory (also $p < 0.01$). At the 60-ms lead time, the 6-dB and 8-dB incremental pulses were inhibitory ($p < 0.01$), and the 2-dB increment provided a near-miss for significant inhibition ($p = 0.051$), while the 4-dB increment did not yield significance ($p = 0.3$). The differences in the inhibitory effects of these three increments at the 60-ms interval were not significant in the analysis of variance, although the analysis of all four intensities at this lead time provided both a significant effect for intensity and a linear trend for this effect. Similarly ANOVA of the effects at 10 ms did not find overall significant differences between 2, 4, and 6 dB, although there was a significant quadratic trend.

These complicated biphasic temporal patterns raise interesting theoretical questions, but present practical problems of interpretation for those who may wish to use very simple tests for rapid screening purposes. It seems that the processes that are variously responsible for facilitation and inhibition must have different time constants, but also that they overlap in time. It is their resolution that determines the vigor in the response at any particular lead time. At any one time, our only

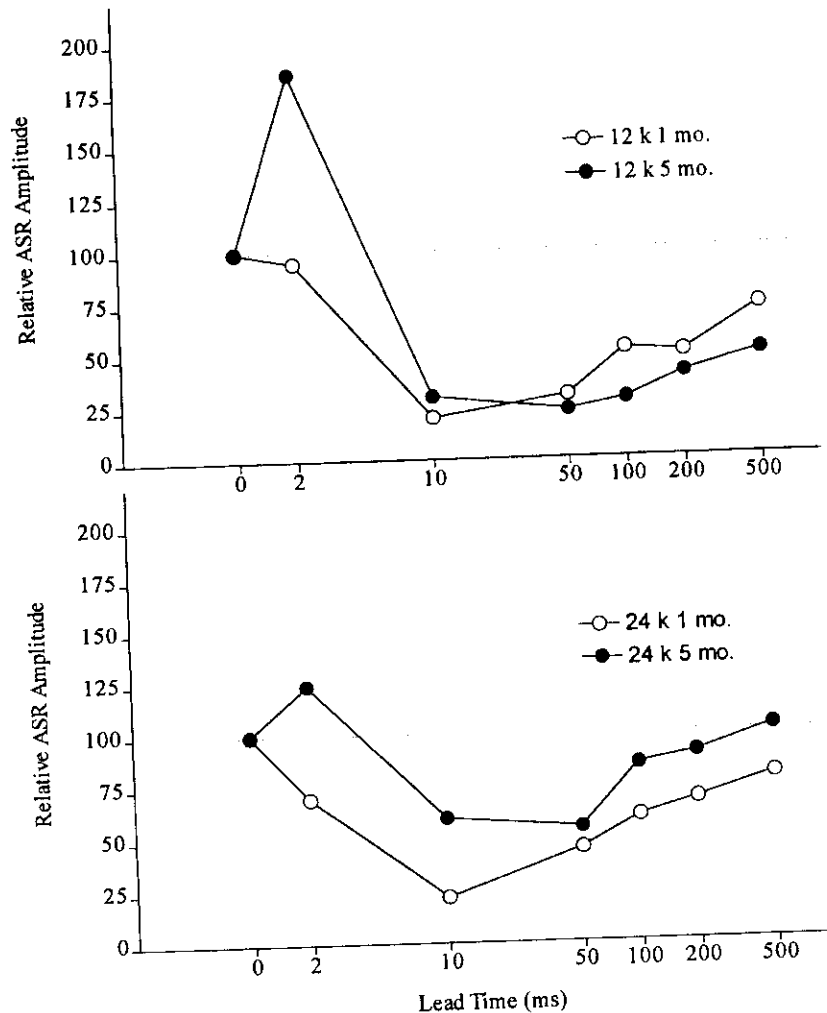


FIGURE 5.11 Mean relative startle amplitudes in two groups of C57BL mice, 1 month and 5 months old, when the 100-dB noise pulse startle stimuli were preceded at lead times of 2 to 500 ms, by 70-dB tone pips of either 12 kHz (above) or 24 kHz (below). Adapted from J.F. Willott and S. Carlson, 1995, *Behav. Neurosci.*, 109, 396-403. With permission.

lead time from 2 to 500 ms. The critical findings in this second report are represented in Figure 5.11. The data at the bottom is for the 24-kHz prepulse, revealing the reduction in peak inhibition and the retarded latency of the peak, as well, perhaps, as the suggestion of reflex augmentation at the 2-ms lead time. This is as if the stimulus had been presented to a younger mouse at a lower intensity; in this respect, peripheral damage seems reasonably described as a loss in gain at the auditory periphery. The data at the top are for the 12-kHz prepulse and agree with those previously obtained by Willott et al. (1994b) in showing that at the 100-ms lead time and beyond, the older mouse shows more inhibition than the younger mouse. But what is to be made of the differences obtained at the brief lead times of 2 ms and, to a lesser extent, at 10 ms? One thought is that this effect is simply another demonstration that the low-frequency stimulus is more effective in the older mouse, not only more effective as an inhibitor as shown here at lead times of 50 ms and beyond and also in Willott et al. (1994b), but now at very brief intervals also more effective in augmenting the startle

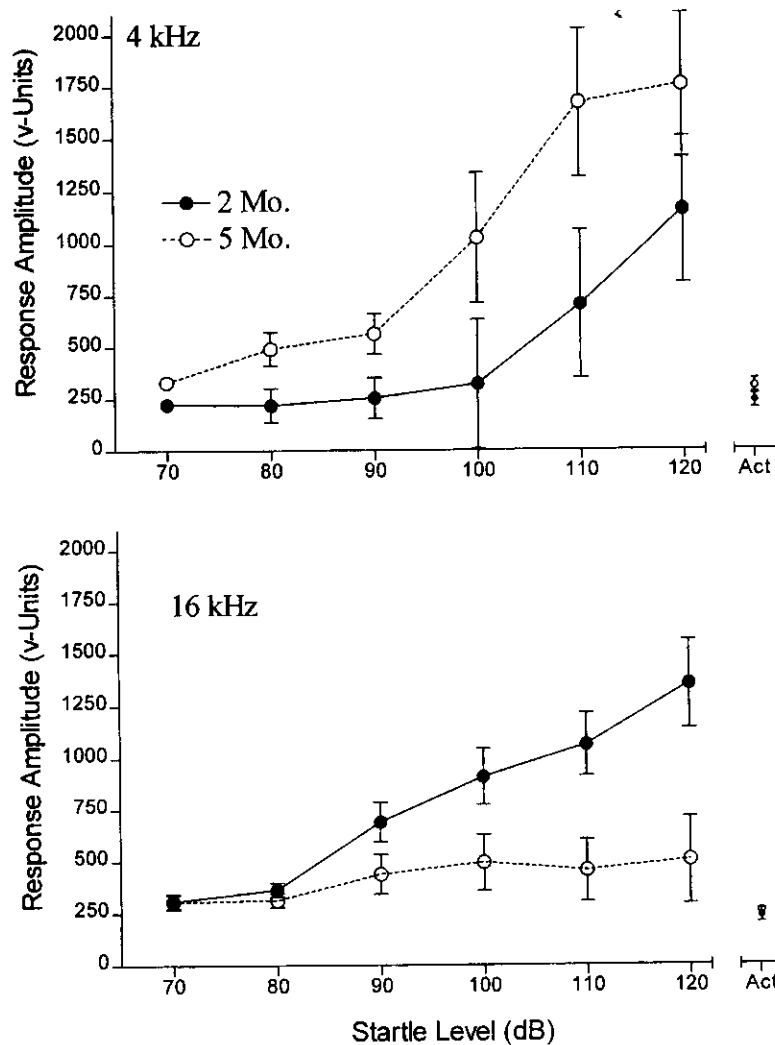


FIGURE 5.13 Mean startle amplitudes (\pm SEM) in two groups of C57BL/6J mice, 2 and 5 months of age, for tonal startle stimuli ranging in level from 70 to 120 dB, at 4 kHz (above) and 16 kHz (below).

Could this slowing in the kinetics of inhibition by itself be responsible for the effect of cochlear degeneration in the C57BL/6J mouse seen in Figure 5.11? This is possible; but we suspect that reflex augmentation may be a direct additional effect of the developing hearing loss, rather than a secondary effect of the change in the kinetics of inhibition. In our work on this phenomenon in the mouse (e.g., Ison et al., 2000), we have replicated the basic details of the phenomenon as reported by Willott and colleagues, but with some small but interesting differences resulting perhaps because the hearing loss in our mice appears to be more pronounced at any given age. In particular, one difference is that for our older mice, the periods of reflex facilitation for 4 and 8 kHz may last for 40 to 50 ms, rather than under 10 ms; and a second difference is that our older mice show sensitized startle responses to low-frequency tone bursts. This latter effect is evidenced in Figure 5.13. The data were collected in two groups of 12 C57BL/6J mice run at 2 or 5 months of age. The startle stimuli were 15-ms tone pips with 5-ms rise and decay times, given at levels of 70 to 120 dB and at tonal frequencies of 4 and 16 kHz. There were 11 blocks of 7 trials each

(6 levels plus a "no-stimulus" activity control) given at each frequency on different test days. For the 16-kHz stimuli in the lower graph, the data are conventional, showing that the older mice with high-frequency hearing loss jumped less. For the 4-kHz stimuli, however, the data are remarkable, as the older mice jumped more vigorously at all intensities of stimulation, except that at the highest level of 120 dB the younger mice were finally catching up. It is interesting but perhaps not surprising that for the young mice, the correlation between peak startle at 16 kHz and the ABR threshold at 16 kHz was $r = -0.58$; as might be expected, the young mice with the most sensitive hearing at 16 kHz responded more vigorously to the startle stimulus at 16 kHz. It is more interesting and more surprising for our conventional views of the effects of hearing loss, that for older mice the correlation between peak startle at 4 kHz and the ABR threshold at 16 kHz was $r = +0.73$; that is, old mice with the least sensitive hearing at 16 kHz startled more vigorously to the 4-kHz startle stimulus. The data are persuasive in showing that peripheral sensitivity changes for seeing and hearing profoundly influence both the strength and timing of central excitatory and inhibitory mechanisms.

A steady accumulation of recent electrophysiological data show that sensory loss changes receptive fields at all levels of the auditory system, as well as effecting both decrements and increments in central inhibition and excitation. This is variously seen in increased spontaneous neural noise and a combination of depressed early components and enhanced later components of auditory evoked potentials. This topic is too rich in scope to discuss here, but Willott and Carlson (1995) provide an extended discussion of research that relates it to the present context of reflex elicitation and modification. They also provide useful speculation on the potential connection between these observations and the more typical complaints of the hearing-impaired listener, which are, of course, not that their startle behavior is dysfunctional, but rather that they have problems understanding those elements of speech that depend on the precise timing among phonemes, especially in noisy and reverberant environments.

This emphasis on timing — so important in speech and also so important in understanding reflex elicitation and modification — provides the transition to the last topic of this chapter, the effects of brief gaps in noise on acoustic startle in the mouse. Temporal processing is critically important for hearing speech because of the intrinsic time-dependent nature of the speech signal, in which the fine structure of the speech envelope on a scale of some few milliseconds determines whether one phoneme or another was intended (Kewley-Port, 1983). Temporal processing is also critical for responding to startle stimuli; a drop in the envelope of a background noise lasting for these same few milliseconds can have an enormous influence on reflex expression. These powerful effects can be demonstrated (1) when a quiet period of a particular duration is inserted into the background noise and presented at various lead times, its inhibitory effect varying with lead time; or (2) if the noise is turned off prior to the startle stimulus, and its inhibitory effect varies with the length of the quiet period; or (3) if gaps of different durations are presented at a fixed interval prior to the startle and inhibition varies with gap duration. All of these effects of a gap are inhibitory, and our extensive work using decrements in noise as the preliminary stimulus we have never found them to produce reflex facilitation. In this respect, noise decrements are very different from noise increments in their effect on the startle reflex.

In the first experiment, which illustrates the temporal development of inhibition for a fixed gap as a function of lead time, a gap of 10 ms in a 80-dB background noise was presented at one of 10 intervals before a 115-dB startle stimulus: 10, 15, 20, 30, 40, 60, 100, 150, 180, and 300 ms, measured from the onset of the gap to the onset of the startle stimuli. The gaps had 0-ms rise and decay times, as did the noise startle stimuli. The subjects were 18 CBA/CaJ mice, both male and female, about 10 weeks of age. They received three test days. The data are presented in Figure 5.14, with absolute values for the mean responses at the top and relative responses below. The absolute response values are provided in part to show that young CBA/CaJ mice jump much more vigorously than C57BL/6J mice, and in part to show that the effects of habituation across days are minimal. However, these data, and also the relative data in the lower graph, show that the temporal function

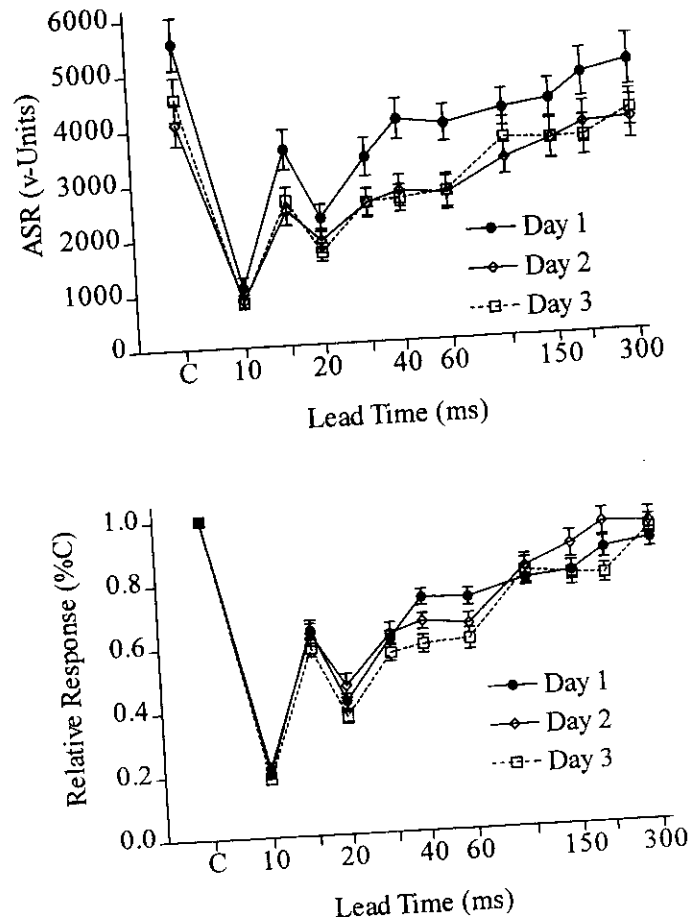


FIGURE 5.14 Mean startle amplitudes (\pm SEM) in a group of mice when the startle stimulus was preceded by a 10-ms gap in a 70-dB background noise at intervals of 10 to 300 ms (above), and relative response means (\pm SEM) (below).

for gaps is remarkable in being biphasic in the CBA/CAJ mouse (and in the rat: for example, Ison et al., 1991; Ison and Bowen, 2000). An immediate decrement is apparent when noise offset has a 10-ms lead time. The response then partly recovers at a lead time of 15 ms, but a second inhibitory trough emerges at 20 ms, and this is followed by a more gradual recovery that may last several hundred milliseconds. It can also be seen that inhibition at the later lead times is improved with training, which does not affect early inhibition; this too is evident in the rat (Ison and Bowen, 2000). In other work in rats, it was found that late inhibition is eliminated by functional decortication (Ison et al., 1991) and reduced by the systemic administration of scopolamine, a muscarinic receptor blocker (Ison and Bowen, 2000), with neither intervention affecting early inhibition. We have thought that response recovery at 15 ms reveals the transition time between the end of a first inhibitory phase and the onset of the second phase at 20 ms from more rostral mechanisms. Now we lean toward the hypothesis that the momentary increase in the response at 15 ms is a facilitatory effect produced by noise onset at the end of the gap riding on inhibition from the prior noise offset at the beginning of the gap, and that early and late inhibition partially overlap in time.

The next experimental paradigm uses noise decrements of 10, 20, 30, and 40 dB from 70 dB to inhibit the startle reflex, with their lead time equal to the duration of the gap, at 1, 2, 4, 6, 8, 10, and 15 ms. The subjects were six C57BL/6J mice, 6 weeks old, and testing continued over

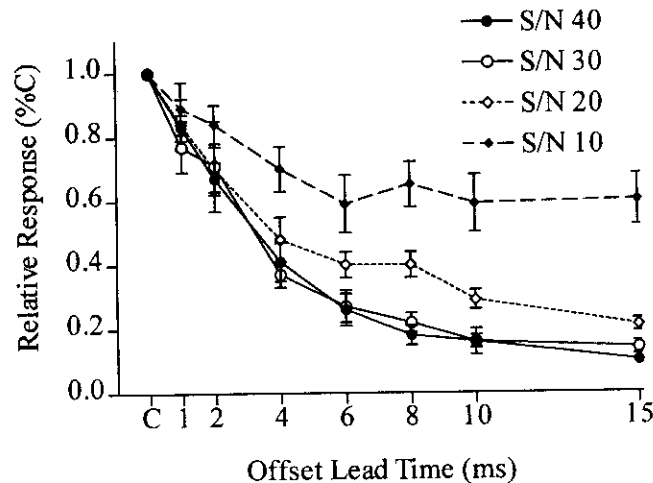


FIGURE 5.15 Mean relative startle amplitudes (\pm SEM) in a group of mice when the startle stimulus was preceded by decrements in a 70-dB background noise, the decrements of 10 to 40 dB, and the lead times of 1 to 15 ms.

4 days with intervening rest days. Figure 5.15 shows the relative response values for each condition. As might be expected, inhibition increased with gap depth up to an asymptote at 30 dB attenuation, and also increased with lead time with an asymptote reached at approximately 8 to 15 ms, depending on gap depth. The most remarkable aspect of the data is that noise decrements presented just 1 ms before startle onset significantly inhibit the response. With large samples (Ison et al., 1998a), 1-ms inhibition is reliable at a 10-dB S/N ratio; startle in the mouse is very sensitive to small perturbations in the background, even when they are extremely brief.

The application of reflex procedures to the study of sensory processing in some measure assumes that they provide an objective measure of "sensation" in the awake and behaving animal. These measures are expected to yield values of, for example, absolute thresholds that agree with those provided by conventional behavioral and psychophysical tests. This is a reasonable assumption, given data that, in humans, threshold measures provided by reflex inhibition agree with those of psychophysics (e.g., Reiter and Ison, 1977; Ison and Pinckney, 1982; Ison et al., 1986), as do the data collected in animals (Young and Fechter, 1983; Wagner et al., 2000). The point of this discussion is that it is unlikely that the 1-ms gaps between noise offset and startle onset are perceptible to the mouse, despite the evidence that it significantly affects startle. The gaps are brief, but moreover they are followed by an intense noise that must provide enormous backward masking. The assertion that they are not perceived would be very difficult to test in mice, but we could test it in human listeners who in other paradigms have about the same gap thresholds as mice. Three young adults were asked to discriminate the same 115-dB noise burst in a background noise from a noise burst that was preceded by noise offset, and we varied the duration between noise offset and startle onset. The detection threshold was 20 ms, not 1 ms. But how does noise offset inhibit startle if it is not perceived? We suspect that the effective mechanism is not one of the neural loops described by Koch and Schnitzler (1997) as being responsible for reflex modification, although their analysis is persuasive for prepulses with moderate and long lead times. Rather, we prefer the hypothesis that noise offset at short lead times has a direct and immediate effect on the efficiency of sensory neurons in the startle pathways. The contrary hypothesis, that it results from a complex mechanism that needs to detect noise offset and then exert inhibitory control over the startle pathways, composed of large fast-acting neurons with few synapses to slow down their activity, just seems impossible, given the brevity of the effective lead times.

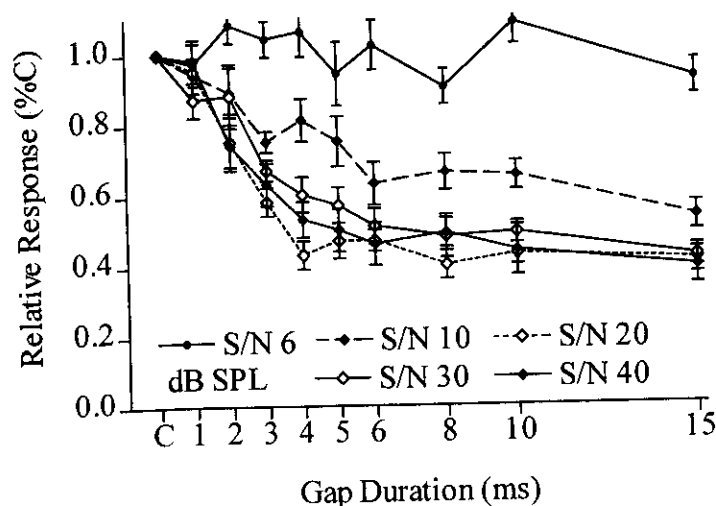


FIGURE 5.16 Mean relative startle amplitudes (\pm SEM) in a group of mice when the startle stimulus was preceded by gaps in a 70-dB background noise, the gaps having durations of 1 to 15 ms, and ending 60 ms before the startle stimulus, and the gaps being decrements in the gap of 6 to 40 dB.

The last procedure showing the effects of gaps in noise on startle inhibition is that of presenting a gap at some moderate interval prior to the startle, in this case 60 ms from gap offset to startle onset. The subjects were 12 CBA/Cal mice, 7 weeks old, and of mixed sex. The tests for gap detection were all given in a background of 70 dB, and the gaps were decrements in noise (6, 10, 20, 30, and 40 dB) of various durations (1, 2, 3, 4, 5, 6, 8, 10, and 15 ms). The experiment was run over 5 test days with different depths in the gap on each day, these separated by a rest day. The relative response data are presented in Figure 5.16. The inhibitory effects of the gaps are similar to those of noise offset, except that 1-ms gaps had no effect, and the functions for S/N values of 20 to 40 dB were equal in rate of growth and in asymptotic levels of inhibition. The 10-dB decrement provided less inhibition than the three larger S/N values, and there was no sign of inhibition with the 6-dB decrement. A similar study of the detection of partially filled gaps was reported by Forrest and Green (1987) in human listeners. They found that the duration threshold for partially filled gaps was minimally affected by the depth of the gap until it was less than about 4 to 5 dB. After this, gap thresholds increased rapidly from about 2 to 3 ms to about 30 s. Figure 5.17 is a scatterplot of gap thresholds of our mice, defining the behavioral threshold as the duration at which inhibition was at least 50% of its maximum. Mean gap thresholds were about equal for 20, 30, and 40 dB decrements, then increased at 10 dB, and were beyond 15 ms at 6 dB.

The power of "reflex modification audiometry" in the animal laboratory is seen in the similarity of these results and those of Forrest and Green. Thresholds in mice defined by prepulse inhibition approximate those measured in humans with the most skillful use of psychophysical techniques; and although there are quantitative differences in the relationship between gap thresholds and gap depth, both show that thresholds are minimally affected by filling the gap until the S/N ratio reaches about 5 dB for humans, and about 10 dB in mice. Psychophysical measures typically focus on the threshold, while reflex modification more naturally examines prepulse inhibition over a range of near-threshold and supra-threshold values. A single measure of threshold can be derived from these behavioral data, but this is not necessarily their primary virtue, as the differences in inhibition for stimuli above the absolute threshold provide useful information about their relative salience. For the present data, it is useful to know that both gap thresholds and asymptotic levels of inhibition are the same for gap decrements of 20 dB and beyond, as this provides further evidence of their perceptual equivalence (as do reaction times for stimulus detection in humans; Virag et al., 2000).

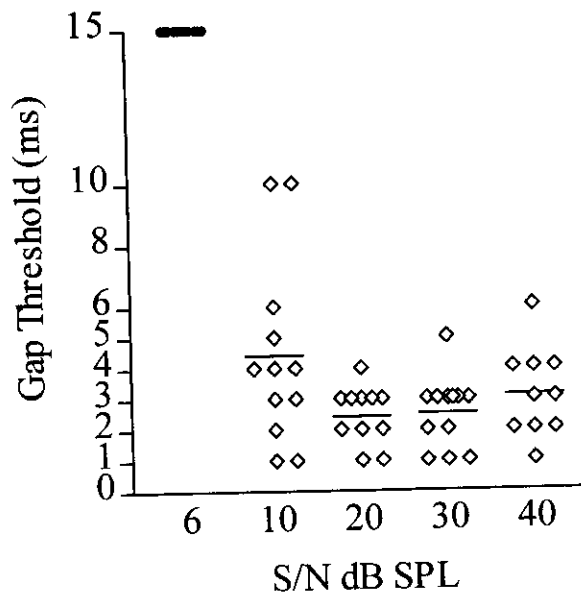


FIGURE 5.17 Gap thresholds (gaps that provide 50% of maximum inhibition) in each mouse for the gaps having different decrements, 6 to 40 dB.

CONCLUSION

We have seen that the experimental analysis of reflex elicitation and reflex modification readily yields observations comparable in their simplicity and their reproducibility to the isolated systems studied in neurophysiology. Yet in their appearance in the alert and behaving animal, they are sensitive to subtle environmental events and to the complex neural activities and their mental correlates occasioned by these events. These procedures have been applied to a range of scientific problems of great theoretical and practical importance. They have provided a unique experimental approach to the description and understanding of basic sensory and perceptual phenomena in laboratory animals, to the investigation of attention and emotionality, and to behavioral and neural plasticity. In addition, the deficit of prepulse inhibition in schizophrenia (Braff et al., 1978) has encouraged the development of an animal model of "sensory-gating deficits" based on changes in prepulse inhibition. This has extended research on startle reflex modification to animal rearing experiments and to studies of anatomical and neurochemical changes in the brain and pharmacological interventions that characterize human psychosis and its treatment (e.g., Swerdlow et al., 1999).

Raymond Dodge (1931) wrote that the study of the varieties of inhibition in "normal human life ...should serve as the connecting link between the nerve muscle preparation and the more elaborate processes with which the psychologist is chiefly concerned." Contemporary work has underscored the value of this linkage, going beyond the nerve muscle preparation of the physiology of that time to contemporary pharmacology and neurochemistry and neurophysiology, and now, in a new chapter, in its connection to molecular biology (Geyer, 1999). A major scientific challenge laid down by the molecular biologist is for neurobiology to uncover the functional significance of different forms of genetic expression in the brain, a new path of research that will enormously illuminate the molecular structure of the brain and its development, with great significance for all forms of normal brain function and brain function in disease and in psychopathology. The tools for this research are best provided in the mouse, in inbred strains, and in transgenic and knock-out mice, for which much genomic evidence is now available and much more soon to follow. The final

consequence of brain activity is behavior and "the more elaborate processes with which the psychologist is chiefly concerned" (Dodge, 1931). The basic research described here in the mouse shows the power of the reflex elicitation and reflex modification procedures in uncovering stable behavioral phenomena with rich theoretical implications, procedures that are readily applied to the analysis of important aspects of normal perceptual, emotional, and cognitive activities in humans and in laboratory animals, to the study of aging in the auditory system, and to psychopathology. It can be expected that the continued development of these mouse models and their application will provide major contributions to our description and understanding of these complex neurobehavioral activities, of their physiological bases, and of their foundations in molecular biology.

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