A Dissertation

entitled

Comparison of Auditory Thresholds obtained with a Conditioned and
Unconditioned Response in Rats

by

Jennifer E. Lee

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Dr. Henry Heffner, Committee Chair

Dr. Stephen Christman, Committee Member

Dr. Stephen Hodge, Committee Member

Dr. Y.T. Lee, Committee Member

Dr. Jason Rose, Committee Member

Dr. Patricia R. Komuniecki, Dean
College of Graduate Studies

The University of Toledo
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There are two types of behavioral approaches for determining if an animal can hear a sound. One is to observe an animal’s spontaneous response (reflex) to the sound; the other is to train (condition) it to respond to the sound. Because reflexive-response thresholds are typically not as low as conditioned-response thresholds, the question of whether the two thresholds are systematically related emerged. The purpose of the current research was to compare absolute thresholds for pure tones in rats obtained with a sensitive unconditioned procedure, prepulse inhibition, to thresholds obtained using a versatile conditioning procedure, conditioned suppression/avoidance. The results have practical and theoretical interest. Practically, we have found that thresholds obtained with prepulse inhibition, though less sensitive, can closely approximate those obtained with conditioned suppression/avoidance if the animals are tested in the same sound field and thresholds are determined by the Linear Interpolation method. Theoretically, these results provide insight into the sensitivity difference between conditioned and unconditioned responses.
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Chapter One

Introduction

Auditory Psychophysics

Animal auditory psychophysics aims to determine the best sensitivity of which animals are capable. Such information allows for comparisons not only between species, but for comparisons between disease states and drug effects within a species. Ideally these determinations would be very fast and require no experience of the subject or the experimenter. However, in audition, responses to sounds that occur naturally—reflexes—do not usually occur to soft sounds, and even become unreliable to much louder sounds upon repeated exposure. Thus, the responses that are easiest to elicit are not the most reliable, nor do they provide the most sensitive measures. For over a century, investigators have sought ways to elicit responses to sounds that occur reliably over long periods, and that occur to the stimuli with the least energy that the ear and nervous system can detect. In nature, such responses occur to sounds that have consequences for survival. Equivalent consequences can be imposed in the laboratory so that a subject becomes motivated to respond consistently to every instance of a sound. These consequences—reinforcers for responding to a sound and punishers when a response is absent—come at the cost of time to train the animal in the nature of the required responses and contingencies. In contrast, unconditioned responses to sound—such as the startle of an animal to a sudden noise—are elicited without the need to spend time in training, but come at the cost of inconsistent responses that require greater stimulus energy for their elicitation.
Thus the tradeoff between unconditioned and conditioned procedures has been between speed and ease versus time and accuracy. To maximize accuracy with a fast procedure, investigators have sought to improve the validity of the unconditioned procedures, as well as the time and simplicity of execution of the conditioned procedures. Currently, among unconditioned procedures, prepulse inhibition—using a low-level sound to inhibit a startle reflex—is a promising approach and in common use. Among the conditioned procedures, conditioned suppression/avoidance—training an animal to cease a response—has proved versatile and reliable as well as one of the more rapidly learned. It is these two representatives of conditioned and unconditioned procedures that are the focus of this study. First a description of each and its use in the study of hearing are warranted.

**Conditioned suppression/avoidance.** Conditioned suppression/avoidance is a procedure in which an animal is trained to make a simple, natural response to receive a reward of food or water. Sporadically, a stimulus is paired with mild electric shock delivered through the reward spout. The animal learns to avoid the shock by breaking contact with the spout whenever it detects the stimulus. The breaking of contact with the spout is then used to indicate that the animal detected the stimulus. In this procedure, each kind of response, breaking or not breaking contact, is followed by a consequence that depends on the stimulus presented (Table 1).

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1 The following sections provide a detailed background of the prepulse inhibition and conditioned suppression/avoidance procedures. Readers already familiar with these may proceed to the rationale for the current investigation, which begins on page 22.
When a sound is presented and the animal breaks contact with the spout, it has avoided punishment, and this is considered a hit. When a sound is not presented and the animal breaks contact with the spout, it loses an opportunity to receive reinforcement, and this is considered a false alarm. When a sound is presented and the animal does not break contact with the spout, the animal receives a mild electric shock, and this is considered a miss. When a sound is not presented and the animal does not break contact with the spout, it gains an opportunity to drink water freely and this is also a correct response. Because it combines punishment with positive reinforcement, this procedure allows for good control over an animal’s performance. Too low a hit rate can be corrected by increasing the shock level; too high a false alarm rate can be corrected by reducing the shock level and/or increasing the rate at which the reward is delivered. Therefore, contingencies can be adjusted to elicit maximum performance even to stimuli of low intensity. Of course, there comes a point at which physical and physiological limits are reached: when the intensity of the signal is reduced to a point at which hit rate and false alarm rate are not distinguishable from one another and performance cannot be improved, even with stronger contingencies (e.g. higher reward and punishment levels). This limit indicates that the animal can no longer detect the stimulus. Conditioned suppression/avoidance has proven useful to assess sensory and perceptual abilities in a wide variety of

<table>
<thead>
<tr>
<th>response</th>
<th>sound</th>
<th>no sound</th>
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<tr>
<td></td>
<td>HIT (avoid shock)</td>
<td>FALSE ALARM (lose opportunity for reward)</td>
</tr>
<tr>
<td>no response</td>
<td>MISS (shocked)</td>
<td>CORRECT REJECTION (reward)</td>
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Table 1. Conditioned Suppression/Avoidance Response Contingencies
animals, including those compromised genetically or by brain damage due to lesions or chemicals. It is also useful for other discriminations, such as sound localization or frequency discrimination.

**Prepulse inhibition.** Prepulse Inhibition, on the other hand, is a procedure in which an animal’s reflexive movement to a startling stimulus is measured and compared to its movement when the startling stimulus is preceded by a weaker stimulus. The startle reflex of the animal is reduced (or inhibited) when a stimulus of relatively low intensity—the prepulse—directly precedes the startling stimulus, hence the name “prepulse inhibition”. The percentage decrease in magnitude of the startle reflex is then used to indicate that the prepulse was detected. For example, if an animal can hear the prepulse tone (e.g. 500 Hz pure-tone at 80 dB SPL), its startle to the following startling stimulus (e.g. white noise at 115 dB SPL) will be less than if there were no prepulse tone presented. If the animal cannot hear the prepulse tone, its startle to the following startling stimulus will be similar to baseline. In this procedure, there are no consequences for the animal’s involuntary responses to the sounds that are presented. Since there is no training involved, prepulse inhibition has been used to quickly assess perceptual abilities in a variety of animals, including species that are difficult to train (e.g. reptiles, amphibians). Although this procedure has been used to determine absolute thresholds in animals, it is limited to this purpose and cannot be used for discriminations between stimuli (e.g. sound localization—left vs. right sounds).
Chapter Two

Hearing

The Study of Hearing with Operant Conditioning and Prepulse Inhibition

A review of the results obtained with these two methods in prior research will help us see weaknesses in the methods and gaps in our understanding of why their results differ.

**Operant conditioning thresholds.** An audiogram can be obtained with conditioned suppression/avoidance, by training an animal to stop drinking when it hears a sound to avoid shock (Heffner & Heffner, 1995). This is a form of associative learning, during which an animal must attend to the sound and emit the voluntary response of breaking contact with the reward spout to avoid the aversive consequence of shock.

Kelly and Masterton (1977) and Heffner, Heffner, Contos, and Ott (1994) used this technique to obtain hearing thresholds for rats. Kelly and Masterton’s (1977) audiogram of the albino rat and Heffner et al.’s (1994) audiogram of the hooded Norway rat are nearly identical (Figure 1). Both audiograms show the same structure, except for a slightly dissimilar “notch” in the middle range of frequencies. Both audiograms show the animal’s best hearing at 8 kHz and 32-38 kHz. Between 1 kHz and 8 kHz, and above 32 kHz, the audiograms vary by less than 3 dB. There are also some minor differences between the two audiograms: the low-frequency limits at 60 dB SPL are just below 530
Hz for the hooded rats and 400 Hz for the albinos, and the high-frequency limits are 68 kHz for the hooded rats and approximately 76 for the albinos. This slight variation at the extremes could be due to strain differences, sound field calibration, or age differences between the two groups of subjects—particularly since hearing loss with age is first apparent and most obvious at the highest audible frequencies. Overall, the similarity of the two audiograms is impressive, especially considering they were done years apart, using very different equipment as well as slightly different test parameters (e.g. signal duration, duration of required response, performance calculation). This similarity
contrasts markedly with the differences observed between conditioned and unconditioned procedures that will be discussed later (e.g. Figure 6).

Thresholds obtained with conditioned suppression/avoidance are not only similar to one another when obtained in different circumstances, but are similar to those obtained with other conditioning procedures. Figure 2 shows good agreement between the audiograms of the big brown bat, obtained using the conditioned suppression/avoidance procedure (Koay, Heffner, & Heffner, 1997) and a go/no-go procedure (Dalland, 1965).
Figure 3 illustrates similar audiograms of the guinea pig, obtained using a positive reinforcement procedure (Prosen, Peterson, Moody, & Stebbins, 1978) and the conditioned suppression/avoidance procedure (Heffner, Heffner, & Masterton, 1971). Conditioned suppression/avoidance, go/no-go, and positive reinforcement procedures are all operant conditioning methods that require the emission of a voluntary, learned response by the animal. In each procedure there are contingencies upon the behavior of the animal, allowing access to reward for correct responses and/or punishment for incorrect responses. Although the rewards and punishers differ in these three different
operant methods, the important point is that there are at least moderate contingencies that
serve to maintain a relatively high hit rate while keeping false alarms and misses
relatively low. The result is good agreement between audiograms obtained with these
operant procedures and thresholds that reveal much greater sensitivity than those
obtained via unconditioned procedures (as discussed in the next section).

**Prepulse inhibition thresholds.** Prepulse inhibition has also been used to obtain
behavioral pure-tone auditory thresholds, even with animals that have been otherwise
untestable. When an animal hears the prepulse tone, its startle to the following stimulus
is partially or completely inhibited. The reduced startle is the response that indicates
detection. Conversely, if the animal does not hear the prepulse tone, then its startle
response will be similar to the startle response when silence precedes the startle stimulus
(baseline). Besides laboratory mammals, this technique has been used to obtain a
behavioral measure of absolute auditory sensitivity of two species of frogs (Simmons,
Moss, & Daniel, 1985). The authors used a mild electric shock as the startling stimulus
and pure tones as the prepulse stimuli. Their reflex audiograms indicate that the bullfrog
can hear from 100 Hz to 3.2 kHz and the green tree frog from 100 Hz to 5 kHz. Due to
the lack of other behavioral methods, the authors compared their results to those obtained
from recordings of multiunit activity from the auditory midbrain of the bullfrog (Figure
4).

Although this study demonstrates that prepulse inhibition can be used with
amphibians, for which other behavioral techniques have not worked, there is no operant
conditioning audiogram for any amphibian against which to validate these results.
Furthermore, since the frog thresholds obtained with prepulse inhibition were compared to those obtained with neural response measures, little can be concluded about the significance of any differences in the results: neural responses and behavioral responses do not purport to measure the same thing. This is evident in the fact that the prepulse inhibition thresholds are 10-20 dB lower than the neural response thresholds at the same frequencies at the auditory midbrain for the bullfrog. The absence of other behavioral measures of hearing for frogs emphasizes the importance of developing procedures that can be used with animals such as amphibians and reptiles. The possibility of using an
unconditioned procedure on animals in these classes—such as prepulse inhibition—would allow for the study of a more diverse array of animals than is possible with the use of operant methods alone: operant methods are useful for obtaining audiograms on mammals and birds, but the auditory apparati of amphibians and reptiles vary markedly within their respective orders and are quite different from those of animals in other classes. However, to evaluate the validity of prepulse inhibition-obtained auditory thresholds, we must turn to studies of animals for which operant conditioning thresholds have been obtained, such as rats and guinea pigs.

Young and Fechter (1983) used the prepulse inhibition technique to obtain pure-tone detection thresholds for hooded rats and guinea pigs. The startling stimulus was a 20 ms white noise burst at 115 dBA. The prepulse tones were also 20 ms in duration and consisted of nine frequencies from 2.5 to 40 kHz in 1/2-octave steps. The mean amplitude of the startle response to each frequency and intensity was analyzed as a percentage of the mean startle amplitude on control trials of the same block within a session (each session consisted of 880 trials split into four blocks of 220 trials; each block included 40 control trials). The authors compared the thresholds they obtained to Kelly
and Masterton’s (1977) audiogram for the rat (Figure 5), and to Prosen et al.’s (1978) audiogram for the guinea pig (Figure 6), both obtained with conditioning procedures. In a later study, the same authors used the same technique and obtained more thresholds for the rat to compare methods of threshold calculation (Fechter, Sheppard, Young, & Zeger, 1988). The authors reported that their prepulse inhibition thresholds agree relatively well with the previously published operant conditioning audiograms regarding the shape of the curve.
However, upon closer examination and comparison to additional operant audiograms, important differences between prepulse and conditioning thresholds are evident. One difference between prepulse inhibition thresholds and the operant conditioning audiograms is that the prepulse inhibition curves show thresholds often more than 10 dB higher, with the degree of difference irregularly distributed across frequency. Figure 7 shows that the prepulse inhibition thresholds for the guinea pig are high in comparison to both of those obtained with operant conditioning procedures (and inconsistently so). For example, at 16 kHz the prepulse inhibition threshold is about 15
dB above operant-obtained thresholds, while at 32 kHz the prepulse inhibition threshold appears to be only 5 dB higher.

Not only do the prepulse inhibition curves differ from the operant-obtained curves, but the prepulse inhibition curves for the rat differ unsystematically from one
Figure 8 shows all three prepulse inhibition audiograms compared to both operant conditioning audiograms for the rat. Some frequencies show almost no difference in threshold while other frequencies appear to be over 20 dB different from those obtained with operant conditioning. For example, at 40 kHz the prepulse inhibition thresholds are about 30 dB different from one another, while at 7 kHz there is only a 10 dB difference among the prepulse thresholds. If the sensitivity differences were consistent—whether between the prepulse and operant thresholds or among the prepulse thresholds themselves—and the audiograms obtained showed the same shape (curve), then the difference might be attributable to equipment variation (i.e. acoustic calibration, standing waves, speaker placement) or stimulus parameters. For example, the first rat prepulse inhibition study (Figure 8 line 1: Young & Fechter, 1983) used brief (20 ms) tones that would be expected to produce 10-15 dB higher thresholds than the 200 ms tones used in the second prepulse inhibition study—200 ms is a duration that should support maximum detectability (Dykman & Ison, 1979). However, the second study (Figure 8 lines 2 & 3: Fechter et al., 1988) did not result in consistently lower thresholds than those in the first study, suggesting that the differences among the prepulse inhibition thresholds were not likely due to stimulus parameters.

Another difference between the prepulse inhibition and operant conditioning thresholds for the rat is the lack of a “notch,” a sharp decrease and then increase in sensitivity at the mid-range of frequencies often seen in the behavioral audiograms of mammals; again, the general shape of the curve derived with prepulse inhibition does not match operant-obtained audiograms. For example, new world monkeys show a decrease
in sensitivity in the 4 kHz region (Coleman & Colbert, 2009); Egyptian fruit bats in the
32 kHz region (Koay, Heffner, & Heffner, 1998). The “notch” is an important component
of hearing in some animals as it takes place at the frequencies that seem to be selectively
filtered by the pinnae as a function of elevation (Wotton, Haresign, & Simmons, 1995).

The size of the pinna, the selective interference induced by the tragus and configuration

Figure 8. Thresholds for the laboratory rat obtained via prepulse inhibition (1 from Young &
Fetcher, 1983; 2 & 3 from Fetcher et al., 1988). The shaded area represents the range of
thresholds from the two operant conditioning procedures: conditioned suppression (Kelly &
Masterton, 1977) and conditioned suppression/avoidance (Heffner et al., 1994).
of folds of the pinna, and the angle of the speaker to these physical properties of the pinna all determine the intensity of the sound that reaches the auditory meatus. The “notch” appears as a departure from the general shape of the audiogram, and this change in sensitivity occurs in a narrow range of frequencies; it is well defined when thresholds are obtained for frequencies that are close together (e.g. 10, 14, 16, and 20 kHz) and less obvious when thresholds are taken at octave intervals (e.g. 8 and 16 kHz). The “notch” was apparent in the thresholds determined by the second prepulse inhibition study (Figure 8 lines 2 & 3: Fechter et al., 1988), however, it did not exist in the thresholds determined by the same authors using the same method 5 years earlier (Figure 8 line 1: Young & Fechter, 1983). We would have expected to see such a decrease in hearing sensitivity at 18 kHz (the frequency tested in the prepulse inhibition studies) because it is very close to the 16 kHz center frequency of the “notch” seen for these animals in operant-obtained audiograms (see Figure 9). Depending on the relative orientation of the speaker to the pinnae, the audiograms of all animals with pinnae should show this “notch,” especially if localization using pinna cues—and discriminating elevation in particular—is important to the species.

In sum, not only are there stark and inconsistent differences in the absolute thresholds obtained with prepulse inhibition and operant conditioning, but a crucial component of hearing for rats—the “notch” that results from pinna directionality—is not visible in the shape of the curve derived with prepulse inhibition. These differences have led researchers to believe that unconditioned responses (such as the startle response in prepulse inhibition), although providing a quick and dirty method, might not be sufficient
indicators of what an animal can hear. Although prepulse inhibition and operant conditioning methods are very different, the threshold differences found up to this point do not necessarily indicate that the use of unconditioned responses itself will always result in higher and less reliable thresholds than the use of conditioned responses. For one thing, the prepulse inhibition studies did not include complete audiograms—that is, thresholds were obtained only for frequencies in the middle range of the animals’ hearing—the entire hearing range was not revealed. Specifically, at 60 dB SPL, a standard used to define an animal’s hearing range, the hooded rat hears from 530 Hz to 68 kHz, a range of 7 octaves. The frequencies tested with prepulse inhibition are from

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**Figure 9.** Behavioral audiograms of the laboratory rat generated using a conditioned suppression (Kelly & Masterton, 1977) and conditioned suppression/avoidance (Heffner et al., 1994) procedure. The “notch” is circled and the frequencies tested with conditioned suppression/avoidance specified.
2.5 kHz to 40 kHz, only a 4-octave span. This limited range of testing makes both the rat and guinea pig audiograms obtained with prepulse inhibition difficult to assess: although the threshold differences are obvious, the reasons for those differences are not.

**Potential reasons for differences between thresholds obtained with prepulse inhibition and operant conditioning.** When speculating about why the prepulse inhibition and operant conditioning studies have produced such different results, it is important to distinguish between *acoustic factors* and fundamental *behavioral factors* inherent in the methods themselves.

One possible explanation for the absolute threshold differences found with prepulse inhibition is that the acoustic parameters were different from those in typical operant conditioning studies. The animals in the prepulse inhibition studies were able to move freely in their cages during testing. The animals’ heads and ears were therefore not in a fixed position in the sound field, making it impossible to specify the intensity of the signal available to the animal. This issue is especially critical because the chambers used in the prepulse studies were not large enough to simulate free-field conditions. Small, echoic chambers allow for the reflection, and thus interference, of two sound waves. This can lead to acoustic “dead spots” and standing waves, which change phase upon reflection off a surface: all phenomena that will allow the intensity to vary in different parts of the sound field through which the animal can move to maximize detectability of a sound. This may not affect the test in the prepulse situation since there is no motivation to maximize detection as there is in operant methods, however, it would compromise the ability of the experimenter to specify the true amplitude of the signals. Another acoustic
difference in the prepulse inhibition studies was the speaker placement. The speaker that
played the startle-inducing white noise was mounted on the sides of the animals’ cages,
and the prepulse speaker—playing the pure-tones to be tested—was mounted above. In
operant conditioning studies, the speaker that plays the pure-tones to be tested is placed
in front of the animal so that the sound is directed at the animal’s ears. This, along with
fixed head placement, is to better-specify the signal intensity that reaches the auditory
meatus. These acoustic differences in the practice of prepulse inhibition are not essential
aspects of the method itself, but might account for the observed threshold differences
between prepulse inhibition and operant conditioning studies.

Another possible explanation for the threshold differences between the prepulse
inhibition and operant conditioning studies is in the inherent differences between the two
methods. Operant conditioning is a form of learning, in which a response that occurs
with some minimum frequency is made to occur more frequently by following it with
reinforcement. The reinforcement elicits motivation to respond correctly and the
punishment that follows incorrect responses elicits motivation to avoid errors. In
contrast, prepulse inhibition involves unconditioned responses. Unconditioned responses
are hard-wired neural reflexes that are not conditioned one way or another; there is no
learning involved in the elicitation of an unconditioned reflex (for which there are no
consequences). For instance, the startle of a naïve rat to a loud sound will result from the
first presentation of that sound\(^2\). On the other hand, a naïve rat in an operant conditioning procedure will not, for example, press a lever for food when it hears a sound until several trials have allowed the animal to learn that response contingency. Furthermore, unconditioned reflexes have been measured in anesthetized animals and after the removal of the cerebral cortex, which is thought to play a vital role in the retention of learned relationships and attention (Forbes & Sherrington, 1914; Granon, Hardouin, Courtiere, & Poucet, 1998; Grossberg, 1995; Ison, McAdam, & Hammond, 1973). Hence unconditioned reflexes are not subject to motivation to respond correctly to a stimulus since there has been no necessary learning of any stimulus-response relationship. By contrast, conditioned responses require attention and motivation. Because operant conditioning involves the use of reinforcement, such as the reward of food or water, and/or the punishment of electric shock, an animal undergoing this procedure must be both attentive (to the stimulus in question) and motivated (to obtain reinforcement and/or avoid punishment). An animal undergoing a prepulse inhibition procedure is not likely motivated to attend to the stimulus since there are no consequences for its behavior.

Behavioral and physiological studies of animals indicate that attention enhances detection and reaction time, increases arousal, and even increases the sensitivity of individual neurons (Corbetta, Miezin, Dobmeyer, Shulman, & Peterson, 1990; Moran & Desimone,

\(^2\) This is not to say that startle magnitude will not change with repeated presentations of the startling stimulus. Habituation will occur: startle on initial trials is greater than startle on trials at the end of a session. However, inhibition of that startle by a prepulse—the reduction in startle to a loud noise by a preceding tone—will occur whether baseline startle has habituated or not. Prepulse inhibition testing thus requires that baseline startle magnitude be obtained frequently throughout the session so prepulse-inhibited startles can be compared to uninhibited startles that occur around the same time.
Thus the motivation to respond correctly in operant conditioning procedures could explain the lower thresholds found with conditioning than with prepulse inhibition, probably through increased arousal and attention. Since unconditioned responses and conditioned responses are very different behaviors, it could be that prepulse inhibition will always elicit higher thresholds than can be obtained with an operant conditioning procedure such as conditioned suppression/avoidance.

This difference between prepulse inhibition and operant conditioning methods—the relevance of attention due to the motivation to respond correctly—might account for the differences between the thresholds that have been obtained with each procedure. This is a tempting conclusion. However, because of the acoustic differences among the published studies (i.e., uncertain signal intensity at the auditory meatus), it is premature. With both acoustic and motivational factors as potential causes of the observed threshold differences, an exploration of results when these factors are systematically manipulated is in order.

**Current investigation.** Because prepulse inhibition uses an animal’s naturally occurring reflexes, it offers a potential advantage over conditioned suppression/avoidance and other conditioning procedures, especially for species that are difficult to train or whose physiology does not readily permit restriction of food or water. Operant conditioning requires the maintenance of motivation with consistent reinforcement, whereas the inhibitory effect of a prepulse is unaffected by weeklong rest periods or “unreinforced” exposures to the stimulus. However, the use of prepulse inhibition in the
study of hearing up to this point has not satisfied auditory psychophysicists: the thresholds obtained with prepulse inhibition have been higher than those obtained with conditioning, and even worse, these absolute threshold differences have been inconsistently distributed across frequency. Although unconditioned and conditioned responses are characteristically different, the reasons for the differences in thresholds obtained via each may or may not be due to the nature of the responses themselves. They may rather be due to acoustic factors, such as sound level measurement, individual differences, such as the use of different animals across studies, or even incomplete testing in prepulse procedures (which did not test the entire hearing range of the animals). The possibility that the observed threshold differences are due to essential behavioral differences in the methods (i.e. attention and motivation) has been mere speculation up to this point.

In this study, we have held the acoustic factors constant and compared the two behavioral methods directly. Since the same animals were tested in the same acoustic environment with each procedure, a difference in the thresholds obtained via each has made it possible to conclude whether such a difference is due to an inherent difference between the two methods. This study sought to determine whether there is a systematic relationship between prepulse inhibition and conditioned suppression/avoidance thresholds across frequency, how the magnitude of this difference compares to that in previous studies, and whether the difference between them can be reduced by using the same test parameters such as signal duration and head placement in both procedures. The acoustic factors were controlled by modifying the prepulse inhibition and conditioned
suppression/avoidance techniques so that they resemble one another. First, unlike previous prepulse inhibition studies, the entire hearing range of the hooded rat was tested and a complete audiogram was obtained with both methods. The prepulse speaker was placed in front of the animal so that it was directed at the animal’s ears in both procedures. The pure-tones consisted of one pulse of the same duration in both procedures, to avoid the effects of multiple pulses (typically used in conditioned suppression/avoidance) and temporal integration (as in prior prepulse studies). The animals were deprived of water and drinking from a waterspout during testing so that their heads stayed in a fixed position to allow for accurate sound measurements in both procedures. This also made the physiological state of the animal (thirsty) similar in both procedures (which has not been the case in previous prepulse studies). Lastly, the same animals were tested using identical equipment and sound-field parameters with both methods, although the conditioned suppression/avoidance thresholds were obtained last and some of the high frequency thresholds may have been affected by age-related hearing loss.
Chapter Three

Method

Subjects

The subjects were three young adult male Long-Evans hooded rats (Sprague-Dawley). For the prepulse inhibition procedure, the rats were 10 months old at the start of testing and 18 months old at the end of testing. For conditioned suppression/avoidance, the rats were 18 months old at the start of testing and 26 months old at the end of testing. All subjects were maintained on a 12-h light-dark cycle in the animal colony room with *ad libitum* access to food in their home cages and received their water during the test sessions. Supplements of water or apple were given as needed to maintain a healthy body weight. Rats were tested daily during the early evening hours when they are normally active. The use of animals in this study was approved by the University of Toledo Animal Care and Use Committee.

Behavioral Apparatus

All testing was conducted in a carpeted audiometric booth (Eckel AB-200EG; 1.02x0.81x1.75 m), the walls and ceiling of which were lined with egg-crate foam. The equipment for stimulus generation and behavioral measurement was located outside the chamber and the rats were observed via closed-circuit television. Within the chamber was an animal test cage (37x22x23 cm) constructed of 1.26-cm wire mesh, mounted on top of a stabilimeter. The stabilimeter consisted of a digital weigh scale (Mainstays 33615) with a voltage output. The voltage output of the stabilimeter was routed to an oscilloscope (Tektronix TDS210) located outside of the sound chamber. Two speakers
were placed in the sound chamber: the pure-tone (prestimulus) speaker was mounted approximately 46 cm in front of the cage, and the white noise (startle) speaker was mounted approximately 46 cm above the cage. Both the test cage and stabilimeter were placed at the height of the animal’s ear level. An abrupt voltage change (startle reflex magnitude) was triggered and read from the oscilloscope (Tektronix TDS210). A waterspout (2 mm diameter brass tube, topped with a 4x6 mm oval lick plate) was mounted vertically such that it projected into the front of the cage at 8 cm above the floor. The spout was attached, with plastic tubing, to a 60-cc water pump (NE 1000, New Era, Wantagh, NY) that was housed outside the chamber so prevent the noise of its operation from disturbing the animal. During testing, a rat drank from the waterspout, the tip of which was at the height of the animal’s mouth and 1 cm in front of the test cage to minimize obstructions between the animal’s ears the loudspeaker. A contact circuit was connected between the waterspout and bottom of the test cage to detect when the animal contacted the spout and to activate the water pump. Requiring the rat to maintain mouth contact with the spout served to keep its head in a fixed position within the sound field.

For the conditioned suppression/avoidance procedure only, a shock generator was connected between the waterspout and test cage. The shock was adjusted for each individual to the lowest level that produced a consistent avoidance response, which consisted of breaking contact with the waterspout. Shock levels ranged from 35 V (0.18 mA) to 74 V (0.34 mA). A 25-W light, mounted 0.5 m below the cage, turned on and off with the shock to provide feedback for a successful avoidance and to indicate when it was safe to return to the spout. A schematic drawing of the general testing apparatus for both
procedures is shown in Figure 10.

**Acoustical Apparatus**

Pure-tone prestimuli, generated by a signal generator (Zonic A&D 3525), were gated through a rise/fall gate (Coulbourn S84-04) to provide a 200 or 300 ms signal with rise/fall times of 20 or 50 ms, respectively. Different signal durations and rise/fall times were used depending on the frequency (number of cycles per second): this was to make sure that there were at least 10 cycles in the rise/fall duration for any frequency (fewer cycles per second in the signal means a longer rise/fall time is required to include 10 cycles) to prevent onset and offset clicks. Specifically, 250 and 500 Hz were presented...
for 300 ms with a 50 ms rise/fall, and the rest of the frequencies (1 to 70 kHz) were presented for 200 ms with a 20 ms rise/fall. Signal intensity was controlled by an attenuator (Hewlett Packard 350D) and attenuated as needed for threshold determination. The output of the attenuator was fed to an amplifier (Crown D75), monitored with an oscilloscope (Tektronix TDS210), and routed to the tone (prepulse) loudspeaker in the sound chamber. A ribbon tweeter speaker (Panasonic EAS-10TH400C) was used for high frequencies (2 kHz and above) and a woofer (Infinity RS2000) for low frequencies (below 2 kHz).

The startling stimulus consisted of a single, abrupt (0 ms rise-fall) 20 ms white noise burst at an intensity of 115 dB SPL. White noise startle bursts were generated by a noise generator (Coulbourn S96-03), and fed through an amplifier (Radio Shack MPA 200) to a piezoelectric speaker (Motorola KSN1000) mounted 46 cm above the test cage. Thresholds were obtained for all rats at the following frequencies: 0.25, 0.50, 1, 2, 4, 8, 16, 32, 64, and 70 kHz. Unattenuated levels of these pure tones varied as a function of frequency and of the particular speaker involved. Selection of the frequencies for testing was based on the existing behavioral audiograms obtained for these animals and thus allowed comparison to the previous studies that used different methodologies.

**Sound Level Measurement**

Sound level measurements were taken by placing the microphone in the position normally occupied by the rat’s head and ears while it drinks from the spout, and pointing

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3 One of the frequencies tested—16 kHz—lies in the center of decreased sensitivity (the “notch”) typically seen for these animals. The threshold at 16 kHz was thus expected to be higher than both 8 kHz and 32 kHz (see Figure 10).
it directly at the loudspeaker. Prestimulus and startle intensities were measured daily using a 6.35 mm microphone (Bruel and Kjaer 4939), corrected for free-field with the protection grid on, with a preamplifier (Bruel and Kjaer 2669) and measuring amplifier (Bruel and Kjaer 2608). The output of the measuring amplifier was then routed to a spectrum analyzer (Zonic A&D 3525) to monitor the speaker output for harmonics or distortion. Care was taken to produce a homogenous sound field (within +/-1 dB) in the area that was occupied by the animal’s head and ears as it drank from the spout.

Prepulse Inhibition Procedure

A thirsty rat was accustomed to drinking from the waterspout. Trials consisting of the presentation of the prepulse and startle sounds (prepulse trials), or the startle sound alone (control trials), were only initiated when the rat was in contact with the spout. Requiring the rat to maintain contact with the waterspout served to orient it toward the loudspeaker and activate the water pump to dispense a steady trickle of water.

The two trial types (prepulse and control) alternated such that every other trial was a control trial and every other trial was a prepulse trial. On control trials, the startle stimulus was presented, and a rat’s whole-body startle amplitude was monitored for a 50 ms period, the onset of the recording period coinciding with the onset of the startling stimulus. On prepulse trials, a pure-tone prestimulus, as described above, was presented beginning either 300 or 400 ms before the noise burst (depending on the stimulus duration), such that there was a 100 ms interstimulus interval between prestimulus offset and startle noise onset. The interstimulus interval was selected because it was found to be optimal in producing inhibition of the rat’s startle reflex (Hoffman & Ison, 1980;
Young & Fechter, 1983). Trials were separated by time-locked intertrial intervals of 10 s, such that the animal had to be in contact with the spout for 10 s before a trial was administered, and the 10 s started immediately when the animal returned to the spout after a trial was finished. The intertrial interval was selected because it was found to be optimal in producing inhibition of the rat’s startle reflex and the most consistent startle magnitude across trials during pilot testing. Each test session included one frequency that was tested at several intensities such that each intensity included enough trials (about 15) for statistical analysis.

The intensity of the tones was successively reduced (in 5 or 10-dB steps) beyond the point at which the rat no longer demonstrated inhibition of the startle response (i.e. when the voltage change on prepulse trials did not differ significantly from the voltage change on control trials, p>.01). This included the testing of intensities from above to below previously published thresholds. During testing, threshold was estimated as the intensity at which there was no difference between prepulse and control startle magnitudes. Testing was considered complete for a particular frequency when the threshold estimates, obtained in at least three different sessions, were within 3 dB of one another (which typically took about 7 sessions). Once all frequencies were tested, certain frequencies were selected (based on any discrepancies between the obtained thresholds and those of previously published audiograms) and rechecked to ensure reliability.

**Conditioned Suppression/Avoidance Procedure**

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Although overall startle does habituate throughout a session, the difference between initial startle amplitude and later startle amplitude within a block of trials (about 15 for each intensity) is negligible: startle amplitudes for a block of trials are not significantly different (p>.05).
After the prepulse inhibition procedure was complete, the subjects were tested on the conditioned suppression/avoidance procedure. The rat was trained to break contact with the spout whenever it detected a tone to avoid impending shock (Heffner & Heffner, 1995).

Test sessions were divided into 2 s trials separated by 1.5 s intertrial intervals. Approximately 22% of the trial periods contained a tone pulse (warning signal), whereas no sound was presented in the remaining trial periods (safe signal). The warning trial consisted of a single 200 or 300 ms (depending on frequency as described above) pure-tone pulse and was followed by a mild electric shock 1.8 s after stimulus offset. The shock (300 ms) was delivered between the spout and floor of the cage and was both avoidable and escapable. The shock light was illuminated concurrently with the shock.

A contact circuit was used to detect whether the rat was in contact with the spout during the last 150 ms of each trial. If the rat broke contact for more than half of the 150 ms response period, a detection response was recorded. This response was classified as a hit if the trial contained a tone (i.e. a warning signal) or as a false alarm if the trial was silent (i.e. a safe signal). The hit and false alarm rates were then determined for each stimulus intensity, with a single intensity presented in a consecutive block of 6-10 warning trials (with approximately 24-40 associated safe trials). Finally, the hit rate was corrected for false alarms to produce a performance measure (Heffner & Heffner, 1995) according to the formula: Performance = Hit rate − (Hit rate × False alarm rate). This measure proportionately reduces the hit rate by the false alarm rate associated with each intensity (i.e. each block of trials) and varies from 0 (no hits) to 1 (100% hit rate with no
false alarms).

The intensity of a tone was successively reduced in 5 or 10-dB steps until the rat no longer responded to the warning signal above chance (i.e. the hit and false alarm rates did not differ significantly, \( p > .05 \), binomial distribution). Threshold was estimated as the intensity at which the performance measure equaled 0.50, which was usually obtained by linear interpolation. As in the prepulse procedure, testing was considered complete for a particular frequency when the thresholds obtained in at least three different sessions were within 3 dB of one another. Once an audiogram was completed, certain frequencies were selected (based on any discrepancies between the obtained thresholds and those of previously published audiograms) and rechecked to ensure reliability. To compare, additional estimates of threshold were calculated after data collection.

**Threshold Determination**

A threshold is determined from a set of data based on 1. how threshold is defined, and 2. how that threshold is calculated mathematically. Note that the typical definition of threshold, the midway point between chance performance and perfect detection, is not an appropriate criterion in the case of prepulse inhibition—startle inhibition is not an all-or-none phenomenon. Startle amplitude psychometric functions do not mirror the typical functions relating response probability to stimulus intensity, so the 50% performance criterion for threshold used in the conditioned suppression/avoidance procedure does not apply. In fact, prepulse inhibition experiments on humans indicate that auditory stimuli resulting in 50% inhibition are much louder than detection thresholds (Reiter, Goetzinger, & Press, 1981). In this study, rats rarely demonstrated a 50% inhibition of the startle
response. Young and Fechter (1983) also found that rats rarely demonstrate more than 15% inhibition to a stimulus if they have shown any less inhibition to a stimulus of a higher intensity. Since the unconditioned responses elicited by prepulse inhibition and conditioned responses by conditioned suppression/avoidance are so different from one another, so must be the way in which threshold is defined and calculated.

**Prepulse inhibition threshold determination.** Prepulse inhibition data were first compiled into the average startle amplitude on prestimulus trials and the average startle amplitude on control trials. This was done for each intensity of each frequency for each individual animal. Both the mean and median were calculated as averages, their later use depending on the method of threshold determination employed (discussed in next section). The data were then plotted and scrutinized via functions relating the extent of startle inhibition to prestimulus intensity (e.g. Figure 11). Inhibition of the startle reflex is thus expressed as a percentage of the average startle reflex on control trials. Such functions are common to all prepulse inhibition studies (cf. Young & Fechter, 1983; Fechter et al., 1988; Simmons et al., 1985).
Threshold was then estimated for prepulse inhibition in six ways, corresponding
to six different threshold determination methods. Some of the methods differ regarding
how threshold is defined, and some only in how it is calculated. The different threshold
determination methods can be summarized as: 1. the Linear Interpolation method with a
15% criterion, where threshold is defined as the stimulus intensity producing 15%
inhibition of startle as determined by linear interpolation; 2. the Linear Interpolation
method with a 10% inhibition criterion; 3. the Spline-Fitting method, where threshold is
defined as the stimulus intensity producing 10% inhibition as determined by use of a
spline function; 4. the Statistical Significance method, where threshold is defined as the lowest intensity at which startle amplitude is statistically different from control startle amplitude (p<.01); 5. the Statistical Significance Midpoint method, where threshold is defined as the midpoint between that lowest stimulus intensity and the next higher intensity; and 6. the Function-Intersect method, where threshold is defined as the point at which the function showing prepulse startle amplitude intersects that of control startle amplitude. Each threshold determination method is described in detail below.

1. Linear Interpolation with 15% criterion. One definition of threshold for prepulse inhibition is the stimulus intensity producing 15% inhibition of startle, as determined by linear interpolation between the most intense stimulus producing less than 15% inhibition and the next higher stimulus intensity. In other words, threshold is the interpolated intensity at which the prepulse stimulus reduces the startle response to 85% of its average amplitude (Figure 11). The criterion of 15% inhibition (or 85% of control startle amplitude) was found to be most reasonable by Young and Fechter (1983) considering the variability of rat startle responses: 15% inhibition lay beyond the range of fluctuations of response amplitudes around threshold, such that there were seldom instances in which a stimulus produced less than 15% inhibition and a less intense stimulus produced more than 15% inhibition.

2. Linear Interpolation with 10% criterion. This threshold determination is identical to the one described above except that threshold is defined as the stimulus intensity producing 10% inhibition of startle (as opposed to 15%). A criterion of 10% ____________

5 A comparison of threshold determination methods and discussion of the validity and reliability of each method can be found in the discussion section.
inhibition was found to be most reasonable for threshold determination in the guinea pigs and frogs (Simmons et al., 1985; Young & Fechter, 1983). 10% inhibition for the guinea pigs was chosen by Young and Fechter (1983) for the same reason 15% inhibition was chosen for the rats as described above. Simmons et al. (1985) used the 10% inhibition criterion for both species of frogs because it represented a level at which consistent statistically significant changes in reflex amplitude from the control level were seen at all prestimulus frequencies and for all individual animals.

3. Spline-Fitting. Another way of determining threshold is with a Spline-Fitting model, as used by Fechter et al. (1988). In this case, the skewness of the data is first evaluated to choose the best measure of central tendency (i.e. median), followed by a transformation to normalize the data (e.g. Tukey square-root). A separate smooth spline function is then calculated for each animal at each tone frequency. Threshold is defined as the stimulus intensity producing 10% inhibition of startle, as determined by the intersection of the spline function with the horizontal line representing 90% of the median square-root of the control trial amplitude (Figure 11).

4. Statistical Significance. Yet another way of determining threshold is to use the lowest prestimulus intensity at which the mean startle amplitude is statistically significantly different from mean control trial startle amplitude, as determined with a paired samples t test (p<.01).

5. Statistical Significance Midpoint. Similarly, the midway point between that lowest prestimulus intensity and the next highest prestimulus intensity (having not resulted in a statistically significant difference) can be used as an estimate of threshold.
The midway point between these two intensities is interpolated.

6. **Function-Intersect**. A final threshold estimate is the point at which the psychometric function showing startle reflex amplitude on prepulse trials intersects the function corresponding to startle reflex amplitude on control trials (Figure 12). This would be using the lowest prestimulus intensity at which there was no difference in startle amplitude between prepulse trials and control trials, without converting the startle amplitudes into relative startle amplitudes or designating a percentage inhibition criterion.

![Figure 12. Example prepulse inhibition psychophysical curves. Mean startle reflex amplitude on control trials and prepulse trials for each intensity of 4 kHz for one subject. The two lines intersect where there is no difference between reflex amplitude on control trials and prepulse trials. Control trial startle amplitude and tone intensity decreases as the total number of trials increases.](image)

**Conditioned suppression/avoidance threshold determination**. Unlike prepulse inhibition functions, conditioned suppression/avoidance psychometric functions typically
show asymptotic performance up to a certain point, at which performance drops steeply to chance (Figure 13).

![Graph showing performance vs. tone attenuation](image)

**Figure 13.** Example conditioned suppression/avoidance function. Performance for each intensity of 4 kHz is calculated as hit rate - (hit rate x false alarm rate) for one subject. The dotted line at 50% performance indicates the typical criterion for threshold determination.

Threshold was determined for conditioned suppression/avoidance in three ways, corresponding to three threshold determination methods that differ regarding how threshold is defined and/or calculated. The different threshold determination methods for conditioned suppression/avoidance can be summarized as: 1. the Suppression Ratio, where threshold is defined as the stimulus intensity that is detected half the time, resulting in 50% performance as calculated with the suppression ratio; 2. the Corrected Hit Rate, where threshold is also defined as the intensity resulting in 50% performance, but performance is calculated with the corrected hit rate; and 3. Signal Detection Theory’s \(d'\), where threshold is defined as the stimulus intensity that is not detectable, as
indicated by a d’ of zero. Each threshold determination method is described in detail below\(^6\).

1. Suppression Ratio. Threshold is typically defined as the stimulus intensity that is detected half the time, which is indicated by 50% performance on a detection task. There are a number of ways to calculate performance for conditioned suppression/avoidance, one of which is with the suppression ratio: \( \frac{W}{S+W} \) (Annau & Kamin, 1961; Estes & Skinner, 1941). This ratio expresses the rate of drinking in the presence of the stimulus divided by the sum of the drinking rate in the presence and absence of the stimulus; \( W \) (warning score) is the drinking rate during tone presentation and \( S \) (safe score) is the drinking rate when there is no tone. With this formula, perfect detection results in a score of 0.0 (no drinking response) and failure to detect is 1.0 (constant drinking response). Table 2 is an example of an animal’s conditioned suppression/avoidance performance calculated with the suppression ratio.

\(^6\) A comparison of threshold determination methods and discussion of the validity and reliability of each method appears in the discussion section.
Since threshold is defined as 50% performance, the calculation of threshold then uses linear interpolation to locate the theoretical intensity at which performance would equal the midpoint between 0% and 100%.

### Corrected Hit Rate

Another way to calculate performance for conditioned suppression/avoidance is with a hit rate that is proportionately reduced by the false alarm rate: Corrected hit rate = observed hit rate – (observed hit rate x false alarm rate) (Heffner & Heffner, 1988). Unlike the suppression ratio, this calculation yields scores from 0 (failure to detect) to 1.0 (perfect detection). With threshold defined as 50% performance, the calculation of threshold then uses linear interpolation to locate the theoretical intensity at which performance would equal the midpoint between 0% and 100%. Table 3 is an example of an animal’s conditioned suppression/avoidance performance calculated with the corrected hit rate.
3. Signal Detection Theory's d'. A final estimate of auditory sensitivity that can be applied to conditioned suppression/avoidance is borrowed from signal detection theory. Hit (H) and false alarm (F) rates are taken as a pair (H, F). Consider that the perfect animal’s performance is (1,0), another animal has some other scores where H=F, and an abnormally nervous animal has (1,1). Intuitively, the best animal maximizes H (and thus minimizes the miss rate) and minimizes F (and thus maximizes the correct rejection rate). Thus the larger the difference between H and F, the better the animal’s sensitivity. The statistic d' is a measure of this difference and is calculated as the difference between the z-scores\(^7\) of these two rates:

\[ d' = z(H) - z(F) \]

\(^7\)A range of values cast as a normal distribution with standard deviations about the mean. The mean is set to 0, over 99% of values lie within 3 standard deviations of the mean. Each value is then some number of standard deviation units above or below the mean. The transformation of any value to its corresponding z-score allows for comparison of measures using different ranges of absolute values.
Table 4 depicts the performance of an animal in the conditioned suppression/avoidance procedure and how \( d' \) is calculated.

<table>
<thead>
<tr>
<th></th>
<th># trials breaks contact</th>
<th># trials maintains contact</th>
<th>total # of trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>signal</td>
<td>9</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>no signal</td>
<td>2</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

\[
d' = z(H) - z(F)
\]
\[
= z(.9) - z(.2)
\]
\[
= 1.28 - (-.84)
\]
\[
= 2.12
\]

Table 4. Example performance on conditioned suppression/avoidance and calculation of \( d' \). \( D' \) is a measure of sensitivity that ranges from 0 (no detection) to infinity.

In this case, threshold is not defined as the intensity of a stimulus such that it is detected 50% of the time. Rather, the intensity at which \( d' \) equals 0 is the intensity at which the animal cannot tell the difference between the presence or absence of a tone (i.e. the stimulus is detected 0% of the time). The calculation of threshold then uses linear interpolation to locate the theoretical intensity at which \( d' \) would equal 0.
Chapter Four

Results

Prepulse Inhibition Results

Figure 14 shows prepulse inhibition audiograms for the average of all three rats; each line representing the audiogram resulting from one of the six different methods of threshold determination. Individual data from each animal show the same relationships between the different threshold determinations as the averages (for a list of individual and average thresholds, see Appendix A). Sessions in which an animal was not drinking consistently from the waterspout or that did not contain enough trials for statistical significance were discarded from analysis, leaving a total average of 1,824 trials per animal (>180 per frequency) for threshold determination.
Thresholds are nearly identical for each of the six threshold determination methods below 1 kHz and above 64 kHz. However, between 1 and 64 kHz, the thresholds differ significantly. The Spline-Fitting method, in which each animal’s data is fit to a cubic spline function, resulted in the highest (least sensitive) overall thresholds. On the other hand, the Function Intersect method resulted in the lowest absolute thresholds: this method used the lowest intensity at which there was no difference

Figure 14. Comparison of all threshold definitions for prepulse inhibition results for the average of all three animals. Each line depicts an audiogram when threshold is defined as: the interpolated intensity at which prepulse trials result in 15% inhibition of the average startle on control trials (in blue), the same for a 10% inhibition of average startle (green), the use of a cubic spline function of the median square-root startle amplitude (yellow), the lowest prestimulus intensity at which there was no significant difference between control and prepulse startle magnitudes (red), the midway point between that lowest intensity and the next highest intensity (purple), and the intersection of the psychometric function depicting startle reflex amplitude on control trials with that of on prepulse trials (orange). A detailed explanation of how to calculate threshold with each of these definitions can be found in the methods section.
between prepulse and control startles. Each of the six methods produced an audiogram with a similar shape, except between 8 and 64 kHz. In this region, only two of the six methods showed the “notch” around 16 kHz: 10 and 15% inhibition defined threshold as the tone intensity producing 10 and 15% inhibition of startle, respectively. As expected, the Statistical Significance and Statistical Significance Midpoint methods produced audiograms that mirror one another closely in shape. These methods defined threshold as the lowest intensity at which prepulse startle is significantly different from control startle (p<.01), and the midway point between that intensity and the next highest intensity, respectively.

Prepulse inhibition demonstrates that the rat’s range of hearing, at the 60 dB level, extends from just above 500 Hz to 64 kHz. Generally, frequencies of 250 Hz and 70 kHz produced minimal reflex inhibition at intensities below 60 dB SPL, indicating that the rats’ sensory thresholds are high at those frequencies. Prepulse inhibition shows the rat’s maximal sensitivity (around 20 ±10 dB) to tones ranging from 8 to 32 kHz. From this region of maximal sensitivity, sensitivity declines at a rate (slope) of about 11 dB/octave on the low-frequency side and 16 dB/octave on the high-frequency side, although the slopes are not smooth. Generally, prepulse inhibition shows the rat’s audiogram to resemble the overall U-shaped audiogram seen in most vertebrates, with the inclusion of the “notch” typically seen for animals that have pinnae (cf. Wotton et al., 1995). A detailed comparison of the six methods of threshold determination and of the current audiogram to previously published curves generated from prepulse inhibition can be found in the discussion section.
To see if frequency affected the amount of startle reflex inhibition, the average percent inhibition was calculated for each animal at each frequency at relative intensities of 5, 10, and 20 dB above threshold (Figure 15; individual and average data shown in Appendix B). Although the average percent inhibition at these above-threshold intensities is quite variable (range = 16.6% to 44.5% inhibition, SD= 8%), frequencies from 1 to 4 kHz produced the most inhibition (33.6% to 44.5%) and frequencies from 8 to 32 kHz produced the least inhibition (16.6% to 30%), the difference being statistically significant (t=7.9, p<.0001). The 8 to 32 kHz region of the audiogram is an area of increased sensitivity, however, whether there is a relationship between this and the lower percent inhibition to stimuli above threshold at those frequencies is unknown. Recall that percent inhibition is calculated by dividing the prepulse startle magnitude (v) by control startle magnitude (v).
Figure 15. Average percent inhibition as a function of frequency for relative intensities of 5, 10, and 20 dB above threshold. The difference in inhibition between the 1-4 kHz region and the 8-32 kHz region is statistically significant, $p<.0001$. Intensities 10 and 20 dB above threshold were not tested for frequencies at which it was not possible to produce a clean signal (free of distortion and/or harmonics).

**Conditioned Suppression/Avoidance Results**

Figure 16 shows conditioned suppression/avoidance audiograms for the average of all three rats; each audiogram line representing the audiogram resulting from one of the three different methods of threshold determination. Individual data from each animal are consistent with the averages plotted (Appendix C). Sessions in which an animal was not drinking consistently from the waterspout or that did not contain enough trials for threshold calculation were discarded from analysis, leaving a total average of 304 sessions per animal (>30 per frequency) for threshold determination.
The three different methods of calculating threshold each resulted in very similar audiograms. The highest thresholds resulted from the use of the Corrected Hit Rate, where threshold is defined as 50% performance and performance is calculated by $H-HxFP$. The lowest thresholds resulted from the use of $d'=0$ to estimate threshold. The Suppression Ratio, where performance is calculated by $W/(S+W)$, resulted in absolute thresholds that were close to those from $d'=0$. The thresholds differed the least in the high end of the audiogram (above 32 kHz) and the most in the middle range of hearing (from 8 to 32 kHz), although these differences are negligible, especially in comparison to the differences between the prepulse inhibition methods.

Figure 16. Comparison of all threshold definitions for conditioned suppression/avoidance for the average of all three animals. Each line depicts an audiogram when threshold is defined as: 50% performance calculated with the suppression ratio, 50% performance calculated with the corrected hit rate, and the point at which the standardized values of hit rates and false alarm rates do not differ from one another. A detailed explanation of how to calculate threshold with each of these definitions can be found in the methods section.
These results show that the rat’s range of hearing, at the 60 dB level, extends from just below 500 Hz to 64 kHz. Frequencies of 250 Hz and 70 kHz produced minimal avoidance, indicating the highest thresholds. The rat’s maximal sensitivity is to tones of 8 kHz (around -5 dB) and 32 kHz (around 5 dB). From this region of maximal sensitivity, sensitivity declines at a rate (slope) of about 14 dB/octave on the low-frequency side (from 250 Hz to 8 kHz) and about 30 dB/octave on the high-frequency side (from 32 to 70 kHz), although the low-frequency slope is not smooth. Generally, conditioned suppression/avoidance shows the rat’s audiogram to resemble the smooth, U-shaped audiogram seen in most vertebrates, with the inclusion of the “notch” typically seen for animals that have pinnae. A detailed comparison of the three methods of threshold determination, and of the current audiogram to previously published audiograms that used conditioned suppression/avoidance, can be found in the discussion section.
Chapter Five

Discussion

Conditioned Suppression/Avoidance Thresholds

Three methods for determining thresholds from the conditioned suppression/avoidance data have been described and their results displayed. The scrutiny of any procedure for determining sensory thresholds rests in part on its logical defensibility. Thus the objective is to: A. discuss the efficacy of each threshold determination method and compare the threshold estimates to one another, and B. choose the best estimate (the most valid and reliable) to comprise the audiogram that will be compared to those of previously published studies that used conditioned suppression/avoidance to generate rat audiograms.

A. Conditioned suppression/avoidance threshold determination methods.

Suppression Ratio: \( W/(S+W) \). One problem with the use of the suppression ratio to calculate performance on the conditioned suppression/avoidance task is that it does not result in a valid threshold estimate when used for an animal that exhibits atypical behavior. Typically, as the intensity of the signal decreases and the stimulus becomes less detectable, hit rates begin to fall rapidly while false alarm rates increase only slightly. However, one animal often displayed a different pattern in which hit rates fell slightly while false alarm rates increased markedly. At times, this animal was able to maintain a high hit rate by increasing its false alarm rate to as high as 50%. When high hit rates are accompanied by high false alarm rates, the suppression ratio can give misleading results. This is illustrated by the extreme example of a hit rate of 100% (or \( W=0 \)), which will
yield a perfect performance ratio (0.0) for any false alarm rate less than 100% (or S>0).

In this case, safe scores of both 1.0 (no false alarms) and 0.01 (99% false alarms) result in a performance ratio of 0.0. This implies perfect detection, even though a safe score of 0.01 and a warning score of 0.0 are not significantly different from one another and both indicate chance performance. Table 5 presents a performance calculation using the standard suppression ratio for a nervous animal that rarely maintained contact with the spout regardless of stimulus presentation.

<table>
<thead>
<tr>
<th></th>
<th># trials breaks contact</th>
<th># trials maintains contact</th>
<th>total # of trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>sound</td>
<td>25</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>no sound</td>
<td>24</td>
<td>1</td>
<td>25</td>
</tr>
</tbody>
</table>

\[ W = \text{drinking rate during tone presentation} \]
\[ = 0/25 = 0.0 \]

\[ S = \text{drinking rate in the absence of the tone} \]
\[ = 1/25 = .04 \]

\[ \text{suppression ratio} = \frac{W}{(S + W)} \]
\[ = 0.0 \]

\[ \text{performance} = 0\% \]

Table 5. Example performance calculation using the suppression ratio. In this case the animal rarely maintained contact with the spout (regardless of tone presentation), yet the suppression ratio results in a score of 0% which indicates perfect detection.

Therefore, although it is tempting to assume that when an animal does not hear the tone it will make and break contact with the spout randomly (so that performance is at chance), this assumption is incorrect: it does not take response bias into account.

Suppose the animal in the previous example broke contact with the spout every two seconds. In this case it had a hit rate near 100%, meaning the proportion of tone presentations upon which the animal broke contact with the spout was close to perfect.
This does not mean that the animal was able to detect the stimulus—it would not have had to listen—it also had a false alarm rate near 100%. In other words, the proportion of silent trials during which the animal broke contact with the spout was also nearly as high as possible. To compare this response strategy with an opposite one, consider another animal that rarely broke contact with the spout (as happened during several testing sessions). This animal then had a correct rejection rate near 100%, but a very low hit rate. Thus, the hit rate becomes meaningful when interpreted with the animal’s response bias—its tendency to maintain or frequently-break contact with the spout. An ideal threshold calculation therefore attributes responses to a combination of sensitivity and bias: sensitivity is what we are interested in, bias is what we have to consider to reveal sensitivity accurately. Table 6 presents the lowest and highest frequencies audible at SPLs from 30 to 70 dB for each of the three conditioned suppression/avoidance audiograms. Although the suppression ratio resulted in lower absolute thresholds than the corrected hit rate, its neglect of response bias brings its validity into question. In addition, the thresholds derived from the suppression ratio were more variable than those from the corrected hit rate (SD=3.2 dB; Appendix D), bringing the reliability of the suppression ratio into question as well.
Corrected Hit Rate: H-H(FP). The false alarm rate can indicate response bias, and the corrected hit rate proportionately reduces the hit rate by the false alarm rate. With the corrected hit rate, 1.0 (perfect detection) is a score that only results from a hit rate of 100% and a false alarm rate of 0%. For the animal that exhibited unusually high false alarm rates, this calculation of performance made it less likely that the 50% performance score (which was used as a marker of threshold) was due to chance. Table 7 depicts the performance of our nervous animal (backing away from the spout every 2 seconds) calculated with a corrected hit rate.

<table>
<thead>
<tr>
<th>dB SPL</th>
<th>W/(S+W)=.50</th>
<th>H-H(FP)=.50</th>
<th>d'=0</th>
<th>W/(S+W)=.50</th>
<th>H-H(FP)=.50</th>
<th>d'=0</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>.19*</td>
<td>0.25</td>
<td>.18*</td>
<td>70.5*</td>
<td>70.0</td>
<td>70.4*</td>
</tr>
<tr>
<td>60</td>
<td>0.34</td>
<td>0.40</td>
<td>0.33</td>
<td>67.8</td>
<td>67.0</td>
<td>67.8</td>
</tr>
<tr>
<td>50</td>
<td>0.49</td>
<td>0.56</td>
<td>0.49</td>
<td>65.0</td>
<td>64.0</td>
<td>65.0</td>
</tr>
<tr>
<td>40</td>
<td>0.66</td>
<td>0.74</td>
<td>0.66</td>
<td>59.4</td>
<td>56.5</td>
<td>59.4</td>
</tr>
<tr>
<td>30</td>
<td>0.85</td>
<td>0.94</td>
<td>0.85</td>
<td>52.1</td>
<td>48.8</td>
<td>51.9</td>
</tr>
</tbody>
</table>

Table 6. Lowest and highest frequencies audible at SPLs from 30 to 70 dB (*values are estimated).
Considering the conservativeness and validity of the corrected hit rate as a performance calculation compared to the suppression ratio, it is impressive that the thresholds resulting from this method are almost as low as those resulting from the suppression ratio (the differences ranging from 0 to 10 dB). Although the corrected hit rate resulted in thresholds that are higher than those of the other two threshold determination methods, the difference is negligible, especially in the high frequencies. Moreover, the corrected hit rate proved the least variable (and thus most reliable) threshold determination method for conditioned suppression/avoidance (SD=2.8 dB).

**Signal Detection Theory's d' = 0.** Signal detection theory posits that the world comes through the ears as a continuously changing spectrum of sound, and the job of an animal (both in nature and conditioned suppression/avoidance) is to divide that spectrum accurately into signal and noise. In conditioned suppression/avoidance, the signal is the sound the experimenter initiates and manipulates; in nature this could be any sound of

<table>
<thead>
<tr>
<th></th>
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<th># trials maintains contact</th>
<th>total # of trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>sound</td>
<td>25</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>no sound</td>
<td>24</td>
<td>1</td>
<td>25</td>
</tr>
</tbody>
</table>

\[
\text{hit rate} = \frac{25}{25} = 100\%
\]

\[
\text{false alarm rate} = \frac{24}{25} = 96\%
\]

\[
\text{corrected hit rate} = \text{hit rate} - (\text{hit rate} \times \text{false alarm rate})
\]

\[
= 1 - (1 \times .96) = .04
\]

\[
\text{performance} = 4\%
\]

Table 7. Example performance calculation using a hit rate corrected for false alarm rate. In this case a performance of 4% indicates that the stimulus was well below threshold (close to no detection). This is in contrast to the same responses resulting in a performance that indicates perfect detection when calculated with the suppression ratio (as depicted in Table 7).
interest, such as the thud of a pouncing predator. The noise is an always-present combination of sound waves from a variety of sources (e.g. murmur of the building’s air conditioning system, sound of the heart pumping blood, rustling blades of grass across a windy plane). Thus there are two distributions of sound intensities: one is for noise alone, and one is for noise plus signal (Figure 17).

![Figure 17. Signal detection model example. The observed sound intensity increases from left to right. The peaks of the curves correspond to the most frequently observed value, and because the observations are distributed symmetrically around these two points, they are also the mean value of each set of observations. Here the mean of the noise distribution is lower than the signal-plus-noise distribution, indicating that when a signal is present the overall observed intensity of sound is greater than when there is only background noise.](image)

Conditioned suppression/avoidance presents signals and noise to subjects, who try to detect all (and only) the signals. As the signal decreases in intensity and approaches threshold, the noise and signal-plus-noise distributions increasingly overlap, such that their means become closer together. If the signal is at an intensity so low that it is not detected by the animal, there is no difference between the noise and signal-plus-noise distributions, and the distance between their means is 0. Conversely, if the signal is so loud that even at its lowest intensity it is above the highest-intensity background noise,
the two distributions are completely separate and the distance between their means is large.

To characterize an animal’s performance on the conditioned suppression/avoidance task, signal detection theory uses hit and false alarm rates to estimate the distance between the noise and signal-plus-noise distributions. The statistic $d'$ is a measure of the distance between the means of the noise and signal+noise distributions. $D'$ uses the z-scores of the hit (H) and false alarm (F) rates in its calculation.

Whether in their original proportions or their z-scores, when $H = F$, then $d' = 0$. In other words, when hits and false alarms occur equally often, the value of $d'$ indicates that—according to the animal—there is no difference between background noise and signal. This is true regardless of the animal’s response bias (whether it breaks contact with the spout constantly or rarely). In this study, the use of $d' = 0$ to estimate threshold resulted in thresholds that are lower than those derived from the corrected hit rate. This is to be expected, because a $d'$ of zero represents the point at which the animal can no longer distinguish between the presence or absence of the tone. On the other hand, the corrected hit rate defines threshold as 50% performance, such that the animal can distinguish between the presence and absence of the tone half of the time. Unfortunately, $d' = 0$ resulted in the most variable (least reliable) thresholds of all conditioned suppression/avoidance threshold determination methods (SD=3.9 dB).

Even though it resulted in the lowest absolute thresholds, $d' = 0$ will not be used to comprise the conditioned suppression/avoidance audiogram for this study, in part because of the high variability among thresholds derived with this method. Instead, the corrected
hit rate proved most appropriate, as it resulted in the most valid and reliable thresholds. The corrected hit rate is a robust measure that is most commonly used for obtaining thresholds from conditioned suppression/avoidance data, and thus will be used as the basis of all further comparisons.

**B. Conditioned suppression/avoidance audiogram.** The average conditioned suppression/avoidance audiogram from the current study is shown in Figure 18 along with the average thresholds from key prior conditioned suppression/avoidance studies (Heffner et al., 1994; Kelly & Masterton, 1977). In this study, conditioned suppression/avoidance has demonstrated the rat’s ability to respond to frequencies from 250 Hz to 70 kHz, their best hearing ranging from about a half-octave above 250 Hz to just below 70 kHz at the 60-dB level. The current conditioned suppression/avoidance audiogram also reveals the “notch” commonly seen for these animals from 8 to 32 kHz.
The largest differences between the current audiogram and previous conditioned suppression/avoidance curves are in the threshold values (rather than shape or form of the audiogram), although these differences are minimal in comparison to those among the prepulse inhibition curves (next section). Table 8 presents the lowest and highest frequencies audible at SPLs from 30 to 70 dB for each of the three conditioned suppression/avoidance audiograms. The current audiogram reveals equally sensitive thresholds in the high frequencies (over 64 kHz), more sensitivity (lower thresholds) in the low frequencies (under 1 kHz), and less sensitivity (higher thresholds) in the middle range of frequencies, especially between 8 and 32 kHz. The higher thresholds in the

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**Figure 18. Average conditioned suppression/avoidance audiograms of the hooded rat (J, this study; H, Heffner et al., 1994), and albino rat (K, Kelly & Masterton, 1977).**
current audiogram are likely due to the onset of presbycusis, a sensorineural hearing loss associated with age that begins in the high frequencies. The animals in the current study were 18 months old at the start of testing for conditioned suppression/avoidance—an age when hearing sensitivity to frequencies of 16 kHz and higher can begin to decline rapidly until as much as 30 dB in sensitivity is lost by the age of 34 months (cf. Fetoni, Picciotti, Paludetti, & Troiani, 2010; Gratton, Bateman, Cannuscio, & Saunders, 2008).

Conversely, the animals used in the prior studies were only 12 months old at most during testing. Despite any presbycusis effects, the current audiogram is only 10 dB less sensitive than either of the previous audiograms at any given frequency, and all three audiograms vary on average by less than 5 dB between 1 and 8 kHz. In addition, all three audiograms unanimously show the point of best hearing at 8 kHz, followed closely by a point of second-to-best hearing at or near 32 kHz.

Overall comparison of the three audiograms for conditioned suppression/avoidance reveals that they are very similar, especially regarding the shape of the curves.

<table>
<thead>
<tr>
<th>dB SPL</th>
<th>Lowest audible frequency (kHz)</th>
<th>Highest audible frequency (kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K</td>
<td>H</td>
</tr>
<tr>
<td>70</td>
<td>0.30</td>
<td>0.29</td>
</tr>
<tr>
<td>60</td>
<td>0.040</td>
<td>0.53</td>
</tr>
<tr>
<td>50</td>
<td>0.50</td>
<td>0.63</td>
</tr>
<tr>
<td>40</td>
<td>0.70</td>
<td>0.75</td>
</tr>
<tr>
<td>30</td>
<td>0.90</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Table 8. Lowest and highest frequencies audible at SPLs from 30 to 70 dB (J, this study; H, Heffner et al., 1994; K, Kelly & Masterton, 1977) (*values are estimated).
All three audiograms show the same general structure: a rapid increase in sensitivity from 250 Hz to 1 kHz, a gradual increase in sensitivity from 1 to 4 kHz, a sharp increase in sensitivity from 4 to 8 kHz followed by an equally sharp decrease in sensitivity from 8 to 16 kHz, an increase in sensitivity from 16 to 32 kHz, and finally a rapid decrease in sensitivity above 32 kHz.

The agreement among the conditioned suppression/avoidance curves is impressive, especially considering they were done years apart with slightly different sound fields, test parameters, and training methods. For example, the current study used a single 200 ms tone, which is considerably briefer than the 10 s tone used by Kelly and Masterton (1977) and four 400 ms pulses used by Heffner et al. (1994). The sound chamber used in the current study was considerably smaller than those used in both prior studies as well. However, the combination of positive reward with mild electric shock punishment was shared among all three conditioned suppression/avoidance studies. This combination allows for such good control over an animal’s behavior that it is conducive to eliciting the best possible performance out of its subjects, thus yielding highly replicable results.

**Prepulse Inhibition Thresholds**

Six methods for determining thresholds from the prepulse inhibition data have been described and their results displayed. The efficacy of any one of these methods rests in part on the comparison of its results to those of conditioned suppression/avoidance, and in part on its logical defensibility. The objective, then, is to: A. discuss the validity of each threshold determination method and compare the obtained threshold
estimates to one another, and B. choose the best estimate (the most reliable and similar to conditioned suppression/avoidance) to comprise the audiogram that will be compared to previously published prepulse inhibition thresholds.

A. **Prepulse inhibition threshold determination methods.**

*Linear Interpolation.* Thresholds estimated by linear interpolation between the most intense stimulus producing less than 15 and 10% inhibition\(^8\) and the next higher stimulus intensity use unbiased estimates of the true mean response at each intensity. They are unbiased in the sense that they do not make assumptions about the relationship between startle response and prestimulus intensity: they use only the raw data, and do so without fitting the data to a theoretically ideal trend (as with the Spline-Fit method). Although unbiased, they lack precision: little of the actual experimental data (only two points at each intensity: the inhibition at the intensities directly above and below threshold) enter into each mean estimate (Fechter et al., 1988), and responses indicating threshold are interpolated. Also, the imprecision of the mean is inherent in the way in which it describes central tendency. This is evidenced in the fact that mean control trial amplitude values often do not coincide with actual control trial values, as happens for any data set that is highly variable. Despite this, the Linear Interpolation method of threshold determination resulted in the least variable (and therefore most reliable) prepulse inhibition thresholds, as determined by the standard deviation of each animal for each

---

\(^8\) A lower inhibition (e.g. 5%) is not used because as intensity approaches threshold, prepulse startle approaches and sometimes exceeds baseline (control) startle magnitude. Because startle is highly variable, there are instances in which a stimulus produced less than 5% inhibition and a less intense stimulus produced more than 5% inhibition.
frequency tested (Appendix E). In other words, the thresholds for each rat at each frequency varied from one another less, on average, when the Linear Interpolation methods were used to estimate threshold than with any of the other threshold determination methods. Using a 15% inhibition criterion with Linear Interpolation resulted in the second-to-lowest average standard deviation (SD=4.3 dB), and the 10% inhibition criterion resulted in the lowest average standard deviation (SD=4.2 dB) of all threshold determination methods used for prepulse inhibition. Neither the 10% or 15% inhibition criteria resulted in the lowest absolute thresholds when compared to the other prepulse inhibition threshold determinations (although 10% inhibition was the second-to-lowest, next to the Function-Intersect method). Table 9 presents the lowest and highest frequencies audible at SPLs from 30 to 70 dB for each of the six prepulse inhibition audiograms. Lastly, the 10% inhibition audiogram agreed the most with the conditioned suppression/avoidance audiogram regarding form and shape⁹, as determined in part by calculating the correlation (r=.882, p<.001: Appendix F).

⁹ A detailed comparison of the prepulse inhibition and conditioned suppression/avoidance audiograms can be found at the end of the discussion section.


**Spline-Fitting.** In a study of threshold determinations, fitting the data to straight lines between the means and applying linear interpolation (as described in the previous paragraph) resulted in significantly higher threshold estimates (less sensitivity) than fitting a spline function to the square-root transformation of the median (Fechter et al., 1988). This is in part because the median is a measure of central tendency that is less vulnerable to the skewness of raw data than the mean. Theoretically, the use of a smoothing spline function allows for a more precise threshold estimate than Linear Interpolation because the function itself intersects directly with the horizontal line representing some amount of inhibition (in our case, 10%). It is also considered more precise because the function uses all the data to estimate a value at any one point on the curve, rather than solely the mean estimate at that point. However, this precision comes at the expense of bias: spline functions are used when the relationship between two variables (in this case startle amplitude and prestimulus intensity) is assumed to be

<table>
<thead>
<tr>
<th>dB SPL</th>
<th>Lowest audible frequency (kHz)</th>
<th>Highest audible frequency (kHz)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15% inhb</td>
<td>10% inhb</td>
</tr>
<tr>
<td>70</td>
<td>0.42</td>
<td>0.41</td>
</tr>
<tr>
<td>60</td>
<td>0.61</td>
<td>0.60</td>
</tr>
<tr>
<td>50</td>
<td>0.77</td>
<td>0.76</td>
</tr>
<tr>
<td>40</td>
<td>0.91</td>
<td>0.90</td>
</tr>
<tr>
<td>30</td>
<td>4.35</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Table 9. Lowest and highest frequencies audible at SPLs from 30 to 70 dB (*values are estimated).
smooth and continuous. Fechter et al. (1988) based their use of a spline function on the theoretical concept that reflex inhibition is a continuously graded response. Curiously, the current data did not demonstrate the benefits of the Spline-Fitting method. The variability of thresholds estimated with Spline-Fitting was higher than that of both 10% and 15% Linear Interpolations (SD=4.7 dB). Moreover, the Spline-Fit thresholds were the highest (just above the Statistical Significance method thresholds) of absolute thresholds compared to all the other threshold determination methods.

Statistical Significance and Statistical Significance Midpoint. It should be noted that the sensitivity of a threshold estimate depends on the operational definition of threshold used. Less “sensitive” thresholds (higher threshold estimates) are also more conservative. Conservative threshold estimates make it less likely that a chance reduction in response amplitude, a false positive response, or any similarly aberrant data will exert undue influence on a threshold. Using the lowest intensity at which there is no statistical difference in startle magnitude between prepulse and control trials offers the most conservative threshold estimate compared to the other five methods. This is akin to defining threshold as the intensity of a stimulus such that it is not detectable (a sort of “lower limit”), as opposed to the intensity of a stimulus such that it is detected half of the time. Using the midway point between that lowest intensity and the next highest intensity as threshold is conceptually more similar to traditional definitions of threshold (i.e. 50% detection). The Midpoint is thus a less conservative threshold estimate that results in more “sensitivity” (lower thresholds) than the Statistical Significance method. For the current data, both the Statistical Significance and Midpoint methods resulted in
thresholds that were highly variable (SDs = 5.05 dB and 5.1 dB, respectively). In addition, the absolute thresholds estimated by the Statistical Significance method were high in comparison to the other methods (almost as high as the spline-fit method thresholds). As expected, the Midpoint method resulted in threshold estimates that were slightly lower than the Statistical Significance method, however not as low as those of the Function-Intersect method. Lastly, the Statistical Significance method resulted in thresholds that correlated the least with those of conditioned suppression/avoidance compared to the other prepulse inhibition threshold methods (although in general, all correlations were high: r=.849, p<.001).

**Function Intersect.** The Function-Intersect method of threshold determination defined threshold as the lowest intensity at which there was no difference in startle amplitude between prepulse and control trials. Upon its conception, this was especially appealing: it provides an estimate of threshold that is both unbiased (it does not make assumptions about the relationship between startle response and prestimulus intensity) and somewhat precise (it does not require interpolation).

Also enticing is the fact that this method resulted in the lowest absolute thresholds of all threshold determination methods for prepulse inhibition. Unfortunately, however, this method resulted in the most variable thresholds of all threshold determination methods (SD=6.0 dB). Recall that in the prepulse inhibition procedure, the choice to increase the apparent sensitivity of a threshold measure is accompanied by the risk that chance reductions in response amplitude at a given tone intensity (a.k.a., false positives) will exert excessive influence over that threshold. Many thresholds influenced by deviant
responses will collectively comprise an audiogram that varies greatly from animal to animal. In that sense, the Function-Intersect method lacks reliability.

A point worth noting is that the only two prepulse inhibition threshold determinations that revealed the “notch” around 16 kHz are the 10 and 15% Linear Interpolation methods. That both these methods revealed the “notch” is not surprising considering they calculate threshold the same way (and only differ regarding the % inhibition criterion), however why the “notch” was not revealed by any of the other methods is unknown. One possibility is that the Statistical Significance methods are not sensitive enough (they are the most conservative), the Spline Fitting method not accurate enough (since the data is fit to an ideal trend), and the Function Intersect method too sensitive, such that chance reductions in startle magnitude exert excessive influence on threshold calculation.

Even though it resulted in the lowest absolute thresholds, the Function-Intersect method will not be used to comprise the prepulse inhibition audiogram for this study due to the high variability among thresholds derived with this method. Instead, Linear Interpolation with a 10% inhibition criterion can be used, as it resulted in the next-to-lowest of absolute thresholds, the least variability among thresholds of all threshold determination methods, and the highest correlation with conditioned suppression/avoidance.

B. Prepulse inhibition audiogram. The average prepulse inhibition audiogram from the current study, with threshold estimated by Linear Interpolation representing 10% inhibition, is shown in Figure 19 along with the average thresholds from the prior
prepulse inhibition studies (Fechter et al., 1988; Young & Fechter, 1983). In this study, prepulse inhibition has demonstrated the rat’s ability to respond to frequencies from 250 Hz to 70 kHz, their best hearing ranging from just above 500 Hz to just below 70 kHz at the 60-dB level. The current prepulse inhibition audiogram also reveals the “notch” commonly seen for these animals between 8 and 32 kHz.

The prior prepulse inhibition studies tested a limited range of frequencies, making it difficult draw many overall conclusions from the current comparison. The current audiogram spans over 8 octaves, whereas the prior prepulse inhibition studies only covered slightly over 4 octaves. However, given what we have, we can make a

Figure 19. Average prepulse inhibition audiograms of the rat (current study, using 10% inhibition thresholds; 1 from Young & Fechter, 1983; 2 & 3 from Fechter et al., 1988).
comparison of the thresholds for frequencies ranging from 2 to 40 kHz— the range tested in the previous prepulse inhibition studies.

The largest difference between the current audiogram and the previous prepulse inhibition curves is in the absolute thresholds themselves (though the shapes of some are similar). The absolute thresholds obtained in the current study are similar to those from Young and Fechter (1983) (Figure 19, line 1), slightly higher (by anywhere from 0-10 dB) than the first set of thresholds obtained by Fechter et al. (1988) (Figure 19, line 2), and considerably higher (by 5-30 dB) than the second set of thresholds obtained by Fechter et al. (1988) (Figure 19, line 3). This is somewhat surprising considering the tones in the current study were of a longer duration (200 ms) than those in the Young and Fechter (1983) study (20 ms). In this case one would have expected the current study to reveal lower thresholds, such as those from Fechter et al. (1988) who also used longer duration (200 ms) tones.

Although the absolute thresholds are more similar to those from Young and Fechter (1983), the shape of the current audiogram more closely resembles the curves from Fechter et al. (1988). The current audiogram and both curves from Fechter et al. (1988) show a rapid increase in sensitivity beginning around 3 kHz, a point of best hearing at 8 kHz, a decrease in sensitivity between 8 and 16 kHz, an increase in sensitivity until a second point of best hearing around 32 kHz, and finally a decrease in sensitivity above 32 kHz. This modest agreement of shape in the middle range of frequencies is the greatest similarity between the current audiogram and any of the
previous studies. Mostly, the current audiogram differs unsystematically from the other prepulse inhibition curves, which also differ unsystematically from one another.

The lack of similarity between the current audiogram and the previous curves is thus to be expected, considering the inconsistency of previous prepulse inhibition results and the novelty of the current application of prepulse inhibition to obtain auditory thresholds. This study employed several changes to the typical prepulse inhibition procedure, some of which are reviewed here. One such change is the use of different subjects, although strain-related hearing differences are not likely significant. Longer prepulse signal durations and rise/fall times were used, which prevented any temporal integration effects that may have affected previous research. The prestimulus speaker was placed in front of the animal and the startle noise speaker overhead, which could account for the prominence of the “notch” in the current audiogram compared to the others that had the speakers in reversed placement. The sound chamber was considerably larger and had thicker sound-insulating material, which makes it less prone to acoustic dead spots or standing waves. Animals were tested individually, as opposed to in groups of four, in which they could have responded to the movements, smells, and sounds emitted by one another. There were just as many control trials as prepulse trials in the current study, as opposed to less than 20% control trials randomly interspersed among prepulse trials in previous research. The interstimulus and intertrial intervals were shorter to decrease startle variability. The threshold definitions and calculations differed, and this seemed to have a grave effect on the thresholds derived with each. The animals in the current study were water-deprived, in a physiologically “thirsty” state, and drank until
satiated during testing. Among various other differences between previous prepulse inhibition studies the current study, having the animals drinking from a waterspout during testing is probably the most important novelty, which may have even affected their startle reflex. Although the act of drinking from the spout could have increased the variability of the rats’ reflexes by changing their state of tension, this served to keep their heads in a fixed position and thus decrease variability in sound intensity measurement. This is especially important for close comparison of prepulse inhibition to conditioned suppression/avoidance. On the other hand, the prior prepulse inhibition studies used a single sound intensity measurement to represent what was, in actuality, a multitude of stimulus intensities that arrived at the animal’s ears as it moved freely in the cage during testing.

**Prepulse Inhibition vs. Conditioned Suppression/Avoidance**

The conditioned suppression/avoidance and prepulse inhibition audiograms are shown together in Figure 20. The two audiograms agree well regarding the general shape and range of audibility in the rat. The only disagreement between the two audiograms is regarding the sensitivity of each measure. Prepulse inhibition resulted in less sensitivity (higher thresholds) for the entire range of the audiogram except in the high frequencies (above 32 kHz). In fact, prepulse inhibition appears to be more sensitive in the frequencies above 32 kHz than conditioned suppression/avoidance. This is most likely due to presbycusis, since the prepulse inhibition procedure was completed before conditioned suppression/avoidance. Indeed, when the two highest frequencies that might have been affected by presbycusis are removed from analysis, the correlation between
conditioned suppression/avoidance and prepulse inhibition increases (from $r=.882$ to $r=.961$: Appendix G). Apart from absolute sensitivity differences, the shapes of the curves are very similar. In some places (i.e. .50, 2, and 4 kHz) prepulse inhibition thresholds are about 10 dB higher than conditioned suppression/avoidance, and in other places (i.e. 1, 16, and 32 kHz) they are only higher by 5 dB or less. However, in general, a change in one line parallels a change in the other. This is impressive, considering the vast
difference between the two methods as well as the difference in age of the animals in each test. The slopes of the audiograms, representing the change in intensity per frequency range (e.g. dB/octave), agree closely, until the high frequencies above 32 kHz, at which point the prepulse inhibition audiogram has a much steeper slope than conditioned suppression/avoidance. This is likely due to presbycusis as well. To see how the current prepulse inhibition audiogram correlates with the prior conditioned suppression/avoidance audiograms, which were not subject to the effects of presbycusis, correlations were also calculated for the thresholds from Kelly and Masterton (1977) and Heffner et al. (1994). Figure 21 shows all four correlations: the highest of all being the correlation between the current prepulse inhibition audiogram with all frequencies included and the conditioned suppression/avoidance audiogram from Heffner et al. (1994) (r=.987, p<.00001).
Several factors (all discussed as relevant to the purpose of this research) might contribute to the sensitivity difference between the obtained prepulse inhibition and conditioned suppression/avoidance audiograms. In the past, threshold differences among audiograms have been attributed not only to different behavioral and threshold determination methods, but also to differences in the accuracy and precision of the sound measurements. For example, differences in the heterogeneities of the sound field near the animal’s ears, differing degrees of movement of the animal’s ears in the sound field during responding, differing background noise levels, and differing levels of the masking noises inadvertently made by the animal itself while responding may all have likely
contributed to the obtained threshold differences of previous research. Other differences have included the subjects, the number of subjects tested at a time, water deprivation, signal duration, rise/fall times, sound chamber acoustics, speaker placement, and more. However, the current research has held all of these factors constant, nullifying sound field issues as a source of the differences in thresholds obtained with each measure. That the current prepulse inhibition audiogram compares well with the conditioned suppression/avoidance audiogram is most likely due to holding the animal’s head in a fixed position, as well as using the same sound parameters and testing environment throughout the investigation.

One might wonder if part of the sensitivity difference in the audiograms of each method could result from differences in the threshold determination methods—that is, how the raw data are translated into a threshold. Since conditioned suppression/avoidance and prepulse inhibition include such different behavioral tasks, and therefore different response types, a common threshold calculation is not possible. Although the calculation of threshold might account for some of the discrepancies between prepulse inhibition and conditioned suppression/avoidance audiograms, this argument is peripheral to the main point. The threshold calculations cannot be the same because the behavioral methods are not the same. What the two procedures did have in common was almost everything else: the stimulus properties (duration, rise/fall, etc.), the sound field and measurement (sound chamber, equipment, etc.), the subjects (although they were older for the second experiment), even the “thirsty” state of the animals. Considering all the parameters that were identical for both experiments, it seems most obvious that the
difference between the two audiograms is related to the inherent sensitivity of the two behavioral techniques. This includes that in conditioned suppression/avoidance, the animals are trained observers. Trained observers are arguably more attentive observers. This also includes the different motivation levels of the animals due to differences in reward and punishment training: in one instance the animals received no consequence for their behavior one way or another; in the other instance the animals were punished for an incorrect response.

Obviously, prepulse inhibition is less sensitive than conditioned suppression/avoidance for determining auditory thresholds in animals. The results of this study suggest that this is inevitable: animals trained via conditioned suppression/avoidance are motivated to perform at their best to avoid impending punishment, which is not the case in prepulse inhibition. However, this conclusion alone does not necessarily compromise the merit of prepulse inhibition, or the use of an unconditioned response as a means of obtaining auditory thresholds in animals. Rather, the possibility of calibrating unconditioned responses for estimating sensory thresholds becomes an important question.

Since the thresholds obtained with each method do not vary in a manner that is precisely and consistently systematic (i.e., the absolute threshold differences and direction of differences are not consistent across frequencies), a specific calibration of unconditioned responses is not possible. However, it would be safe to generalize that thresholds obtained via prepulse inhibition are going to range from 5 to 10 dB higher than those obtained from an operant conditioning procedure, especially one that uses both
reward and punishment to maximize an animal’s motivation to perform. The data presented in this study suggest prepulse inhibition can assuredly provide rapid evaluations of auditory detection thresholds. Even if the animal is drinking during prepulse inhibition, which does not allow for as many trials per session as previous prepulse inhibition experiments in which the animal was not drinking, it does not require months of training on an operant task before data can be collected. In that sense, prepulse inhibition is conducive to the study of sensory abilities when time is limited. However, an important point of this research is that one should use caution when using an unconditioned response to estimate sensory thresholds: one must distinguish among the many possible threshold determination methods, and keep in mind the lack of sensitivity compared to operant thresholds. Given that, unconditioned responses can offer advantages of speed and ease. They should suffice to indicate whether there has been an overall change in sensory ability over time, or change due to drug effects, etc. Unconditioned responses should also suffice to assess thresholds in compromised subjects that may not have the cognitive or motor skills required by operant tasks.

This study directly compared two behavioral methods of determining what an animal can hear: one that uses an unconditioned response—prepulse inhibition, and another that uses a highly trained response—conditioned suppression/avoidance. The question of interest was whether the large discrepancies in the audiograms obtained with each of these methods in prior research were due to an inherent difference between the uses of unconditioned and conditioned responses, as opposed to other factors that differed from study to study. The answer is twofold: unconditioned responses are inherently less
sensitive than conditioned responses, however the vast disagreement among previous audiograms was mostly due to technical differences unrelated to the response type of each method. The current study presented modifications of the prepulse inhibition procedure such that it more-closely resembled conditioned suppression/avoidance regarding head placement, sound field parameters, signal duration, and more. The most important finding is that prepulse inhibition can be improved for detection studies by employing such modifications, most notably holding the animal’s head fixed in the sound field. Although this study has shown an unprecedented resemblance between unconditioned and conditioned response thresholds, the similarity of the audiograms obtained with these two methods is still inferior to that among various operant conditioning methods. However, prepulse inhibition should be appropriate for animals that cannot learn an operant procedure, or when time restrictions do not permit extensive training. Unconditioned responses are adequate indicators of sensory ability as long as: the proper threshold calculation is used, the head is held fixed relative to the sound field, and thresholds are considered, on average, 5-10 dB less sensitive than those obtained from conditioned responses. Given these criteria, this study suggests that unconditioned responses can be used to obtain sensory thresholds in animals.
References


## Appendix A

### Average and Individual Prepulse Inhibition Thresholds

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<th>Rat</th>
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<th>FncT Insect</th>
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|     | 0.5             | 67            | 66.8          | 67      | 67      | 66.5       | 66.3        |
|     | 1               | 36            | 34.8          | 44      | 40      | 35         | 32          |
|     | 2               | 48.8          | 46.8          | 49.9    | 53      | 48         | 43          |
|     | 4               | 32            | 29.8          | 33.5    | 48.5    | 43.5       | 18.5        |
|     | 8               | 6.82          | 15            | 25      | 28      | 23         | 8           |
|     | 16              | 24.4          | 22            | 30.8    | 31      | 26         | 6           |
|     | 32              | 10.6          | 8             | 35      | 30      | 25         | 0           |
|     | 64              | 33.3          | 32            | 32.6    | 35      | 30         | 29.3        |
|     | 70              | 59.3          | 58.8          | 60.5    | 65      | 62.5       | 57.5        |

| C   | 0.25            | 78.9          | 77.5          | 80      | 80.5    | 78.25      | 72.8        |
|     | 0.5             | 66.5          | 66.3          | 66.5    | 67      | 66.5       | 67          |
|     | 1               | 31.9          | 30            | 32.1    | 40      | 35         | 17.3        |
|     | 2               | 34.4          | 33            | 33.8    | 43      | 38         | 26          |
|     | 4               | 33            | 31.5          | 28.2    | 28.5    | 23.5       | 29.5        |
|     | 8               | 6.07          | 15.5          | 27.4    | 18      | 13         | 12          |
|     | 16              | 26.4          | 23            | 28.3    | 31      | 26         | 4           |
|     | 32              | 20.8          | 15            | 34.5    | 20      | 15         | 7           |
|     | 64              | 31.2          | 28            | 31.2    | 25      | 20         | 27.8        |
|     | 70              | 62.2          | 61.5          | 62.6    | 65      | 62.5       | 61          |

**Average Prepulse Inhibition Thresholds in dB SPL**

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<th>Frequency (kHz)</th>
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<th>10% Inhib (%)</th>
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**Individual Prepulse Inhibition Thresholds in dB SPL**

82
Appendix B

Percent Inhibition above Threshold

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Percent Inhibition = 100 * (1 - [prepulse v / control v])
## Appendix C

### Individual and Average Conditioned Suppression/Avoidance Thresholds

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</tr>
<tr>
<td></td>
<td>70</td>
<td>70.6</td>
<td>72.5</td>
<td>70</td>
</tr>
</tbody>
</table>

Average Conditioned Suppression/Avoidance Thresholds in dB SPL

<table>
<thead>
<tr>
<th>Frequency (kHz)</th>
<th>$w/(s+w)=0.5$</th>
<th>$h-h(fp)=0.5$</th>
<th>$d^* = 0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>65.98</td>
<td>70</td>
<td>65.24</td>
</tr>
<tr>
<td>0.5</td>
<td>49.19</td>
<td>52.67</td>
<td>48.84</td>
</tr>
<tr>
<td>1</td>
<td>21.78</td>
<td>26.5</td>
<td>21.47</td>
</tr>
<tr>
<td>2</td>
<td>17.96</td>
<td>25</td>
<td>20.67</td>
</tr>
<tr>
<td>4</td>
<td>12.97</td>
<td>17.17</td>
<td>12.95</td>
</tr>
<tr>
<td>8</td>
<td>-3.64</td>
<td>-0.83</td>
<td>-8.27</td>
</tr>
<tr>
<td>16</td>
<td>12.93</td>
<td>17.83</td>
<td>12.71</td>
</tr>
<tr>
<td>32</td>
<td>2.33</td>
<td>8</td>
<td>4.31</td>
</tr>
<tr>
<td>64</td>
<td>46.46</td>
<td>50</td>
<td>46.18</td>
</tr>
<tr>
<td>70</td>
<td>68.37</td>
<td>70.5</td>
<td>68.87</td>
</tr>
</tbody>
</table>

Individual Conditioned Suppression/Avoidance Thresholds in dB SPL
## Appendix D

### Conditioned Suppression/Avoidance Standard Deviations

<table>
<thead>
<tr>
<th>frequency (kHz)</th>
<th>$W/(S+W) = .50$</th>
<th>$H - (H\times FP) = .50$</th>
<th>$d' = 0$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>3.221</td>
<td>3.122</td>
<td>3.725</td>
</tr>
<tr>
<td>0.50</td>
<td>4.288</td>
<td>4.252</td>
<td>4.478</td>
</tr>
<tr>
<td>1</td>
<td>2.803</td>
<td>1.500</td>
<td>3.557</td>
</tr>
<tr>
<td>2</td>
<td>4.453</td>
<td>1.323</td>
<td>3.063</td>
</tr>
<tr>
<td>4</td>
<td>1.412</td>
<td>1.041</td>
<td>0.947</td>
</tr>
<tr>
<td>8</td>
<td>3.046</td>
<td>4.163</td>
<td>7.865</td>
</tr>
<tr>
<td>16</td>
<td>3.562</td>
<td>3.253</td>
<td>3.324</td>
</tr>
<tr>
<td>32</td>
<td>1.900</td>
<td>1.803</td>
<td>1.400</td>
</tr>
<tr>
<td>64</td>
<td>1.965</td>
<td>3.606</td>
<td>6.769</td>
</tr>
<tr>
<td>70</td>
<td>5.164</td>
<td>3.905</td>
<td>3.839</td>
</tr>
<tr>
<td></td>
<td>$m=3.181$</td>
<td>$m=2.797$</td>
<td>$m=3.897$</td>
</tr>
</tbody>
</table>

Conditioned Suppression/Avoidance Threshold Standard Deviations in dB SPL
### Appendix E

**Prepulse Inhibition Standard Deviations**

<table>
<thead>
<tr>
<th>frequency (kHz)</th>
<th>15% inhb</th>
<th>10% inhb</th>
<th>SplnFt</th>
<th>StSig</th>
<th>StSig Mid</th>
<th>Fnet Insect</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.901</td>
<td>0.866</td>
<td>0.601</td>
<td>2.598</td>
<td>2.598</td>
<td>4.423</td>
</tr>
<tr>
<td>0.50</td>
<td>0.382</td>
<td>0.289</td>
<td>0.292</td>
<td>0.577</td>
<td>1.443</td>
<td>0.433</td>
</tr>
<tr>
<td>1</td>
<td>2.385</td>
<td>3.166</td>
<td>5.919</td>
<td>5.774</td>
<td>5.774</td>
<td>9.063</td>
</tr>
<tr>
<td>2</td>
<td>7.804</td>
<td>8.087</td>
<td>8.293</td>
<td>5.774</td>
<td>5.774</td>
<td>8.544</td>
</tr>
<tr>
<td>4</td>
<td>1.250</td>
<td>1.090</td>
<td>2.674</td>
<td>10.000</td>
<td>10.000</td>
<td>5.568</td>
</tr>
<tr>
<td>8</td>
<td>2.517</td>
<td>3.041</td>
<td>2.784</td>
<td>5.774</td>
<td>5.774</td>
<td>2.309</td>
</tr>
<tr>
<td>16</td>
<td>2.255</td>
<td>0.577</td>
<td>8.090</td>
<td>0.000</td>
<td>0.000</td>
<td>4.867</td>
</tr>
<tr>
<td>32</td>
<td>12.393</td>
<td>13.454</td>
<td>2.706</td>
<td>10.000</td>
<td>10.000</td>
<td>15.695</td>
</tr>
<tr>
<td>64</td>
<td>5.198</td>
<td>3.512</td>
<td>7.862</td>
<td>10.000</td>
<td>10.000</td>
<td>0.804</td>
</tr>
<tr>
<td>70</td>
<td>7.844</td>
<td>7.844</td>
<td>7.560</td>
<td>0.000</td>
<td>0.000</td>
<td>7.848</td>
</tr>
<tr>
<td><strong>m=4.293</strong></td>
<td><strong>m=4.192</strong></td>
<td><strong>m=4.678</strong></td>
<td><strong>m=5.050</strong></td>
<td><strong>m=5.136</strong></td>
<td><strong>m=5.955</strong></td>
<td></td>
</tr>
</tbody>
</table>

Prepulse Inhibition Threshold Standard Deviations in dB SPL
Pearson correlations between prepulse inhibition thresholds and conditioned suppression/avoidance thresholds (p<.01). All threshold definitions result in an audiogram that is highly correlated with conditioned suppression/avoidance; 10% Inhibition has the highest and Statistical Significance (t test cut-off) has the lowest correlation.

<table>
<thead>
<tr>
<th>rat</th>
<th>15% inhb</th>
<th>10% inhb</th>
<th>SplnFt</th>
<th>StSig</th>
<th>StSig Mid</th>
<th>Fnet Inset</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.876</td>
<td>0.871</td>
<td>0.805</td>
<td>0.807</td>
<td>0.815</td>
<td>0.88</td>
</tr>
<tr>
<td>B</td>
<td>0.826</td>
<td>0.891</td>
<td>0.843</td>
<td>0.86</td>
<td>0.858</td>
<td>0.88</td>
</tr>
<tr>
<td>C</td>
<td>0.917</td>
<td>0.883</td>
<td>0.906</td>
<td>0.88</td>
<td>0.878</td>
<td>0.862</td>
</tr>
<tr>
<td>m=.873</td>
<td>m=.882</td>
<td>m=.851</td>
<td>m=.849</td>
<td>m=.850</td>
<td>m=.874</td>
<td></td>
</tr>
</tbody>
</table>
Appendix G

Pearson Correlations without High Frequencies

<table>
<thead>
<tr>
<th>rat</th>
<th>15% inhb</th>
<th>10% inhb</th>
<th>SplnFt</th>
<th>StSig</th>
<th>StSig Mid</th>
<th>Fnct Insect</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.938</td>
<td>0.945</td>
<td>0.945</td>
<td>0.91</td>
<td>0.912</td>
<td>0.915</td>
</tr>
<tr>
<td>B</td>
<td>0.973</td>
<td>0.969</td>
<td>0.969</td>
<td>0.946</td>
<td>0.955</td>
<td>0.953</td>
</tr>
<tr>
<td>C</td>
<td>0.734</td>
<td>0.97</td>
<td>0.928</td>
<td>0.985</td>
<td>0.982</td>
<td>0.907</td>
</tr>
<tr>
<td>m=.882</td>
<td>m=.961</td>
<td>m=.947</td>
<td>m=.947</td>
<td>m=.950</td>
<td>m=.925</td>
<td></td>
</tr>
</tbody>
</table>

Pearson correlations between prepulse inhibition thresholds and conditioned suppression/avoidance thresholds (p<.01) when frequencies that might have been affected by presbycusis (64 & 70 kHz) are removed from analysis. In this case correlations increase by an average of 7.21%.