Chapter 2
Behavioral Tests for Tinnitus in Animals

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1 Introduction

Tinnitus refers to the perception of sound in the absence of external sound. Although this can include the perception of internal sounds, it is most often used to designate the perception of sound in the complete absence of acoustic stimulation, which is the way it is used here (e.g., McFadden, 1982; Penner & Jastreboff, 1996). Of the various causes of tinnitus, the best known are exposure to loud sound and the ingestion of large doses of ototoxic drugs, such as salicylate, which is the active ingredient of aspirin, or quinine, which is a former treatment for malaria and a current flavor component of tonic water. Interest in tinnitus has increased in recent years, aimed primarily at finding a treatment, but understanding this disorder may also give some insight into the neurological basis of the perception of sound. Because carefully controlled studies of neurological disorders are best conducted with animals, this has created a need for a way to determine if an animal has tinnitus.

Devising a behavioral test to determine whether an animal has tinnitus presents problems not encountered in routine animal psychophysics. Determining an animal's ability to detect or discriminate *physical* sounds involves training it to make a specific response in the presence of a particular sound and to make a different response, or no response at all, in the absence of that sound (e.g., Klump et al., 1995). For example, an animal can be trained to press a lever when a tone is presented and the intensity of the tone varied to determine the animal's detection threshold. Confidence that the resulting threshold is valid is obtained by demonstrating that the animal consistently presses the lever to suprathreshold intensities (has a high "hit" rate), rarely presses when no physical tone is present (has a low "false positive" rate), and that its ability to detect the tone declines sharply around

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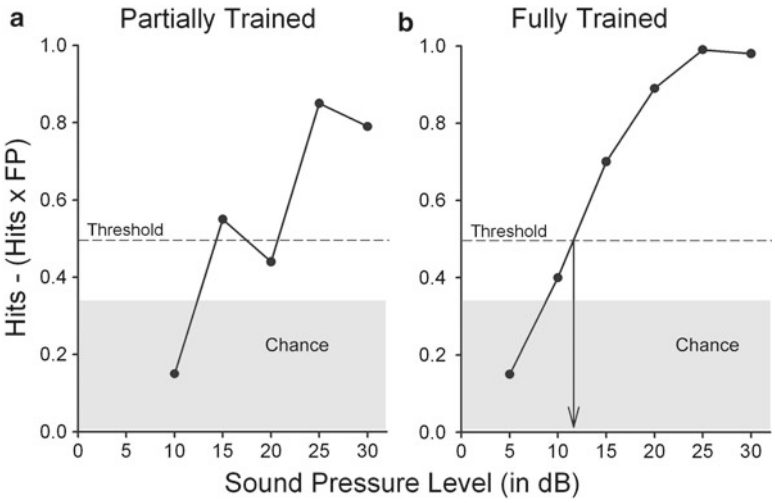

Fig. 2.1 Illustration of how an animal's psychophysical function for the detection of a sound changes with training. A score of 1.0 indicates a 100% hit rate with a 0% false positive (FP) rate; a score of 0 indicates no hits. (**a**) Performance of a partially trained animal that has not yet learned to listen to low-level sounds; its performance does not consistently change with the sound level with the result that thresholds are ambiguous. In addition, failure to achieve near perfect performance at higher levels leads to low confidence in the results. (**b**) Performance of a fully trained animal with a monotonic relation between performance and sound level with a sharp decline in performance around threshold. Several weeks of training are usually necessary for an animal to become a reliable observer. The horizontal dashed line indicates the 50% definition of threshold; the shaded area indicates chance level of performance

threshold (Fig. 2.1); it can take several weeks of training for an animal to become a reliable observer. Moreover, by conducting the tests in an environment that is free of extraneous stimuli (i.e., a sound-proof room), it is possible to demonstrate that the animal is responding to the sound being presented and that its responding is not affected by other stimuli.

However, training an animal to respond to its tinnitus presents two problems not found when working with physical stimuli. The first is inducing the tinnitus; although it is well established that loud sound and salicylate cause tinnitus in humans, there is significant variability between subjects (McFadden, 1982). Specifically, humans differ in whether a particular treatment induces tinnitus, what the characteristics of the tinnitus will be (e.g., noise-like or tonal and, if tonal, what pitch), and how long it persists. Thus, unlike the situation with physical stimuli, one cannot be certain that a treatment will induce tinnitus in an animal and what the characteristics of the tinnitus will be. The second problem is that human studies indicate that treatments used to induce tinnitus may affect hearing in other ways; for example, loud sound and ototoxic drugs can cause a hearing loss and may also cause physical sounds to be perceived as distorted (e.g., Davis et al., 1950; Cazals, 2000).

Thus, it is necessary to rule out the possibility that an animal's responding is affected by changes to its hearing other than tinnitus.

This chapter reviews the procedures that have been devised to test animals for tinnitus. Because one of the first questions about tinnitus in animals is how well it matches what we know about tinnitus in humans, this chapter begins with a brief description of tinnitus in humans caused by exposure to loud sound and salicylate, as well as other auditory effects of these treatments.

2 Human Studies

The human literature consists primarily of studies of patients with preexisting tinnitus, with a much smaller number of studies in which tinnitus was experimentally induced, typically by loud sound or salicylate (McFadden, 1982; Cazals, 2000). The studies in which tinnitus was induced are important, not only because the relation between the tinnitus-inducing agent and the resulting tinnitus can be determined, but also because these studies often include additional measurements regarding the effects of the tinnitus-inducing agent on hearing. Studies of preexisting tinnitus have been particularly important in describing the general characteristics of tinnitus, such as how it interacts with physical sounds.

2.1 Exposure to Loud Sound

A small number of studies have exposed humans to loud sound and observed the resulting tinnitus. One early study, conducted by Hallowell Davis and his colleagues (1950), is worth describing in some detail because it is often overlooked. Using themselves and Harvard students as subjects, Davis and his colleagues would expose an ear to a loud sound and observe the resulting changes in sensitivity, loudness, and pitch perception. Subjects were tested once or twice a week, with time allowed for recovery between tests, thus providing multiple observations with replication on the same subjects. In commenting on the tinnitus that accompanied the hearing loss, they noted that tinnitus resulting from exposure to a loud tone was more likely to have a "definite and constant pitch" than that resulting from exposure to broadband noise. Moreover, the pitch of the tinnitus typically occurred at the high-frequency edge of a sharply localized hearing loss, an observation suggesting that tinnitus occurs when a section of the basilar membrane is rendered partly or completely unresponsive to sound, with the pitch of the tinnitus corresponding to the less affected portion of the basilar membrane at the high-frequency end of the damaged section (the idea that tinnitus can be an "edge" effect has been noted by others, e.g., Fowler, 1941; McFadden, 1982; Moore et al., 2010). Judging from the illustrations in their report, the pitch of the tinnitus was perceived to match a tone 1–1.5 octaves above the frequency of the exposing tone. It may be noteworthy that the tinnitus was

well above the frequency of maximum hearing loss, which was about 0.5 octave above the frequency of the exposing tone.

The hearing loss that resulted from exposure to loud sound was the main focus of the Davis et al. study, and several of their findings are pertinent to understanding tinnitus and evaluating animal models. First, an individual's preexposure audiogram did not vary much and could usually be replicated within 5 dB. Second, although exposing an ear to the same loud sound on more than one occasion tended to produce the same hearing loss each time, with the maximum hearing loss occurring at the same frequency, there could be significant variation. Third, exposing different subjects to the same loud sound could produce very different hearing losses, a result that has been observed in animals (e.g., Heffner & Harrington, 2002). Because the induced tinnitus may depend on the specific hearing loss, these results suggest that there is likely to be variation in the occurrence and pitch of tinnitus in subjects exposed to the same loud sound.

Finally, Davis and his colleagues noted that exposure to loud sound produced more than a hearing loss; it also distorted the perception of physical sounds. For example, the exposure could cause a pure tone to sound "rough," "noisy," or "buzzing"; it could also cause a single tone to sound like two tones presented in combination, which they referred to as "doubles." In addition, the pitch of a tone in the exposed ear might be shifted in comparison with its pitch in the unexposed ear, a phenomenon referred to as "diplacusis." It should be noted that Davis and his colleagues reported that the hearing loss, and presumably the accompanying effects, disappeared within a few days or at most a week. Thus, they observed no permanent effects for the exposures they used, which consisted of tones of 500 Hz, 1000 Hz, 2000 Hz, and 4000 Hz, as well as noise resembling aircraft noise, at intensities ranging from 110 to 130 dB sound pressure level (SPL) and durations ranging from 1 to 64 minutes (with the higher intensities presented for shorter times).

Since 1950, there have been two studies of tinnitus induced by exposure to loud sound that systematically looked at the relationship between the pitch of the resulting tinnitus, the exposing stimulus, and the hearing loss (Loeb & Smith, 1967; Atherley et al., 1968); interestingly, the authors of these studies were apparently unaware that Davis and colleagues (1950) had previously reported on tinnitus. The study by Loeb and Smith found, as had Davis et al, that the median pitch of tone-induced tinnitus was higher than both the exposing tone and the frequency of maximum hearing loss. On the other hand, Loeb and Smith found that the median pitch of tinnitus induced by exposure to octave-band noise (which was not investigated by Davis et al.) was only slightly higher than the center frequency of the stimulating noise and *below* the frequency of the maximum hearing loss (Table 2.1). All but 3 of the 86 cases studied by Loeb and Smith reported tonal tinnitus (the authors did not say which stimuli produced noise-like tinnitus).

The study by Atherley et al. exposed 57 subjects to 1/3-octave filtered noise, 51 of whom developed transient tinnitus. They found the median pitch of the tinnitus to be slightly higher than the center frequency of the exposing noise band in 43 of the 50 cases for which pitch matches were obtained, results similar to those found by Loeb and Smith for octave-band noise (Table 2.1). Again, like Loeb and Smith,

Table 2.1 Relations between frequency of the exposing sound, frequency of maximum hearing loss, and pitch of tinnitus in humans

Exposing sound	Maximum hearing loss relative to exposing sound	Pitch of tinnitus relative to	
		Exposing sound	Maximum hearing loss
Tone[a]	0.5 oct. above	1–1.5 oct. above	Not specified
Tone[b]	0.35–0.96 oct. above	1.04–1.76 oct. above	0.08–1.41 oct. above
1/3–oct. noise[c,d]	0.42–0.81 oct. above	0.12–0.58 oct. above	0.19–0.22 oct. below
1–oct. noise[b,d]	0.60–1.91 oct. above	0.04–0.61 oct. above	0.48–1.87 oct. below

[a] Davis et al., 1950.
[b] Loeb & Smith, 1967.
[c] Atherley et al., 1968.
[d] Center frequency of the noise bands used in the calculations. oct, octaves. The values are averages; there was notable individual variation in hearing loss and pitch of tinnitus

Atherley et al. found that the median pitch of the narrowband noise-induced tinnitus was below the frequency of the maximum hearing loss.

One important question on which there is conflicting evidence is whether exposing one ear to a loud sound results in tinnitus that is lateralized to that ear, to the other ear, or to both, a point not addressed in the three previously mentioned studies. Specifically, two studies reported that tinnitus is not always lateralized to the exposed ear. Theilgaard (1951) reported that of 189 exposures, tinnitus was lateralized to the unexposed ear in 33 exposures, with the remaining 156 exposures lateralized to the exposed ear. However, Thompson and Gales (1961), who exposed the ears of their 4 subjects multiple times, reported that 3 of the 4 subjects typically reported tinnitus lateralized, not to the exposed ear, but to the unexposed ear, with the remaining subject lateralizing tinnitus to one or the other ear or to both. Although the earlier report by Davis et al. (1950) did not comment on the perceived location of the tinnitus, one of the authors later stated that the tinnitus was always lateralized to the exposed ear (J. E. Hawkins, Jr., personal communication to H. Heffner, February 25, 2003). A possible explanation for these disparate results may lie in the time allowed between exposures. All three studies (Davis et al., 1950; Theilgaard, 1951; Thompson & Gales, 1961) involved exposing each ear on multiple occasions. The procedure used by Thompson and Gales involved exposing the left ear first and the right ear 1–2 hours later. In contrast, Davis and his colleagues waited at least several days between exposures (Theilgaard did not report the time between exposures). Thus, it is possible that the perception of tinnitus in the ear contralateral to the exposure might be due to the exposure reinstating tinnitus in the previously exposed ear (that tinnitus can be reinstated by a stimulus that in itself would not cause tinnitus has been suggested by Heffner [2011]).

In summary, human studies indicate that exposure to a loud sound sufficient to induce tinnitus would be expected to have the following results:

1. The exposure will produce an immediate hearing loss; if the exposure does not cause permanent damage, both the hearing loss and tinnitus will subside in a few days.

2. The tinnitus is more likely to have a definite and constant pitch if it is produced by exposure to tones or narrowband noise rather than broadband noise.
3. The exposure may affect the perception of physical stimuli, causing them to sound distorted, at least during recovery from the temporary portion of the hearing loss.
4. The median pitch of tone-induced tinnitus is higher than the frequency of the exposing tone; the median pitch of noise-induced tinnitus is usually near or slightly higher than the center frequency of the exposing noise band.
5. The median pitch of tone-induced tinnitus is higher than the frequency of the maximum hearing loss; on the other hand, the median pitch of noise-induced tinnitus is lower than the frequency of maximum hearing loss.
6. There is considerable individual variation in both the hearing loss and the pitch of the tinnitus induced by a loud sound.
7. It is likely that exposing one ear to loud sound will cause any resulting tinnitus to be lateralized to that ear, given that tinnitus has not been recently induced in the other ear.

2.2 Effect of Salicylate

In contrast to exposure to loud sound, a number of studies have examined the effect of salicylate on hearing and the auditory system (McFadden, 1982; Cazals, 2000) because salicylate, in the form of aspirin, is widely used as an analgesic and because its effects on hearing are believed to be temporary. The most noticeable effects of high doses of salicylate, usually administered orally, are tinnitus and hearing loss, both of which increase during the initial days of treatment and then level off, fluctuate, or decrease; the effects are reversible and typically disappear a few days after treatment is stopped (Cazals, 2000). The pitch of the tinnitus is usually described as a high-frequency tone or noise, although it is occasionally lower; one study found pitch matches ranging from 14.5 kHz down to 900 Hz, with the loudness of the tinnitus matched to external tones of greater than 60 dB (Day et al., 1989). The degree of hearing loss varies with the amount of salicylate, but the relationship between plasma salicylate levels and hearing loss is not perfect and there is much individual variation (Cazals, 2000). Some studies indicate that the hearing loss is equal at all frequencies whereas others have found that the loss is greater at high frequencies (cf. McCabe & Dey, 1965; Myers & Bernstein, 1965). No relationship between the pitch of the tinnitus and the hearing loss has been observed, possibly because the audiograms have not been sufficiently detailed, as they are typically conducted in octave steps, or because they did not extend into the high-frequency range above 8 kHz (McFadden, 1982). As previously mentioned, both effects are typically reversible, with the tinnitus subsiding and hearing returning to preexposure levels in 1–3 days after salicylate has been discontinued.

Besides inducing tinnitus and hearing loss, salicylate has been found to affect the perception of sound; the most prominent example is a hypersensitivity that

causes some sounds to be especially irritating, a phenomenon also referred to as hyperacusis (more on this later). Salicylate also affects other auditory functions such as frequency selectivity, temporal integration, and gap detection (Cazals, 2000).

Two final points are especially noteworthy. First, the effect of salicylate is highly variable; not only do the hearing loss and tinnitus vary between individuals with the same blood levels of salicylate, but the blood levels of salicylate among individuals given the same dosage may also differ noticeably (Cazals, 2000). Thus, animals given the same dose of salicylate would be expected to vary in their tinnitus. Second, salicylate crosses the blood–brain barrier, giving it the potential to cause tinnitus by acting directly on the central auditory system. However, elderly people with hearing loss resulting from loss of hair cells in the cochlea that encode high frequencies (presbycusis) do not develop tinnitus when given salicylate (Mongan et al., 1973; Schuknecht & Gacek, 1993). This suggests that it is the effect of salicylate on hair cells that causes tinnitus and any direct central effect of salicylate is not sufficient, although it may still play a role.

2.3 Interaction Between Tinnitus and Physical Sounds

An important question is whether tinnitus interacts with physical sounds. Over the years, there have been several reports of interactions between tinnitus and external sounds; one of the earliest and most detailed is that of R. L. Wegel (1931). Studying his own tinnitus, Wegel observed that his tinnitus interacted with external tones that were close in pitch to his tinnitus to make the sound "impure and discordant," although only at intensities close to threshold. He also observed that tones close in pitch to his tinnitus not only rendered the tinnitus inaudible, but were themselves inaudible—in other words, the tinnitus and the external tones cancelled each other out. Finally, Wegel stated that external tones could interact with his tinnitus to produce "mushy" beats.

Although other researchers have also found similar interactions between tinnitus and external tones, it is now believed that these occur only in cases in which the ear itself is generating a physical sound (McFadden, 1982; Penner & Jastreboff, 1996; Penner, 2000). Specifically, it is well established that the ear is not just a passive receiver, but that it can spontaneously generate sounds, referred to as spontaneous otoacoustic emissions (SOAEs). However, most tinnitus is not associated with SOAEs, which may account for why other researchers were unable to replicate Wegel's findings (e.g., Davis et al., 1950). Indeed, it has been emphasized that in some ways tinnitus is not like an external sound, especially when it comes to masking (McFadden, 1982; Penner & Jastreboff, 1996). For example, tinnitus can sometimes be masked by sounds that would not mask an external tone of similar pitch, and the intensity necessary to mask the tinnitus does not always relate to the tinnitus in the same way it relates to the masking of external tones.

There are, however, two well-established ways that tinnitus and external sounds do interact, although it should be noted that in both cases the external sound affects

the perception of tinnitus and not the other way around. One effect is that tones close in pitch to one's tinnitus will temporarily suppress the tinnitus, an effect that can make it difficult to match an external tone to the pitch of one's tinnitus; this is referred to as "residual inhibition" (e.g., McFadden, 1982). The other is that the intensity of a broadband noise used to mask tinnitus must be continuously increased over time to maintain the masking; this is in contrast to masking an external tone in which the level of the broadband noise remains relatively constant, and is an example of how tinnitus does not behave as does an external sound of equivalent loudness (Penner & Jastreboff, 1996).

3 Determining Auditory Sensitivity in Animals

Because the procedures for inducing tinnitus also cause a hearing loss, it is often necessary to obtain a measure of auditory sensitivity to rule out the possibility that changes in the performance of an animal after exposure to a tinnitus-inducing agent are the result of the hearing loss rather than the tinnitus. Because behavioral measures are time consuming, the threshold shifts are usually measured electrophysiologically. Thus, before describing the procedures for detecting tinnitus, it is helpful to review the correspondence between electrophysiological and behavioral measures of hearing loss.

3.1 Electrophysiological Measures of Auditory Sensitivity

The most commonly used electrophysiological measure of auditory sensitivity is the auditory brain stem response (ABR) because it is a relatively simple procedure to use. Unlike a behavioral assessment, which can take weeks or months to complete, the ABR allows an estimate of auditory sensitivity to be made on a sedated animal in a few hours. Although speed is a real advantage, it is necessary to determine if the results are sufficiently accurate for the purposes of the study.

A recent study comparing behavioral and ABR measures of threshold shift in rats exposed to loud sound found that the correspondence between the two measures depended on two factors: first, whether it was the initial threshold shift (the temporary plus permanent), or just the permanent threshold shift that was being measured; second, whether the stimulus to be detected was a tone or an octave-noise band (Heffner et al., 2008). Specifically, the tone-evoked ABR estimated the initial pure-tone threshold shifts to within ±5 dB only 11% of the time and the permanent threshold shifts 55% of the time, with large errors being common for both. Better correspondence between the ABR estimates and behavioral threshold shifts was found an octave (20- to 40-kHz) noise band, with the ABR estimating the initial threshold shifts to within ±5 dB 25% of the time and the permanent threshold shifts 89% of the time, with much smaller errors.

The finding that the ABR estimates the permanent pure-tone threshold shift to within ±5 dB about 60% of the time is not unusual, as comparisons of behavioral and evoked-potential thresholds recorded from the inferior colliculus after sensorineural damage found a similar degree of correspondence (Henderson et al., 1983; Davis & Ferraro, 1984; for a review, see Heffner et al., 2008). Although tone-evoked measurements of hearing do not give an accurate estimate of threshold shift, it may be possible to improve their accuracy by using octave noise rather than tones to evoke the ABR (Heffner et al., 2008).

4 Behavioral Procedures for Determining if Animals Have Tinnitus

There are currently eight behavioral procedures that have been used to test animals for tinnitus; they are discussed in the approximate order in which they were developed. In addition to describing the procedures, they are evaluated on the following points:

1. Would the tinnitus-inducing agent used cause tinnitus in humans?
2. Would the procedure detect tinnitus in humans?
3. Has the procedure been tested by simulating tinnitus with physical sounds?
4. Would the test be affected by an accompanying hearing loss?
5. Would the test be affected by hyperacusis?
6. Has the procedure been used to determine the pitch of tinnitus?
7. Are the results of the procedure consistent?
8. Does the procedure require group testing or can tinnitus be assessed in individual animals?
9. Can the procedure follow an animal's tinnitus over time?

Before beginning, it should be noted that interpreting these studies is complicated by the fact that there is no standard way in which the results are presented. In some studies, a high score indicates a negative response, that is, the animal is not responding to the stimulus, which could be either an external sound or its tinnitus; in others, it means the opposite. Adding to the confusion is that a positive sign of tinnitus could be either a high or a low score, depending on whether tinnitus was induced before or after training. These factors must be kept in mind when viewing the graphical presentations of the results.

4.1 Conditioned Suppression Procedure of Jastreboff

The first behavioral test of tinnitus in animals, developed by Jastreboff and his colleagues, uses the conditioned suppression procedure (Jastreboff et al., 1988). This consists of allowing a thirsty animal to drink from a water spout in the presence of

a background sound and then suppressing its drinking when the background sound is turned off by following the "silent" interval with electric shock.The effect of tinnitus on this task depends on when the tinnitus was induced. Animals in which tinnitus is induced *after* training are expected to continue perceiving a sound (their tinnitus) when the background sound is turned off and thus be less likely to suppress their drinking during testing (when the shock is discontinued). On the other hand, animals in which tinnitus is induced *before* training come to associate their tinnitus that they hear during the silent intervals with shock and are more likely to suppress their drinking when the shock is discontinued. This basic approach has been used by several laboratories, as described in the text that follows.

4.1.1 Jastreboff and Colleagues

The behavioral procedure used by Jastreboff and his colleagues was developed to test for tinnitus in rats given salicylate (e.g., Jastreboff et al., 1988; Jastreboff & Brennan, 1994). Although the details of their method have varied somewhat, the basic procedure is as follows. A thirsty rat is placed in a test cage for two or more daily sessions and accustomed to licking a water spout to obtain water in the presence of a broadband noise. Next, it is trained to stop licking whenever the broadband noise is turned off for 60 s by presenting a brief foot shock at the end of the "noise off" or silent interval. Training consists of one or more daily sessions in which the rat is presented with five silent intervals in each session. The number of licks the animal makes during the 60 s when the background noise is turned off is compared to the number it made during the preceding 60-s sound-on interval and the animal is trained until the number of licks during the silent interval is less than 25% of the number of licks in the preceding interval. The entire training procedure requires as few as seven daily sessions, by which time the animal is reliably discriminating silence from sound (e.g., Jastreboff & Brennan, 1994). For testing, the animals are exposed to a tinnitus-inducing agent and tested for five or more sessions with each session containing five silent intervals. Note that the animals are tested "in extinction" (the shock is turned off), which means that they eventually learn to continue licking when the background noise is turned off, at which point they can no longer be tested.

The results have shown that rats given salicylate after training are more likely to continue drinking during silent intervals than control animals given saline, suggesting that the animals given salicylate develop tinnitus and thus no longer experience silence, which was associated with shock, during the noise-off intervals (Fig. 2.2). Indeed, there is a dose–response relationship such that the effect of salicylate on behavior increases as a function of dosage, suggesting that the more salicylate the more salient the tinnitus and the less likely an animal is to stop drinking when the background sound is off (Jastreboff & Brennan, 1994). On the other hand, rats given salicylate before training are less likely than control animals to continue drinking during silent intervals, suggesting that they develop tinnitus during training and came to associate it with the shock (Fig. 2.2); this would work if the background

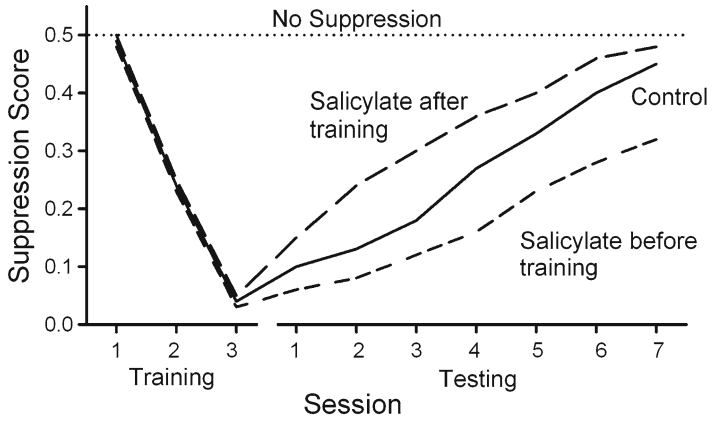

Fig. 2.2 Hypothetical example of the results using Jastreboff's conditioned suppression procedure. Rats are trained to stop drinking when a background sound is turned off by following the silent intervals with shock. The suppression score is the number of licks during the silent interval divided by the number of licks in the preceding sound interval plus the number of licks in the silent intervals, that is, During/(Pre + During). A score of 0.5 indicates no suppression whereas a score of 0.0 indicates complete suppression. During testing, the shock is turned off and the animals eventually stop suppressing. Animals given salicylate *before* training have learned to associate their tinnitus with shock and take longer than control animals to stop suppressing. Animals given salicylate *after* training generalize to their tinnitus from the background sound and take less time than control animals to stop suppressing. (After Penner & Jastreboff, 1996.)

noise masked the animals' tinnitus so that it was more apparent during the silent intervals.

To demonstrate that animals have developed tinnitus, it is necessary to rule out alternative explanations of their behavior. One of the first is the possibility that the salicylate has some general effect on behavior, such as a change in thirst, motivation, or an analgesic effect that reduced sensation of the foot shock However, these explanations are easily ruled out because giving salicylate before training has the opposite effect of giving salicylate after training; the first causes the animals to be less likely to drink whereas the second causes them to be more likely to drink when the background sound is turned off (Jastreboff et al., 1988). Thus, the results do not seem to be due to any general motivational effect of salicylate on the tendency of animals to drink or to avoid foot shock. One point that may be noted is that the animals given salicylate before training suppress more than the untreated control animals, suggesting that their tinnitus was a more effective signal for shock than was silence (Fig. 2.2); that a sound can make a more effective signal for shock than silence was supported by a test in which a 7-kHz tone was also found to be more effective than silence in causing rats to suppress their licking (Jastreboff et al., 1988).

Jastreboff and his colleagues have addressed three other questions regarding their procedure: What might be the effect of hearing loss? Would the animals be expected to generalize from the background sound to their tinnitus? Is the effect of

salicylate restricted to auditory stimuli? The impact of hearing loss was addressed by showing that reducing the SPL of the background sound by 20 dB did not cause the animals to test positive for tinnitus (Jastreboff, 1990); thus a hearing loss of up to at least 20 dB would not be expected to affect the results. That animals trained with broadband noise as a safe signal would generalize to tonal tinnitus was addressed by showing that presenting a tone when the broadband sound was turned off (i.e., simulating tinnitus) caused untreated animals to respond much as did the salicylate-treated animals (Brennan & Jastreboff, 1991: Jastreboff et al., 1988). Finally, salicylate had no effect on rats that had been trained to stop licking when a light (instead of noise) was turned off (Jastreboff et al., 1988); thus, salicylate does not have a general effect on an animal's performance, but, instead, its effect is specific to auditory tasks.

Jastreboff's procedure has also been used to estimate both the apparent loudness and the pitch of tinnitus resulting from salicylate. Apparent loudness is estimated by comparing the responses of animals given different doses of salicylate with the responses of animals given different intensities of a tone simulating tinnitus. The expectation is that the perceived loudness of tinnitus in salicylate-treated animals can be determined by matching their score (i.e., the amount they differ from the control group) to the score of the animals in the simulated tinnitus test (Jastreboff & Brennan, 1994). Thus, for example, the perceived loudness (or salience) of tinnitus in a group of animals given salicylate was considered to be 60 dB because their average score matched that of a group of untreated animals for whom a 60-dB, 10-kHz tone was turned on during the silent intervals.

The pitch of the animals' tinnitus was determined by administering salicylate to them before training so that any tinnitus they developed would be paired with shock; they were then presented with tones of different frequencies in the expectation that tones similar in pitch to their tinnitus would cause greater suppression of licking (Brennan & Jastreboff, 1991). The results showed that the suppressing effect of tones increased as their frequency was increased from 7 to 11 kHz, leading the authors to suggest that the tinnitus in rats caused by salicylate was 10 kHz or higher (Fig. 2.3). However, because the animals were not tested above 11 kHz, to determine if higher frequencies caused even less suppression, it is possible that the pitch of the tinnitus may actually be higher. The possibility that these results were affected by the hearing loss caused by salicylate, which increases with frequency (Brennan et al., 1996), was ruled out by showing that rats given salicylate after training, which would have had the same hearing loss, differed in their response to the tones from those animals given salicylate before training (Fig. 2.3).

4.1.2 Other Investigators Using Jastreboff's Procedure

Jastreboff's conditioned suppression procedure has been modified and used by other researchers, two examples of which are presented here. First, the procedure has been used with two modifications to detect tinnitus in hamsters exposed in one ear to a loud sound: avoidable shock was used, which would make it more difficult for

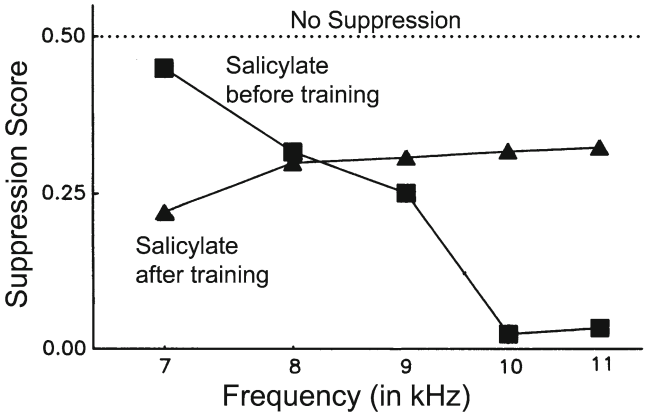

Fig. 2.3 Estimation of the pitch of tinnitus in rats given salicylate using the Jastreboff conditioned suppression procedure. Rats trained to suppress licking when a background noise was turned off were then tested by presenting a tone when the noise was turned off. Rats given salicylate before training learn to associate their tinnitus (audible during silent periods) with shock. Their suppression to 10- and 11-kHz tones suggests that those frequencies are similar in pitch to their tinnitus. Rats given salicylate after training (at the beginning of testing) associated neither their tinnitus nor the tones with shock and suppressed less to the tones. Because the animals were not tested at higher frequencies, beyond the range in which the tones had a suppressing effect, it is not known if the pitch of the tinnitus is higher than 11 kHz. See Figure 2.2 for a description of the suppression score. (Modified from Brennan & Jastreboff, 1991.)

the hamsters to learn that the shock was turned off during testing as they usually avoided the shock during training, and the hamsters were given extensive training to increase the possibility that they would respond to any tinnitus they might develop (Heffner & Harrington, 2002). The test consisted of training hamsters to discriminate silence from broadband noise and tones ranging in frequency from 8 to 24 kHz, the pitch range over which they might be expected to develop tinnitus after being exposed to the loud sound (a 124-dB, 10-kHz tone). In addition, the location of the loudspeakers through which the sounds were presented was varied because preliminary testing indicated that changing the location of the sound source would otherwise affect the animals' response—an important consideration as the animals would likely develop tinnitus only in one ear. The results of this study showed that exposure to the 10-kHz tone made the hamsters more likely to drink during silent intervals, as compared to control animals, indicating that they were hearing a sound, that is, their tinnitus. In addition, the longer the duration of the exposure to the 10-kHz tone, the higher the tinnitus score, suggesting that longer exposures made the tinnitus more salient. The hearing loss resulting from the tone exposure was not considered a factor because only one ear was exposed, leaving the other ear with normal hearing.

The conditioned suppression procedure has also been used by Zheng and his colleagues to look at the effect of various drugs on salicylate-induced tinnitus in rats (e.g., Zheng et al., 2010). They modified the procedure developed by Jastreboff in

two ways. First, because Jastreboff's results suggest that salicylate produces tinnitus similar in pitch to 10- to 11-kHz tones, they use background tones of 8–11 kHz, instead of noise, on the assumption that the animals will be more likely to generalize from the tones to their tinnitus. Second, they screen each animal by testing its response to salicylate to one or more background tones. If an animal tests negative to one tone, they try a tone of a different frequency or intensity; if no tone can be found that causes the animal to test positive for tinnitus, it is assumed not to develop tinnitus and is dropped from the study. Thus, in testing the effects of various drugs on salicylate-induced tinnitus, only animals that have previously tested positive are included in the study.

4.1.3 Conditioned Suppression Summary

In summary, Jastreboff's conditioned suppression procedure is based on training animals to discriminate the presence of a physical sound from its absence by training them to cease drinking when the sound is turned off, an event that signals impending shock (which may or may not be avoidable). The procedure can work two ways: if tinnitus is induced after training, the animals are expected to generalize to it as a safe signal and be more likely to continue drinking when the sound is turned off; if tinnitus is induced before training, the animals will associate it with shock and be less likely to drink when the sound is turned off.

Evaluating Jastreboff's conditioned suppression procedure on the nine points:

1. The tinnitus-inducing agents used with this procedure (salicylate, quinine, exposure to loud sound) would be expected to cause tinnitus in humans.
2. The procedure of having subjects report the presence or absence of sound as a way of determining if they have tinnitus would reveal tinnitus in humans.
3. The procedure has been tested by simulating tinnitus with physical sounds, showing that animals trained to respond to broadband noise will generalize to tones.
4. Hearing loss as a factor has been ruled out by showing that reducing the background sound, to similate a hearing loss, does not cause animals to test positive for tinnitus and by testing animals that have been exposed to loud sound in only one ear, which leaves them with normal hearing in the other ear.
5. Because the animals are trained to discriminate sound from silence, as opposed to responding to the quality of the sound, hyperacusis would not be expected to affect the results.
6. The procedure has been used to determine the pitch of tinnitus induced by salicylate.
7. The results are generally consistent with higher doses of salicylate and increased exposure to loud sound resulting in higher tinnitus scores.
8. Animals are tested in groups, with an untreated control group for comparison; this reduces the statistical power of such studies and requires large numbers of animals.

9. The procedure cannot be used to follow the animals' tinnitus over time because the shock is turned off (they are tested in extinction), which soon causes them to stop responding to their tinnitus.

4.2 Conditioned Avoidance Procedure of Bauer and Brozoski

The behavioral procedure devised by Bauer and Brozoski is derived from that of Brennan and Jastreboff (1991). It involves training animals to discriminate sound from silence and then presenting tones during the silent intervals with the expectation that the animals will respond differently to tones that are similar to their tinnitus than to tones that are not similar (Brozoski & Bauer, 2005, 2008). Specifically, an animal is trained to press a lever to receive food in the presence of 60-dB SPL broadband noise. Next, trials are presented in which the broadband noise is turned off for 60 s, at the end of which the animal receives foot shock. However, the shock is not delivered if the animal reduces the number of lever presses during a silent trial to a specified criterion, such as less than 25% of the number emitted during the preceding 60-s noise interval. Once the animal has learned to discriminate sound from silence, additional trials are presented in which the noise is turned off and replaced by a tone. Tones of different frequency and intensity are presented to determine the frequency at which the average performance of animals exposed to a tinnitus-inducing agent differs statistically from the average performance of unexposed control animals; the frequency at which the two groups differ is considered to match the pitch of the animals' tinnitus. Because the animals are still shocked during testing when their responding during silent trials exceeds the criterion, their response to silence does not extinguish and testing is continued indefinitely. The procedure requires carefully trained animals and can take several months for training and testing (e.g., Brozoski et al., 2007b).

As with Jastreboff's procedure, the response of an animal depends on whether it is exposed to a tinnitus-inducing agent before or after training. In the most commonly used variation, animals are exposed to the tinnitus-inducing agent before training so that any tinnitus they may develop is perceived during the silent intervals and becomes associated with shock (it is assumed that the background noise renders their tinnitus inaudible or at least less audible). Accordingly, tones that resemble an animal's tinnitus are expected to decrease lever presses, as compared to control animals with no tinnitus, although none of the tone trials is ever followed by shock. In the second variation, the animals are exposed to a tinnitus-inducing agent after training; in this case, it is believed that any tinnitus the animals develop will interact with tones similar in pitch to produce a "noisier" sensation, making it sound more like the background noise and cause the animals to be more likely to continue lever pressing than control animals.

The most common tinnitus-inducing agent used in these studies is octave noise centered at 16 kHz and applied to one ear at an intensity of 110–120 dB for an hour (e.g., Brozoski et al., 2007a). Interestingly, the animals do not test positive for tinnitus

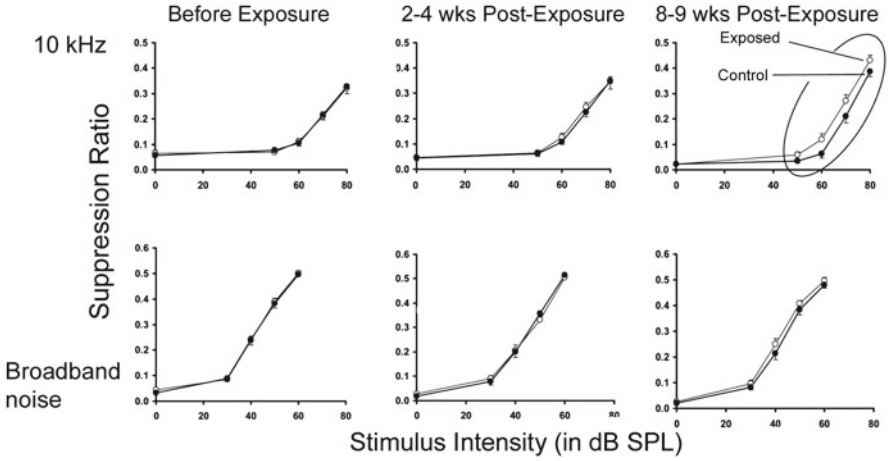

Fig. 2.4 Example of the results obtained with the Bauer and Brozoski avoidance procedure (from Turner et al., 2006). Rats were trained to stop pressing a lever for food when a background sound was turned off (i.e., silence) to avoid shock. The animals were then exposed to a loud sound (16-kHz octave noise, 116 dB, 1 hour) in one ear and tested by replacing the background sound with another sound, such as a 10-kHz tone or broadband noise. Four different intensity levels were used for each sound. Scores were calculated using the same formula as used in Figures 2.2 and 2.3. Thus, a score of 0.5 indicates no suppression of lever pressing whereas a score of 0.0 indicates complete suppression. The exposed rats showed less suppression than the control animals 8–9 weeks later when a 10-kHz tone was presented (top right panel) than when broadband noise was presented (bottom row), indicating that they developed tinnitus that was similar to the 10-kHz tone, but not to the broadband noise. Note that 0 dB actually indicates no sound (silent interval) and the rats were not shocked if their lever presses during a silent interval was less than 25% of their lever presses during the preceding background noise interval. (Modified from Turner et al., 2006.)

until weeks or months after exposure, which is interpreted as indicating that the exposure causes delayed chronic but not acute tinnitus (Fig. 2.4). Animals exposed to the 16-kHz noise have differed from control animals at 10, 16, 18, 20, 22, and 24 kHz (Bauer & Brozoski, 2001; Turner et al., 2006). Overall, 20 kHz is the most common frequency at which the exposed animals differ from the controls, leading to the conclusion that exposure to the 16-kHz octave noise band typically results in tonal tinnitus that most closely matches 20 kHz. The basis of the explanation of why exposed animals sometimes differ from controls on more than one frequency is that tinnitus of those animals might be noise-like and lack a clear tonal quality.

Hearing loss does not seem to be an explanation for the results. Although an initial study indicated that the 16-kHz octave-noise exposure caused a permanent threshold shift in the auditory brain stem response of about 60 dB for tones from 4 to 31.5 kHz, as well as for clicks (Bauer & Brozoski, 2001), later studies indicated a temporary threshold shift in the ABR with recovery to near preexposure levels over time (e.g., Brozoski et al., 2007a). In any case, exposing only one ear leaves hearing intact in the other ear and a control study in which earplugs were inserted in one ear demonstrated that a monaural hearing loss did not affect the response of animals to tones (Bauer & Brozoski, 2001).

The explanation for why the exposed animals differ from the controls on some tones has changed over the years. Initially, it was proposed that the tinnitus caused the animals to perceive the tones as louder or noisier, which would explain why animals exposed to a tinnitus-inducing agent after training would be more likely to maintain lever pressing when the background noise had been turned off and replaced by a tone (Bauer et al., 1999). However, as previously noted, there is no evidence that tinnitus in humans affects the perception of external sounds in this or any other way, although hyperacusis does. More recently, it has been proposed that the animals are likely to respond to external tones that resemble their tinnitus; that would explain why animals exposed to a tinnitus-inducing agent before training, and that then come to associate their tinnitus with shock, would be more likely to suppress to tones that are perceived to be similar to their tinnitus (Brozoski & Bauer, 2005). One way to support this interpretation would be to simulate tinnitus with an external tone to see how readily animals generalize to tones of similar frequency—this has not yet been done.

The most remarkable aspect of this research is the idea that an animal can be exposed to a sound that is too low to cause instantaneous tinnitus, but is sufficient to cause permanent tinnitus that emerges months later. Although there is no documented evidence that this occurs in humans, two lines of evidence may be offered in its support. First, it has been claimed that there are patients who, after years of exposure to loud sound, have developed tinnitus for the first time (e.g., Kaltenbach & Godfrey, 2008). However, such reports must be viewed with caution; do the patients mean that this is the first time they have ever experienced tinnitus or is it the first time their tinnitus has become persistent or distressing? The second line of evidence relies on the observation that exposure to loud sound causes an increase in spontaneous activity in the dorsal cochlear nucleus (DCN), activity that might be the source of tinnitus and that reaches a maximum a week or so after exposure (Kaltenbach & McCaslin, 1996). However, the limited behavioral evidence on this point indicates that the increased spontaneous activity in the DCN is related, not to tinnitus, but to the accompanying hearing loss (Zhang et al., 2004), possibly the result of release from inhibition in the cochlear nucleus due to hair cell damage in the cochlea; there is also evidence that the increased activity could be due to a greater sensitivity to somatic inputs to the DCN after hearing loss (Shore et al., 2008).

Another issue is whether the exposed and control groups are differing by chance. That is, what is the likelihood that two groups of animals, tested over time on half dozen different sounds, might begin to differ statistically on at least one of the sounds. This question could be answered by a control test in which one group of animals is given sham exposures to see if they eventually differ from a control group on some frequency.

Finally, the claim that exposure to the 16-kHz octave-noise results in tinnitus that does not appear until weeks or months later raises a question that has not yet been addressed. According to the Bauer/Brozoski procedure, exposing animals to a loud sound *before* initial training causes them to associate their tinnitus with shock and press a lever less than a control group when presented with a tone similar in pitch to their tinnitus. On the other hand, exposing animals to a loud sound *after* initial training implicitly trains them to use their tinnitus as a cue that it is safe to press the lever and

causes them to lever press more than the control group when presented with a tone that resembles their tinnitus (Brozoski & Bauer, 2008). However, if exposing rats to 16-kHz octave-noise (110–120 dB for an hour) results in tinnitus that does not appear until weeks later, when the animals are in the testing phase, then it would seem that that there was no tinnitus for the group that was exposed before training to associate with shock and their behavior should not differ from the group that was exposed after initial training. Furthermore, because all animals are shocked during testing when their response rate on silent trials exceeds the criterion, both groups are now receiving identical training, which might be expected to override their initial training.

Evaluating Bauer and Brozoski's conditioned avoidance procedure on the nine points:

1. The form of tinnitus being studied has not been observed in humans, as there is little evidence of exposure to loud sound causing tinnitus that does not appear until weeks after the exposure.
2. It is not known if the procedure would reveal tinnitus in humans, as there is no evidence in the human literature for tinnitus modifying the perception of physical sounds. Although it is conceivable that an external sound could be confused with tinnitus, this has not been studied.
3. The procedure has not been tested by simulating tinnitus with external sounds to determine how well animals generalize to different tones.
4. Hearing loss is eliminated as a factor by exposing only one ear to loud sound, leaving the other ear with normal hearing.
5. If exposing an ear to a loud sound makes some sounds appear "noisier," this could be explained by hyperacusis, as opposed to tinnitus interacting with the sounds.
6. The procedure has been used to determine the pitch of tinnitus.
7. The tones to which the tinnitus is pitch matched vary from study to study. Although this may be because of the variable nature of tinnitus, the possibility that the results are random variation needs to be addressed.
8. Animals are tested in groups, with an untreated control group for comparison; this reduces the statistical power of such studies and requires large numbers of animals.
9. The procedure is used to follow tinnitus over time. However, the fact that all animals are shocked during testing when their response rate to silent trials exceeds a criterion would be expected to reduce any differences in the responses to tinnitus between animals that were exposed before versus after initial training.

4.3 Conditioned Avoidance Procedure of Rüttiger

As with the previous procedures, the one devised by Rüttiger and colleagues is based on training animals to discriminate the presence of a background sound from its absence (Rüttiger et al., 2003). Their goal was to devise a procedure that required only mild deprivation (15–18 hours of water deprivation) and used avoidable, as opposed to unavoidable, foot shock (although Jastreboff's procedure can also be used with avoidable shock, e.g., Heffner & Harrington, 2002).

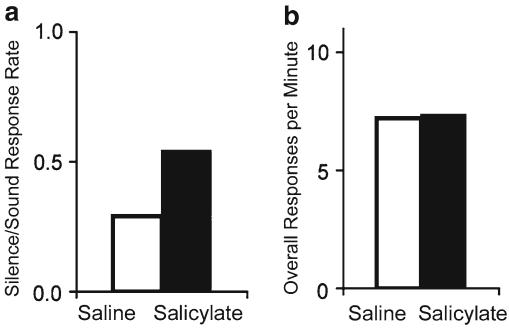

Fig. 2.5 Illustration of the results obtained by Rüttiger and colleagues using the alternation procedure in which rats stop responding when the background sound is turned off. (**a**) The ratio of activity during silent periods versus sound periods is greater in animals given salicylate (filled bar) than when they are given saline (open bar). (**b**) The overall responding per minute is not affected by salicylate (filled bar) as compared to saline (open bar), indicating that the greater response rate of the salicylate animals during silence is not due to a general increase in their response rate. (Modified from Rüttiger et al., 2003.)

Rüttiger's procedure is an alternation task in which an animal is trained to alternate between two drink tubes that dispense a 3% sucrose solution, which rats prefer to plain water. After obtaining a reward from one drink tube, the rat has to go to the other one to obtain the next reward. The animal is then trained to go to the drink tubes only when a 70-dB SPL broadband noise is on by rewarding it when the sound is on and shocking it if it licks a tube when the sound is off. The animal's performance is calculated as the ratio of its response rate during 60-s silent intervals divided by its response rate when the broadband noise is on. An animal is trained until its response rate during the silent intervals is 20% or less of its response rate during the broadband noise intervals.

Once the animal has learned to reliably perform the discrimination, it is put on a partial reward schedule in which only every second or third alternation is rewarded. This is to prevent the animal from extinguishing too quickly during tinnitus testing, which is conducted with both reward and shock turned off; that is, the animals are run in extinction. However, if the tinnitus is transient, as it is expected to be when induced by salicylate, the animals can be retrained and tested again later.

Testing for tinnitus involves comparing the response of the animals after they receive salicylate with the response of the same animals after they receive saline. Specifically, an animal is injected with either saline or salicylate and placed, 3 hours later, into the alternation box for 12–15 minutes. Sound and silent periods are presented as before, but the animal is not shocked and it receives the sucrose solution only for the first 4 minutes of the session to get it to start responding (the data from the first 4 minutes are not used). Responding more often during the silent intervals after receiving salicylate is taken as a sign that the salicylate has caused tinnitus which the rat interprets as a signal that it will be rewarded, and not shocked, for licking the drink tubes (Fig. 2.5). This procedure has been used to detect tinnitus after exposure to loud sound as well as to salicylate (Tan et al., 2007).

Potential alternative explanations of these results have been addressed in control tests (Rüttiger et al., 2003). To begin, the possibility that the results could be explained by a general effect of salicylate on activity was ruled out two ways; the first was by showing that animals given salicylate continue to respond at the same rate as untreated animals; the second was by showing that salicylate had no effect when a light, instead of a sound, was used to signal when it was appropriate to respond.

Another possible explanation is that the hearing loss caused by salicyalte could have affected these results, either by increasing or decreasing activity during silent intervals. That is, if animals are particularly fearful of the shock, a decreased ability to discriminate silent from sound intervals would cause their response rate to decrease. If, on the other hand, the animals are highly motivated to obtain the sucrose solution, their response rate after the reward is turned off might increase in what is known as an "extinction burst" (e.g., Miller, 2006). When the effect of a hearing loss was simulated in untreated rats by reducing the intensity of the broadband noise in stepwise fashion, it was found that the broadband noise could be reduced by 20 dB without significantly affecting their performance; reducing the intensity further caused their response rate during the silent intervals to decrease whereas the overall response rate of rats given salicylate is the same as when they are given saline. Thus, it appears that any reduced ability to hear the broadband noise would not be a factor in this test.

The main reason for determining the effect of reducing the intensity of the broadband noise was to estimate the perceived intensity of the animals' tinnitus. That is, by reducing the level of the broadband noise it was possible to find the intensity at which untreated rats matched the scores of the salicylate-treated animals; the estimate of the tinnitus intensity for rats given 350 mg/kg of salicylate was 28 dB SPL.

Evaluating Rüttiger's conditioned avoidance procedure on the nine points:

1. Humans given the tinnitus-inducing agents used here (salicylate and loud sound) would be expected to develop tinnitus.
2. The procedure of having subjects report the presence or absence of sound as a way of determining if they have tinnitus would also reveal tinnitus in humans.
3. The procedure has not been tested by simulating tinnitus with external sounds.
4. Hearing loss as a factor has been addressed by determining the effect of reducing the level of the broadband noise for untreated animals.
5. Because the animals are trained to discriminate sound from silence, as opposed to responding to the quality of the sound, hyperacusis would not be expected to affect the results.
6. The procedure does not indicate the pitch of the animals' tinnitus; it has, however, been used to indicate the perceived intensity of the tinnitus.
7. The results are generally consistent with previous experiments that have found evidence of tinnitus after similar doses of salicylate and exposure to loud sound.
8. The animals are used as their own controls, making it possible to assess tinnitus in individual animals, although group data are usually presented.

9. The procedure cannot be used to follow the animals' tinnitus over time because both the reward and shock are turned off (they are tested in extinction), which causes them to eventually stop responding to the sound.

4.4 Conditioned Avoidance Procedure of Guitton

Guitton and his colleagues have devised an avoidance procedure to assess rats for tinnitus in which an animal climbs a pole to avoid shock when it perceives a sound (Guitton et al., 2003). The procedure consists of placing a rat in a test box that has a grid floor and a pole; the animal is trained to climb the pole to avoid electric shock delivered through the floor whenever a 10-kHz 50-dB SPL tone is presented. Unlike the previous procedures, the shock is associated with the *presence* of sound rather than its absence. A rat is trained in 10-minute sessions in which the tone is presented 10 times and the animal required to climb the pole to avoid or escape the shock. Training is considered complete when the rat successfully avoid the shock 80% of the time or better in three consecutive sessions; more than one session can be conducted in a day, so training takes only 2–3 days. Having the animal climb a pole to avoid the shock is a novel procedure which may have been chosen to increase the response costs to the animal, thus decreasing its false-positive rate; other avoidance tasks, such as one in which an animal need only cross from one side of a cage to another to avoid shock, have the drawback in that an animal that becomes too fearful of the shock may avoid it by constantly crossing back and forth regardless of whether the sound is on.

The 10-kHz tone was chosen as the training signal because the work of Jastreboff and his colleagues has indicated that the pitch of the tinnitus caused by salicylate may be close to that frequency (although as previously noted, the pitch may be higher). Testing is conducted with the shock delivered when an animal fails to response to the tone and tinnitus is expected to increase an animal's responding during silent intervals. Injecting rats with salicylate caused the animals' average tone detection rate to decline slightly and their false positive rate to increase markedly, results that could be explained by either tinnitus, which resembles the tone that signals shock, or a hearing loss, which makes it difficult for the animal to discriminate the tone trials from the silent intervals (Fig. 2.6). However, increasing the intensity of the tone to compensate for the animals' hearing loss, as estimated by the compound action potential, prevented their detection rate from decreasing, but did not keep their false-positive rate from increasing, a result consistent with the animals having developed tinnitus to which they responded as if it were the warning tone. A control test in which the animals were trained to climb the pole when a 4-kHz tone was presented found that although salicylate reduced the animals' detection rate slightly, it did not increase their responding during silent intervals, presumably because the animals did not generalize from 4 kHz to the higher pitch of their tinnitus (although it may be noted that salicylate causes a noticeable hearing loss at 10 kHz, but little or no hearing loss at 4 kHz, Brennan et al., 1996).

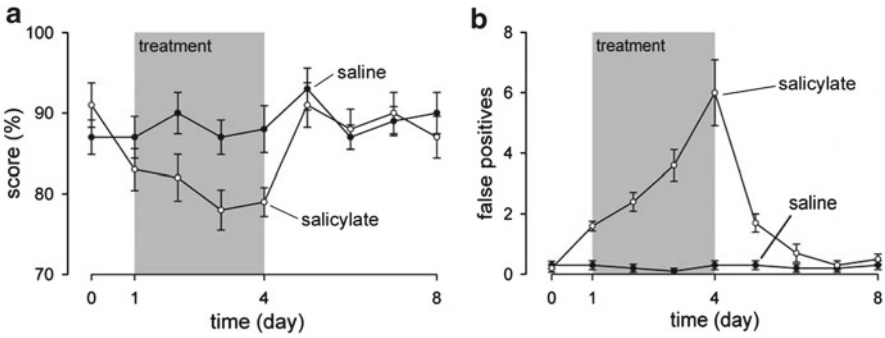

Fig. 2.6 Example of the results obtain by Guitton et al. in which rats climb a pole to avoid shock when a sound is turned on. The results show that salicylate lowers the score (**a**, hit rate), but increases the false-positive rate (**b**, responding during silence), which is interpreted to indicate that the animals perceive sound (tinnitus) during silent periods. (Modified from Guitton et al., 2003.)

Evaluating Guitton's avoidance procedure on the nine points:

1. Humans given salicylate would be expected to develop tinnitus.
2. The procedure of having subjects report the presence or absence of sound as a way of determining if they have tinnitus would reveal tinnitus in humans.
3. The procedure has not yet been tested by simulating tinnitus with different external sounds to determine how well animals generalize to different sounds. Knowing how well the animals generalize to other sounds could lend support for the interpretation that rats trained to respond to a 4-kHz tone do not test positive for tinnitus when given salicylate because it is too different in pitch from the training tone.
4. Hearing loss as a factor was addressed by showing that the results did not change when the level of the 10-kHz tone was increased to compensate for the salicylate-induced hearing loss. (It should be noted that the hearing loss was estimated with the compound action potential [CAP], an imperfect measure of behavioral hearing loss, and distortion produced otoacoustic emissions [DPOAEs], which have not been evaluated as a measure of behavioral threshold shift.)
5. Because the animals are trained to discriminate sound from silence, as opposed to responding to the quality of the sound, hyperacusis would not be expected to affect the results.
6. The procedure has not been used to indicate the pitch of an animal's tinnitus.
7. The results are generally consistent with previous experiments that have found evidence of tinnitus following similar doses of salicylate.
8. Although the procedure can reveal tinnitus in individual animals by demonstrating an increase in false-positive rate after treatment, comparisons are typically made between groups of treated and untreated control animals.
9. Because the animals receive shock on tone trials if they do not respond, the response does not extinguish and the animals' tinnitus could be followed over time.

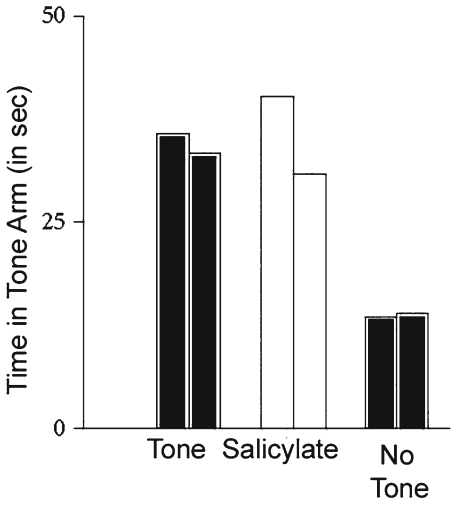

Fig. 2.7 Example of the results of the water T-maze procedure developed by Guitton and Dudai (2007). The paired bars represent the amount of time the rats spent in the "tone" arm during the first and second 50 s of testing. Salicylate caused the rats to spend more time than the control animals in the tone arm when no tone was present, indicating that they were hearing a sound (i.e., their tinnitus). (Modified from Guitton & Dudai, 2007.)

4.5 Water T-Maze Procedure of Guitton

Guitton and colleagues have also devised a two-choice procedure to test for tinnitus that consists of a water T-maze in which rats swim to the left or right side depending on whether or not they perceive a sound (Guitton & Dudai, 2007). This procedure has the advantage of not using either deprivation or electric shock; instead, rats are motivated to find a resting place in the water maze by swimming to a submerged platform. Specifically, rats are placed in the start arm of the T-maze and trained to swim to the right arm when a tone is on and to swim to the left arm when there is no tone. The training stimulus consists of a 10-kHz tone as the researchers expect that tinnitus induced by salicylate or by exposure to a 130-dB, 6-kHz tone will be similar in pitch to that frequency. The rats are trained in 3 daily sessions in which they receive 12 trials per session with the tone and no-tone trials alternated in blocks of 3; this may not be the best choice of trial presentation because an animal could ignore the sound altogether and still perform well by doing a triple alternation (the use of a Gellermann schedule would eliminate this possibility; Gellermann, 1933). In addition, water maze tests are generally conducted with a substance added to the water to make it milky (e.g., powered milk) so that the rats cannot see the submerged platform, a point not mentioned by the authors. Testing is conducted in a single trial session in which an animal is placed in the T-maze with no platform. The primary measure of an animal's performance is the amount of time it spends in each of the two arms over a period of 100 s (which is broken into the first and second 50-s intervals); the first arm chosen by the rat is also recorded, although not always considered in analyzing its performance.

The authors validate the T-maze test by demonstrating that rats given 4 days of injections of salicylate spend more time in the right (tone) arm than in the left (no-tone) arm even though no sound is presented (Fig. 2.7). Similarly, rats whose

cochleas are treated with ifenprodil, which the authors expect will block tinnitus, causes the animals to spend more time in the no-tone than in the tone arm of the maze.

Exposing rats to a 130-dB, 6-kHz tone for 15 minutes (both ears are apparently exposed to the sound) and testing them 2 weeks later, however, resulted in a more complicated situation. As expected, the authors found that rats trained in the T-maze with a 6-kHz tone did not test positive for tinnitus, presumably because any tinnitus they might have had did not match the pitch of the 6-kHz training tone. However, of the 26 animals trained with the 10-kHz tone, only 12 of them tested positive for tinnitus. The authors interpret this as indicating that not all animals develop tinnitus after exposure to loud sound. There is, however, an alternative interpretation.

Exposure to loud sound causes immediate tinnitus that declines over time. Based on what can be gleaned from Davis et al. (1950), it appears that exposures in the range used on the rats in this study may not cause either permanent hearing loss or permanent tinnitus. Similarly, a recent study that used sound exposures somewhat less than used here (110-dB, 10-minute tone exposures) found that rats stopped testing positive for tinnitus within a few days after the exposure (Heffner, 2011). Thus, it is possible that few, if any, of the rats had tinnitus by the time they were tested in the water maze two weeks after exposure. In this case, the observation that roughly half the animals went to one arm of the T and the others went to the other arm might indicate that, as a group, they were responding randomly.

Evaluating Guitton's water T-maze procedure on the nine points:

1. Humans given salicylate would be expected to develop tinnitus. However, the exposures to loud sound may not have been sufficient to produce chronic tinnitus.
2. Requiring subjects to respond left or right depending on whether they perceive a sound would reveal tinnitus in humans.
3. The procedure has not been tested by simulating tinnitus with different external sounds to determine how close in pitch a sound must be to the training sound for an animal to test positive.
4. Hearing loss would not be expected to affect results as no sound is presented during testing.
5. Because the animals are trained to discriminate sound from silence, as opposed to responding to the quality of the sound, hyperacusis would not be expected to affect the results.
6. The procedure could be used to study the pitch of tinnitus by training animals with different tones to determine which result in the highest tinnitus scores.
7. The results are generally consistent with previous experiments that have found evidence of tinnitus after similar doses of salicylate. The results of the exposure to loud sound are inconclusive.
8. Although individual animals may be tested, group data compared to an untreated control group is presented.
9. The procedure cannot be used to follow tinnitus because the animals are tested in extinction, that is, with no platform available to swim to.

4.6 Schedule-Induced-Polydipsia Avoidance Conditioning of Lobarinas

Schedule-induced-polydipsia avoidance conditioning involves training an animal to stop licking a water spout whenever it perceives a sound by shocking it if it licks when an external sound is present; thus, tinnitus is indicated when an animal treated with a tinnitus-inducing agent reduces its responding during silent intervals (Lobarinas et al., 2004). A novel aspect of this procedure is that the animals are not water deprived, but are food deprived and lick a water spout while waiting for food pellets. Because rats in this situation will consume excessive water, their behavior is referred to as polydipsia. Using a schedule of food reward to get the animals to drink (instead of depriving them of water) maintains their licking at a constant rate that does not vary with their level of thirst (although it may vary with the level of food satiation).

Specifically, a food-deprived rat is placed in a test cage and allowed to lick a water spout while food pellets are delivered at the rate of one per minute; if a rat does not spontaneously lick the spout, it is water deprived for a day or two to get it to begin drinking. For testing, a food pellet is delivered and followed by a 30-s period during which an external sound may or may not be presented; the animal is shocked if it licks in the presence of a sound, but not if there is no sound. This 30-s test period is followed by another 30-s period during which a sound is always presented and the animal shocked if it licks during that period; a food pellet is delivered at the end of this period and the next trial begins. The sounds consist of narrowband noise centered on frequencies ranging from 4 to 20 kHz to cover the presumed pitch range of tinnitus. Thus, the rats learn to lick during intervals of silence, but not during sound.

This procedure has been used to test rats for tinnitus after administration of salicylate, quinine, or loud sound (Lobarinas et al., 2006). The low incidence of licking during silence after exposure to a tinnitus-inducing agent is taken as a sign that the animals now hear a sound—their tinnitus (Fig. 2.8). Tests of unilateral exposure to 115-dB SPL narrowband noise for 2 hours were conducted on “a few rats,” the results of one were shown.

No control tests have been conducted to determine whether the procedure might be affected by an accompanying hearing loss. Because the animals are always shocked when the external sound is on, their response rate during sound trials will always be low either because they hear the sound or, if they cannot hear the sound, because they receive a shock every time they lick. Furthermore, it is conceivable that an animal that was shocked during the sound trials because of the salicylate-induced hearing loss prevented it from hearing the sound would cease licking during silent intervals. Indeed, because the animals are not licking to satisfy thirst, it would probably take very few unwarned shocks to suppress their licking. Thus, a hearing loss could cause an animal to test positive for tinnitus.

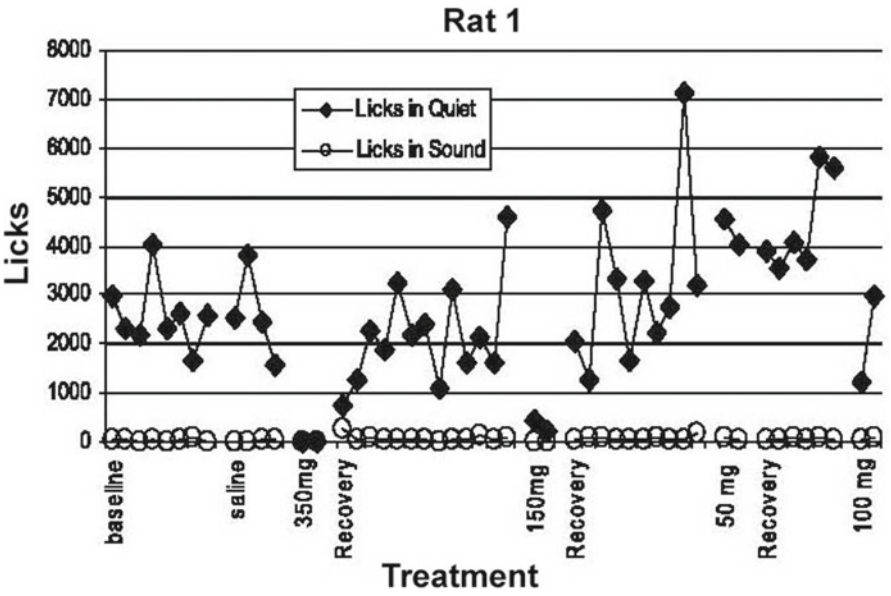

Fig. 2.8 Example of the response of one rat in the schedule-induced-polydipsia avoidance conditioning procedure of Lobarinas and colleagues. The animals were shocked if they licked a waterspout when the background sound was on (open circles). Note that the rat stopped licking during the silent intervals after receiving 350 and 150 mg of salicylate (filled diamonds), suggesting that it perceived its tinnitus as a signal for shock. A lower dose of salicylate (50 mg), like saline, had no effect on performance. (From Lobarinas et al., 2004.)

Evaluating the polydipsia procedure on the nine points:

1. Humans given the tinnitus-inducing agents used here (salicylate and loud sound) would be expected to develop tinnitus.
2. The procedure of having subjects report the presence or absence of sound as a way of determining if they have tinnitus would also reveal tinnitus in humans.
3. The procedure has not been tested by simulating tinnitus with different external sounds.
4. Hearing loss has not been ruled out as a possible confound and, indeed, it is expected that a hearing loss would cause animals to test positive for tinnitus.
5. Because the animals are trained to discriminate sound from silence, as opposed to responding to the quality of the sound, hyperacusis would not be expected to affect the results.
6. The procedure has not been used to determine the pitch of tinnitus.
7. The results are generally consistent with previous experiments that have found evidence of tinnitus after similar doses of salicylate and exposure to the level of loud sound that was used.
8. The test can be conducted on individual animals as well as on groups.
9. The procedure has been used to follow tinnitus over time.

4.7 Startle Reflex Inhibition Procedure of Turner

The startle reflex inhibition procedure involves reducing an animal's startle response to a sudden, loud sound by presenting another stimulus just before the startle sound is presented. A reduction in the amplitude of the startle response when it is preceded by the stimulus indicates that the animal perceived that stimulus. A common use of this procedure has been to determine the audibility of sounds by observing if they reduce the startle reflex. However, to test for tinnitus, the startle stimulus is preceded by a brief gap in a background sound with the idea that an animal's tinnitus will make the gap less salient and therefore less effective in reducing the startle response. Moreover, the degree to which an animal's tinnitus affects gap detection is expected to depend on its similarity to the sound in which the gap is imbedded. In addition to testing for tinnitus, the startle reflex by itself has been used to determine hyperacusis because it is believed that hyperacusis will increase the size of the startle reflex.

4.7.1 Gap Detection Test for Tinnitus

Thresholds for detecting gaps in sound are a common measure of the temporal resolution of the auditory system and have been used in studies of the effects of auditory trauma on hearing. When it was found that exposing rats to loud sound increases their gap detection thresholds, it was suggested that this might be because the exposure caused tinnitus that masked the gaps, making them more difficult to detect (Rybalko & Syka, 2005). The next step was to use gap detection to determine if an animal has tinnitus and, if so, the pitch of the tinnitus (Turner et al., 2006). The hypothesis is that when an animal's tinnitus is similar in quality to the sound in which the gap occurs, it will fill in or otherwise interfere with the animal's ability to detect the gap (Turner & Parrish, 2008). Thus, tinnitus is detected by determining an animal's ability to detect gaps in tones and narrowband noise.

In the startle reflex inhibition tinnitus test, an animal is placed in a test cage in which a low-level background sound is playing, such as 60-dB SPL narrowband noise. A startle sound (e.g., 115-dB SPL, 20-ms broadband noise burst) is presented at random intervals and the animal's startle response is measured by a strain gauge attached to the test cage. The startle sound is either presented alone or is preceded by a gap in the background sound, typically a 50-ms gap beginning 100 ms before the startle stimulus. A reduction in the average startle response that is caused by preceding the startle sound with a gap is used to indicate that the animal perceived the gap (Fig. 2.9).

The pitch of an animal's tinnitus is estimated by presenting gaps in background sounds that differ in frequency. Although pure tones are occasionally used, most studies have used narrowband noise (e.g., narrowband noise with a bandwidth of 1 kHz centered at 4, 8, 10, 16, 24, and 32 kHz), as well as broadband noise, because tinnitus is often described as an impure tone or a tone embedded in narrowband noise (McFadden, 1982). The degree to which gaps in the background sounds reduce

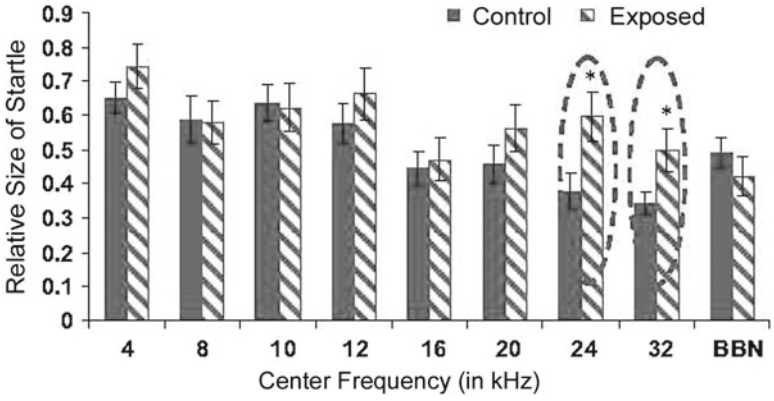

Fig. 2.9 Example of the gap startle reflex inhibition procedure for detecting tinnitus in rats. The bars show the size of the startle reflex relative to the unmodified startle when the startle sound is preceded by a 50-ms gap in the background sound. Background sounds were 1-kHz band noise centered at frequencies from 4-32 kHz, as well as broadband noise (BBN). Solid bars are the results of the control animals; diagonal stripped bars are the results of animals that were exposed in one ear to octave band noise (116 dB, with a peak at 17 kHz). Note that the gaps in the 24- and 32-kHz noise bands were less effective in reducing the size of the startle reflex in the rats given salicylate, suggesting that the animals had tinnitus in the pitch range that made the gaps less salient; the differences between the two groups did not emerge until 16 weeks after exposure. (Modified from Wang et al., 2009.)

the startle reflex can be determined both before and after exposing an animal to a tinnitus-inducing agent; a decrease in the effectiveness of the gap in a particular background sound is believed to indicate that the pitch of an animal's tinnitus is similar to that sound. Thus, for example, a decrease in the effectiveness of a gap in a 10-kHz background sound would indicate tinnitus with a pitch of 10-kHz whereas a decrease in the effectiveness of a gap in broadband noise would indicate that the perceived tinnitus was noise-like (Fig. 2.9).

The startle reflex gap detection task has several advantages (Turner & Parrish, 2008). First, it does not require food or water deprivation or the use of shock. Second, because the animals do not have to learn anything, there is no memory or complex motor component. Third, startle trials may be given at a rate of several per minute with the result that the entire test can be conducted on an animal in less than an hour. Finally, by testing an animal before and after exposure to a tinnitus-inducing agent, it is possible to use each animal as its own control, although, in practice, the performances of animals are usually considered in groups rather than individually.

There are five issues regarding the validity of the startle reflex gap detection task as a measure of tinnitus. The first is whether the procedure would detect tinnitus in humans. As mentioned earlier in this chapter, there are examples in which the perception of one's tinnitus is affected by external sounds, such as the suppression of tinnitus, but there are no known examples in which tinnitus affects the perception of

physical sounds. Although the use of the gap procedure is being investigated in humans, no definitive results have yet been reported (e.g., Hébert et al., 2010).

The second issue is whether the gap detection procedure would be affected by the hearing loss that typically accompanies induced tinnitus. In humans, salicylate is known to increase gap detection thresholds at low SPLs and increasing the sound level to compensate for the hearing loss is necessary to bring the thresholds back to pretreatment levels (McFadden et al., 1984). Similarly, a study using startle reflex inhibition to study the effect of salicylate on gap detection thresholds in rats found that the SPL of the noise in which the gap was embedded had to be increased by 20 dB to bring the rats' performances up to pretreatment levels (Deng et al., 2010). However, the issue of hearing loss in tinnitus studies has been addressed by demonstrating that the audibility of the background sounds in which the gaps are embedded is not affected by salicylate; this is done by using the sounds themselves as prepulse stimuli, that is, presenting a 100-ms burst of the noise, instead of a gap in the noise, before the startle stimulus (Ralli et al., 2010). A more direct approach to the potential effect of hearing loss on the gap procedure would be to determine behaviorally the absolute thresholds of the animals under test and then increase the SPL of the background sound to compensate for the hearing loss caused by the treatment; this, however, has not yet been done.

The third issue is how closely an animal's tinnitus must match the sound in which the gap is imbedded to interfere with its detectability. This question can be approached by determining the ability of physical sounds of various frequencies to degrade gap detection. However, no reports of simulated tinnitus have yet been published for this procedure.

Fourth, the main source of validation of the gap detection procedure comes from two studies that compared its results with those of other tinnitus procedures. The first study compared the gap detection procedure with the avoidance procedure developed by Bauer and Brozoski by testing rats that had been exposed to 16-kHz octave noise at 116 dB for 1 hour (Turner et al., 2006). Testing exposed and control rats on broadband noise and a variety of tones, the avoidance procedure showed a difference between the two groups that emerged after 8–9 weeks on the 10-kHz tone, but not on broadband noise; these results were taken as a sign of tinnitus with a pitch of about 10 kHz. (Although the animals were said to have been tested on other tones, neither the number of tones used nor the results were given). Subsequent testing on the same animals was conducted with the startle reflex gap detection test using three background sounds: the same broadband noise as in the avoidance procedure and two 1-kHz narrow noise bands, one centered at 10 kHz, the other at 16 kHz. The results of the gap detection task showed that the exposed and control groups differed only on the 10-kHz narrowband noise, which, in conjunction with the results of the avoidance conditioning procedure at 10 kHz, was taken as evidence that both procedures were detecting tinnitus that had a pitch of around 10 kHz.

Although the correspondence between the gap detection and the avoidance procedures is noteworthy, questions remain. First, as previously noted, there is no documented evidence in the literature on human studies that exposing a person to a

sound that does not immediately produce tinnitus will result in chronic tinnitus that appears months later. Second, although the authors state that the 16-kHz stimulus they used to induce tinnitus routinely produces signs of tinnitus at 10 kHz, a survey of the literature indicates otherwise; indeed, studies conducted both before and after this one have found signs of tinnitus not at 10 kHz, but at 20 kHz (e.g., Brozoski & Bauer, 2005; Brozoski et al., 2007a,b; Brozoski & Bauer, 2008). Finally, the reported probabilities for the differences between the exposed and control groups for the gap detection and the avoidance procedures were close to the standard $p = .05$ used to reject the null hypothesis ($p = .036$ and .03, respectively) and, although the animals were tested on multiple sounds, it is not stated whether the appropriate statistical corrections necessary for making multiple comparisons were made (e.g., Abdi, 2007). Thus, the results of this study are supportive but not conclusive.

The second comparison of methods was between the gap detection and polydipsia avoidance procedures in which rats were tested before and after being given salicylate (Yang et al., 2007). In the first part of the study, one group of rats was tested using the polydipsia procedure while a different group of animals was tested with the gap procedure. The polydipsia avoidance procedure found that salicylate significantly decreased the animals' licking during periods of no external sound, a result interpreted as indicating tinnitus. The gap detection procedure found that the salicylate reduce the effectiveness of a gap in 16-kHz narrowband noise to reduce the startle reflex, but not gaps in 6- or 12-kHz narrowband noise, which was interpreted as indicating tinnitus with a pitch of around 16 kHz. Thus, both procedures found evidence of tinnitus in rats given the same dose of salicylate. In the second part of the study, four rats were tested using both procedures. The results of this part of the study, shown for one rat, also indicated that salicylate caused tinnitus, with the gap procedure again indicating tinnitus at 16 kHz; although the results of the other three rats were said to be similar, it would have instilled more confidence if those results had also been shown.

Finally, because the gap detection procedure is used to determine the pitch of an animal's tinnitus, it is of interest to compare the results of the various gap detection studies of salicylate with each other as well as with those of other procedures.As previously noted, Jastreboff and his colleagues placed the pitch of tinnitus caused by salicylate at 10 kHz, although it might be higher (Jastreboff & Sasaki, 1994). In comparison, two of the gap detection studies have placed the pitch of salicylate-induced tinnitus at 16 kHz (Yang et al., 2007; Ralli et al., 2010). However, the results of a third gap detection study suggested that salicylate-induced tinnitus was noise like (Turner & Parrish, 2008); specifically, they found that salicylate reduced the effectiveness of gaps in broadband noise, but not in 1-kHz narrowband noise ranging in center frequency from 4 to 32 kHz. As the authors noted, the effect of salicylate is variable and salicylate is known to produce noise-like tinnitus in humans (McFadden, 1982); while true, this means that virtually any outcome of this test can be taken to indicate that it is a test of tinnitus, and any support it provides for a hypothesis is accordingly weakened. Equally interesting was Turner and Parrish's finding that salicylate enhanced the effect of the gaps in the narrowband noises; that is, contrary to previous findings, salicylate caused these gaps to be *more*

effective in reducing the startle response, a result the authors suggest may be a sign of hyperacusis (see later).

Because of its relative ease of use, and the ability to obtain results in as little as a day, the gap detection procedure is becoming widely used, thus making its validation especially urgent (e.g., Engineer et al., 2011; Holt et al., 2010).

Evaluating the gap startle reflex inhibition procedure on the nine points:

1. Humans given salicylate would be expected to develop tinnitus. On the other hand, there is no documented evidence that exposure to loud sound causes tinnitus that does not appear until weeks after the exposure.
2. There is little evidence that tinnitus affects the perception of physical sounds and the possibility that it affects the detection of gaps has not yet been established.
3. This procedure has not been tested with simulated tinnitus to indicate how close in pitch a sound must be before it affects gap detection.
4. Hearing loss may be a factor as salicylate affects gap detection thresholds at low intensities.
5. The startle reflex may be affected by hyperacusis (see later).
6. The procedure has been used to determine the pitch of tinnitus.
7. The pitch of tinnitus after the noise exposure and treatment with salicylate has varied between studies.
8. Animals can be used as their own controls, making it possible to test individual animals.
9. The procedure is designed to follow the animals' tinnitus over time.

4.7.2 Hyperacusis and the Startle Reflex

As noted earlier in this chapter, both salicylate and exposure to loud sound can cause hyperacusis, an oversensitivity to certain sounds making them irritating and unpleasant. Indeed, sounds, especially abrupt sounds (transients) that previously caused no problems are described as clanking, penetrating, aversive, and painfully loud (R. S. Heffner, personal observations). Recently, it has been found that salicylate has at least two effects that may contribute to hyperacusis. First, salicylate increases the amplitude of sound-evoked potentials in auditory cortex (Sun et al., 2009), suggesting that it may make sounds more salient. Second, salicylate increases the amplitude of the startle response to sound (Ison et al., 2007), suggesting, again, that it makes sound more salient. Indeed, hyperacusis was used to explain why one study found that gaps in narrowband noise became more (rather than less) effective in reducing the startle reflex in rats after they were given salicylate (Turner & Parrish, 2008).

Although the evidence that salicylate increases the startle response to sounds by causing hyperacusis is persuasive, there is at least one question that remains to be addressed. Specifically, it is necessary to rule out the possibility that salicylate causes a *general* increase in startle to all stimuli, auditory and nonauditory. This can be done by determining whether salicylate increases the startle response to a nonauditory stimulus such as foot shock, and by determining the inhibitory effect of

nonauditory pre-pulse stimuli such as a flash of light. Thus, we do not yet know if the effect of salicylate on the startle reflex is a general increase in reactivity or is specific to auditory stimuli.

4.8 Sound Localization Procedure of Heffner

The sound localization procedure devised by Heffner and colleagues is based on the idea that exposing one ear to a loud sound will cause tinnitus in that ear and that an animal trained to report whether a sound came from its left or right side will respond, in the absence of a physical sound, as though it perceives a sound (tinnitus) on the side of the exposed ear (Heffner & Koay, 2005; Heffner, 2011). In this test, an animal is trained on a sound localization task to make a left or right response to sounds coming from its left or right side, respectively; correct responses are rewarded with water whereas incorrect responses are shocked. Silent trials, in which no sound is presented, are interspersed among the sound trials; the animal receives neither reward nor punishment for its responses on these trials and its side preference on the silent trials is determined. At this point, feedback on the sound trials is changed so that, randomly, only half of the sound trials are followed by reward or punishment to reduce the possibility that an animal will notice that responses to silent trials are never rewarded or punished.

The animal is then exposed to a loud sound in the ear opposite its side preference on the silent trials and tested to see if it shifts its responding on those trials to the side of the exposed ear; doing so would indicate that the animal perceives a sound (tinnitus) that is lateralized to that side (Fig. 2.10). This is conceptually equivalent to human patients reporting the ear in which they hear their tinnitus. Besides being able to indicate whether an individual animal has lateralized tinnitus, the two-choice procedure would not be expected to be confounded by the hearing loss that accompanies exposure to loud sound, an expectation that has been verified by demonstrating that a conductive hearing loss caused by plugging one ear does not cause a shift in responding on silent trials (Heffner & Koay, 2005). Moreover, because the animals are never given feedback on the silent trials, and their responses on sound trials are given feedback only half of the time, their responding to their tinnitus may not habituate, making it possible to follow the time course of the tinnitus. A key assumption is that exposing an ear to a loud sound will induce tinnitus that is lateralized to that ear—that the tinnitus will neither be lateralized to the *un*exposed ear nor be bilateral (for a discussion of the human evidence on this point, see Section 2.1 of this chapter).

Rats were tested after exposure to tones ranging in frequency from 1 kHz to 45 kHz at 110 dB for 10 minutes with the finding that many of them tested positive for tinnitus for one or more days (Heffner, 2011). In addition, a simulated tinnitus test was given in which low-level (25 dB SPL) 16-kHz 1/3-octave band noise was presented continuously from one side. The results of the simulated tinnitus test indicated that although all six rats responded to the simulated tinnitus on the first day, two failed to significantly shift their responding on one or more of the following

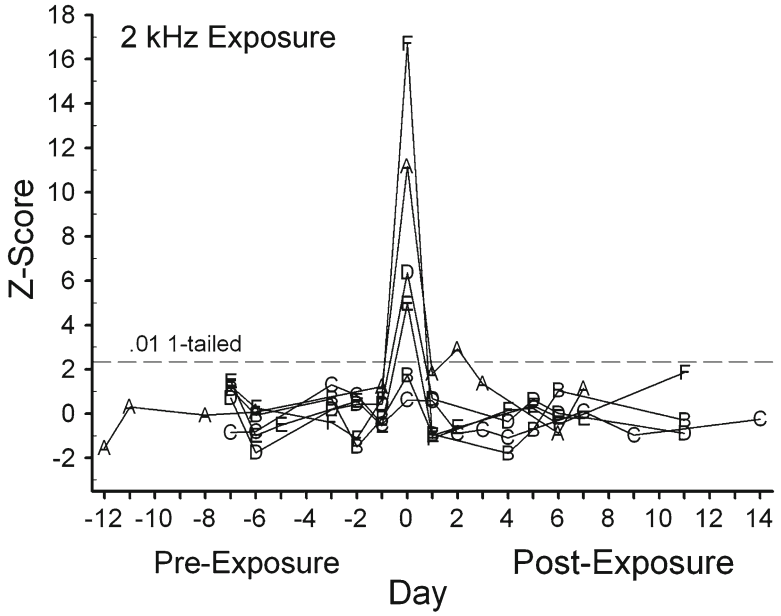

Fig. 2.10 Example of the results of the sound localization procedure for detecting tinnitus in animals. Six rats were exposed in one ear to 2-kHz at 110 dB SPL for 10 minutes. Immediately after exposure (Day 0), four of the six animals shifted their responding on silent trials to the side of the exposed ear with a chance probability of $p < 0.01$ (one-tailed distribution). The shift of their responding on the following days back to their preexposure side preference suggests that their tinnitus had subsided, although simulated tinnitus tests indicate that some animals may cease responding to low-level sounds when their responses are not rewarded or punished. (From Heffner, 2011.)

days; this suggests that animals vary in their reliability of responding, which might be corrected by training the animals to respond to a wider variety and intensity of sounds.

The procedure has revealed an unexpected effect of anesthesia (halothane/nitrous oxide) on tinnitus. That is, whereas anesthetizing unexposed rats did not cause them to test positive for tinnitus, anesthesia alone would sometimes *reinstate* tinnitus in animals that had previously been exposed; that is, a rat that was no longer testing positive for tinnitus would occasionally shift its responding on the silent trials to the side of its previous exposure after being anesthetized even though it was not exposed to any sound at that time (Heffner, 2011). This finding suggests that something that does not cause tinnitus itself may cause tinnitus to reappear in previously traumatized ears, an observation that supports the view that exposing one ear to loud sound could reinstate tinnitus in the other ear if that ear has previously had tinnitus.

Finally, with regard to whether exposing one ear to a loud sound results in tinnitus lateralized to that ear, studies using this procedure to detect tinnitus in rats and hamsters after exposure to one ear (the other ear was never exposed) found evidence of ipsilateral, but not contralateral tinnitus (Heffner & Koay, 2005; Heffner, 2011).

Although this supports the view that exposing one ear to a loud sound does not in itself induce tinnitus in the opposite ear, it does not rule out the possibility that some of the animals that did not test positive for unilateral tinnitus may have experienced bilateral tinnitus.

Evaluating the sound localization procedure on the nine points:

1. The level of sound exposures used in these studies would be expected to cause tinnitus in humans.
2. The procedure is based on exposure to a loud sound in one ear causing tinnitus lateralized to that ear. Although the human evidence on this point is conflicting, there is reason to believe that the tinnitus would be lateralized to the side of the exposed ear. The procedure is not suitable for testing for bilateral tinnitus which may result from exposure to ototoxic drugs.
3. The procedure has been tested with simulated tinnitus, which shows that although all animals respond to it on the first day, some animals are less reliable in reporting the simulated tinnitus on following days. This suggests that animals might be selected for exposure based on the consistency of their responding to simulated tinnitus.
4. Hearing loss is not a factor and, if anything, would work against the procedure as a hearing loss after exposure causes sounds to be perceived primarily on the side of the unexposed ear.
5. Because the animals are trained to indicate the side from which a sound comes, as opposed to responding to the quality of the sound, hyperacusis would not be expected to affect the results.
6. The procedure does not indicate the pitch of the tinnitus.
7. The results appear consistent in that the higher the intensity of an exposing sound, the more likely it is to cause tinnitus (Heffner & Koay, 2005).
8. The sound localization procedure uses each animal as its own control and is ideal for testing individual animals. However, this increase in power comes at the cost of time because it can take over a month to train the animals.
9. The sound localization procedure can be used to follow an animal's unilateral tinnitus.

5 Conclusion

A number of procedures have been devised for detecting tinnitus in animals and a summary is presented in Table 2.2. In selecting a procedure, it is important to consider not only ease of use, but also the degree to which confounding factors such as hearing loss have been ruled out. The procedures for which the most control tests have been conducted are the conditioned suppression procedure of Jastreboff and the sound localization procedure of Heffner. In terms of power, those procedures that use each subject as its own control provide the most power, and the sound localization and startle reflex gap procedures can obtain useful information from single animals. Of the various procedures, the startle reflex gap procedure shows the

Table 2.2 Nine-point comparision of the procedures for detecting tinnitus in animals[a]

Points of comparison	Jastreboff conditioned avoidance	Bauer & Brozoski conditioned avoidance	Rüttiger conditioned avoidance	Guitton conditioned avoidance	Guitton water T-maze	Lobarinas polydipsia avoidance	Turner gap detection	Heffner two-choice sound localization
1. Agent causes tinnitus in humans	Yes	Unknown	Yes	Yes	Yes	Yes	Yes/unknown	Yes
2. Would work on humans	Yes	Unknown	Yes	Yes	Yes	Yes	Unknown	Yes[b]
3. Tested with simulated tinnitus	Yes	No	No	No	No	No	No	Yes
4. Hearing loss not a concern	Yes	Yes	Yes	Yes	Yes	No	No	Yes
5. Hyperacusis not a concern	Yes	No	Yes	Yes	Yes	Yes	No	Yes
6. Used to determine pitch of tinnitus	Yes	Yes	No[c]	No	Yes	No	Yes	No
7. Results generally consistent	Yes	No	Yes	Yes	Yes	Yes	No	Yes
8. Does not require control group	No	No	Yes[d]	Yes[d]	Yes[d]	Yes	Yes	Yes
9. Follow tinnitus over time	No	Yes	No	Yes	No	Yes	Yes	Yes

[a] Note that some of these evaluations may change as further results become become available.
[b] Does not detect bilateral tinnitus.
[c] Used to determine intensity of tinnitus.
[d] Control groups used in some studies.

greatest promise, not only because of its ease of use but also because it may indicate the pitch of an animal's tinnitus. However, there are many questions that must be addressed before it is adopted for use, including whether it is reasonable to expect tinnitus to interfere with gap detection.

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