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Behavioral assessments of auditory sensitivity in transgenic mice

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Abstract

This report summarizes positive reinforcement conditioning procedures for assessing sensory function in transgenic mice. To illustrate these behavioral methods auditory sensitivity was measured in mice lacking $\alpha 9$ acetylcholine receptor subunits ($\alpha 9$ knock-out mice). These receptors are known to play an important role in the efferent pathways that modify cochlear responses to sound stimuli. The strategies of parameter manipulation that led these subjects through their preliminary training stages to stable threshold performances are described in detail. Techniques for estimating and interpreting sensory thresholds are discussed from the perspective of signal detection analyses. This study found no significant differences between $\alpha 9$ knock-out mice and control subjects when hearing thresholds were measured under quiet conditions, as predicted by previous behavioral and electrophysiological evidence.

Keywords: Acetylcholine receptors; Auditory behavior; Hearing sensitivity; Transgenic mice

1. Introduction

Valuable insights into the workings of the sensory nervous system have been gained by linking physiological events to perceptual behaviors in laboratory animals. Although the sensory abilities of a wide variety of species have been assayed by physiologists and animal psychophysicists, the experimental designs of most research fields are dominated by a single established paradigm. In recent years preferences for these long-standing animal models have been eroded by the advantages of studying sensory processing in laboratory mice. Large litters, rapid breeding, and short lifespans make these animals ideal subjects for genetic, developmental, and aging studies of the nervous system. For decades selective breeding has produced mice with perceptual abnormalities that mimic human disease states (e.g. the Snell's waltzer mouse is a model of deafness and imbalance (Deol and Green, 1966)). Now, molecular biologists can create strains of mice in which essential elements of sensory systems have been removed (knock-out), changed (mutant), amplified (over expression), or misexpressed (knock-in) by direct genetic manipulations. These molecular techniques allow remarkable specificity in the isolation and identification of the physiological mechanisms that shape perceptual experience. Crawley (1999) and Crawley and Paylor (1997) recently described a series of tests used to evaluate health and behavioral characteristics of transgenic mice.

This report describes a behavioral method for assessing auditory sensitivity in transgenic mice that lack $\alpha 9$ acetylcholine (ACh) receptors (i.e. $\alpha 9$ knock-out mice (Vetter et al., 1999)). A distinctive functional role for the $\alpha 9$ subunit has been suggested by expressing these cochlear ACh receptors in *Xenopus* oocytes, which results in ligand-gated ionotropic receptor complexes exhibiting the same mixed nicotinic-muscarinic pharmacology of cochlear hair cells (Elgoyhen et al., 1994). When antibodies are used to localize $\alpha 9$ receptors in the cochlea (Park et al., 1997), strong labeling is found near olivocochlear efferent terminals on the base of the outer hair cells (Warr and Guinan, 1979); other structures like the cochlear spiral ganglion or whole brain are not labeled. The specificity of $\alpha 9$ receptor distribution is striking. It is the only known ACh

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receptor that is expressed by cochlear hair cells and it is found nowhere else in the auditory system. These restrictions offer an extraordinary opportunity to modify olivocochlear influences through genetic knock-out of $\alpha 9$ receptors without compromising other aspects of auditory processing.

The release of ACh by olivocochlear efferent neurons acts upon $\alpha 9$ receptors to open calcium-gated potassium channels in the basolateral cell membrane of cochlear hair cells (Fuchs and Murrow, 1992; Blanchet et al., 1996; Evans, 1996). The resulting efflux of potassium hyperpolarizes the hair cells and modifies the mechanical properties of the cochlea that govern sound transduction (Housley and Ashmore, 1991; Dallos et al., 1997). The functional role of this efferent feedback system has been investigated by measuring the effects of olivocochlear lesions on behavioral performances in animals. This surgical approach has failed to demonstrate major changes in auditory sensitivity under quiet testing conditions (Trahoitis and Elliot, 1968; Igarashi et al., 1972), but damaging effects of olivocochlear lesions are apparent when perceptual tasks are conducted in noisy environments (vowel discrimination (Dewson, 1968; Heinz et al., 1998), intensity discrimination (May and McQuone, 1995)). As predicted by this behavioral evidence, $\alpha 9$ knock-out mice show normal auditory thresholds for sound-evoked electrophysiological responses in quiet even though they lack functional efferent feedback (Liberman et al., 1999). This report is the first to describe a behavioral methodology for measuring the effects of olivocochlear deficits on the auditory processing abilities of $\alpha 9$ knock-out mice. The current experimental design tests the specific hypothesis that $\alpha 9$ knock-out mice and normal controls show similar hearing thresholds in a tone detection task. From a more general perspective, these methods easily can be adapted to additional strains of transgenic mice or to other sensory modalities.

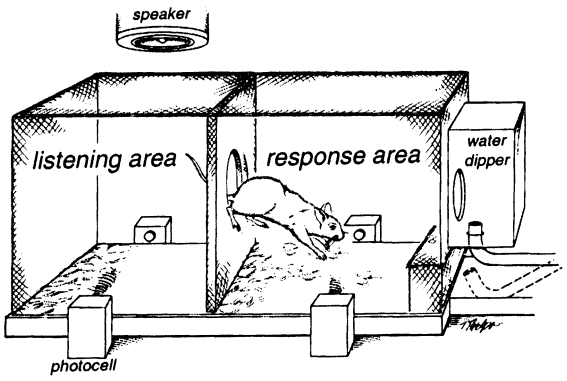

Fig. 1. The two-chambered testing cage. The mouse performs the tone detection task by crossing the barrier that separates the listening and response areas when tones are presented from the overhead speaker. The subject's movements are monitored with photocells and rewarded by activating the water dipper.

2. The testing environment

Functional consequences of genetic manipulations are resolved by comparing the abilities of transgenic animals with normal controls. Experimental designs that use the littermates of transgenic mice as control subjects provide a particularly sensitive measure of genetically-based performance differences. Time constraints inherent in such procedures place severe logistical demands on traditional behavioral approaches. One practical advantage that is gained by studying the auditory behaviors of small animals like mice is that even restricted laboratory space can accommodate several independent testing systems. By automating the psychophysical procedure with personal computers, an investigator not only removes a potential source of experimenter bias from response measures but also gains the capacity to conduct multiple testing sessions simultaneously.

The testing cage shown in Fig. 1 is located inside a sound-attenuation chamber (Lafayette Instruments). The inner walls of the chamber are lined with foam (Sonex) to control acoustic reflections and to prevent external noise from entering the testing environment. As an additional safeguard to maintain a well-calibrated sound field the walls and ceiling of the cage are fabricated from wire-mesh and the bottom tray is filled with pine shavings. The internal dimensions of the cage ($8.5 \times 19.5 \times 10$ cm) are divided into two equal-sized compartments by an inner wall. A mouse can cross into either compartment by jumping through an opening in the partition wall. Photocells (Omron Control Systems) monitor the position of the mouse in the cage. A speaker (Radio Shack, Realistic Super Tweeter) is located over the left compartment, which is the subject's listening area. A water dipper (Coulbourn Instruments) is mounted on the front wall of the right compartment, which is designated the response area. As shown here, the mouse activates the dipper by entering the response area when tones are presented from the speaker.

Commercial hardware for generating calibrated sounds can be purchased as modular components that can be configured to meet the specific demands of the experimental design. Auditory stimuli are produced with a digital waveform generator (Tucker-Davis Technologies, WG2), which also serves as the electronic switch for gating auditory stimuli. Digital signals are passed to a programmable attenuator (Tucker-Davis Technologies, PA4), amplified (Crown D45), and then delivered to the speaker. Stimuli are calibrated by sampling tone levels throughout the listening area without a mouse in the apparatus. Behavioral thresholds are calculated relative to the average of these sound pressure measurements. Premium calibration equipment is extremely expensive, but modern hand-held sound level meters (e.g. a Simpson 899) are an affordable option

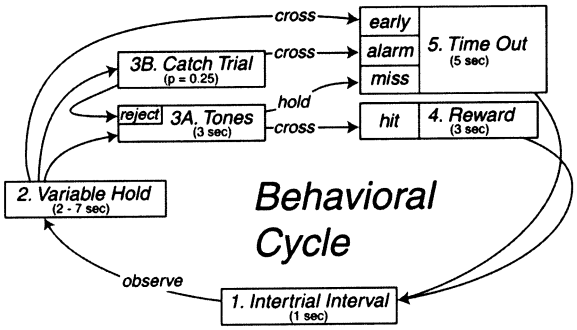

Fig. 2. The behavioral cycle showing final parameters of the tone detection task. The mouse obtains a period of water availability by remaining in the listening area during a silent hold interval and then crossing into the response area during tone presentations. See text for a complete description of behavioral contingencies.

that ensures reliable stimulus control for the smaller laboratory.

3. The tone detection task

A mouse indicates its ability to detect tone bursts by crossing the partition that separates the response and listening areas of the testing cage. Behavioral contingencies of this sound detection task are summarized by the diagram in Fig. 2. A trial begins when the mouse enters the listening area and breaks the beam of the photocells that monitor activity there. The mouse must remain in the listening area for a variable period of silence (2–7 s) then cross into the response area when it hears a sequence of 250-ms tone bursts that repeat at a rate of two bursts per second. If the presence of the mouse is detected by photocells in the response area within 3 s of stimulus onset, the response is scored as a hit and rewarded with a 3-s interval of water availability. Alternatively, if the mouse enters the response area prior to the tones (early response), or fails to cross before the completion of the tone presentations (miss), behavioral contingencies are suspended for a 5-s time-out interval. This temporary interruption of the behavioral cycle reduces the probability of error responses.

On 25% of all trials no sound is presented. These 'catch trials' are used to determine how often a subject inflates its detection scores by entering the response area without first hearing the sequence of tones. An error response during a catch trial (false alarm) produces a time-out interval. If the subject remains in the listening area for the duration of the catch trial (correct rejection), the loudest tones in the stimulus set are presented to create the opportunity for the subject to cross the partition to obtain a water reward. These stimuli are not used in the calculation of behavioral thresholds.

4. Preliminary training

Mice arriving from an external breeding facility must undergo a 2–3 week quarantine period before they are introduced to the main behavioral colony. Behavioral subjects are housed individually, so that their water intake can be closely monitored. The mice are weighed daily during this time to determine the free-water weight of each subject. A moderate level of deprivation is established by limiting the subject's access to water to two 15-min periods that are separated by 12 h. When training begins, the evening watering session is reduced to 5 min and the daytime period is replaced by the rewards that the subject obtains in the behavioral task. The period of free access to water is increased on days in which the subject is not tested (e.g. on the weekend). If a mouse experiences more than a 10% loss relative to its free-water weight on this regimen, evening watering sessions are lengthened.

Mice learn to perform according to the behavioral contingencies shown in Fig. 2 by exposure to six stages of training that emphasize individual components of the tone detection task. Parameter guidelines for each stage of training are suggested in Table 1. The tones

Table 1
Parameter changes for major training stages of the tone detection task

Training stage	Example (Fig.)	State of the behavioral cycle (Fig. 2)						Tone levels (dB SPL)
		1	2	3A	3B	4	5	
		ITI (s)	Var. hold (s)	Tone trl. (s)	Catch trl. (*P*)	Reward (s)	Time out (s)	
1	Fig. 3A	1	1	10	0.01	10	1	55–65
2	Fig. 3B	1	1–2	4	0.05	4	2	55–65
3	Fig. 3C	1	1–4	3	0.15	3	3	55–65
4	Fig. 3D	1	1–7	3	0.25	3	5	55–65
5	Fig. 4	1	2–7	3	0.25	3	5	35–60
6	Fig. 5	1	2–7	3	0.25	3	5	15–50

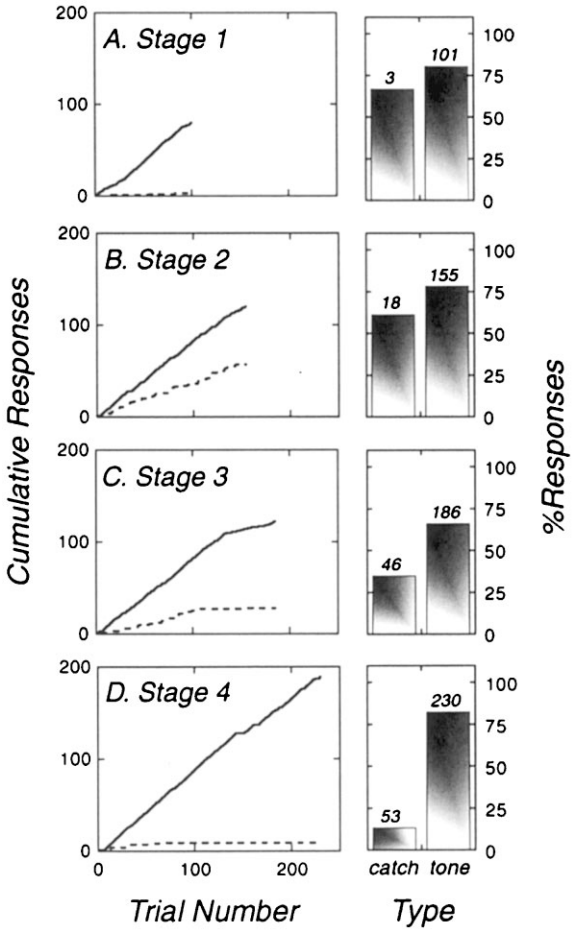

Fig. 3. Response patterns of an $\alpha 9$ knock-out mouse (a9xKo99) during four stages of preliminary training. Cumulative records for representative sessions (left column) plot the subject's hits (solid line) and early responses (dashed line) as a function of trial number. Perfect performance is shown by the unity line in each record. Histograms (right column) summarize response percentages for tone and catch trials during the session. Numerical labels above the histograms indicate the number of trials used in the calculation of response percentages.

that signal trial intervals are presented at easily detected sound pressure levels (55–65 dB SPL) until the completion of preliminary training (stage 4). The hearing thresholds described in this report were obtained with 16-kHz tone bursts, which is a frequency where mice show excellent auditory sensitivity.

The primary objective of stage 1 training is to link the subject's natural exploratory behaviors to the procurement of water rewards in the testing cage. This training can be facilitated by minimizing the number of times that a mouse enters the response area and fails to activate the dipper because the behavioral response is scored as an error. Examination of Fig. 2 identifies three types of response errors that result in time-out intervals instead of rewards (early responses, false alarms, and missed tone trials). To prevent the mouse from entering the response area prior to tone presentations (early), the variable hold interval is reduced to a fixed 1-s duration during the first stage of training. With this short hold in effect, tone presentations begin almost immediately after the mouse enters the listening area. To lessen the possibility that the mouse will fail to leave the listening area before the completion of the tone presentations (miss), the duration of the trial window is expanded to 10 s. Lengthening the trial duration also increases the likelihood that the mouse will enter the response area during catch trials (false alarm), but this error is effectively eliminated by decreasing the probability of a catch trial to 0.01. This small non-zero probability gives the mouse limited exposure to catch trials without disrupting training priorities. In the event that the mouse does produce an error, the time-out interval is reduced to 1 s to ensure that the training paradigm is only briefly interrupted. Correct barrier crossings are not rewarded if the mouse fails to drink from the dipper before the end of the reward interval. This outcome is avoided by lengthening the period of water availability after each correct response to 10 s.

Fig. 3 summarizes some of the behavioral landmarks that were achieved during the training of a representative $\alpha 9$ knock-out mouse (a9xKo99). Cumulative records in the left column of the figure plot the subject's total hits (solid line) and early responses (dashed line) as a function of trial number. The testing sessions that produced these data are spaced at approximately 2-week intervals to illustrate typical changes in response patterns during each of the four stages of preliminary training. Perfect performance is indicated by the unity line in the cumulative records. In Fig. 3A notice how stage 1 parameters make it possible for the mouse to respond without error on most trials. In the right column of the figure the response histograms show the overall hit and false alarm rates for the representative sessions. During stage 1 training, there is little difference in the subject's tendency to respond to tone versus catch trials because behavioral performance has not been brought under adequate stimulus control. This is not a major problem because the low probability of stage 1 catch trials yields only three presentations.

The goal of stage 2 is to train the mouse to enter the response area immediately after it detects tone presentations. This objective is achieved by decreasing the parameters that specify the duration of tone trials and reward intervals. The cumulative records and response histograms in Fig. 3B summarize the results of a stage 2 training session with mouse a9xKo99. After repeated exposure to the shorter trial window, the mouse learns to maintain relatively high detection scores by simply moving back and forth between the listening and response areas. This strategy for timing the entry into the response area gains the subject frequent water rewards but also results in many early responses and high false alarm rates.

Stage 3 trains the mouse to attend to auditory stimuli by undermining the reliability of timed responses. The major parameter change for this stage is an increase in the hold interval that precedes the onset of tone presentations. Because the mouse must remain in the listening area for longer and more variable time intervals to produce tone trials, the accelerated movements used to obtain water rewards during stage 2 become less effective. The cumulative records in Fig. 3C plot the performance of mouse a9xKo99 after 2 weeks of stage 3 training. Although the silent interval preceding tone presentations has increased, the mouse actually exhibits fewer early responses and false alarms relative to stage 2 sessions (Fig. 3B). These results suggest that the subject has learned to adapt to the more temporally demanding testing procedure by listening to auditory cues.

Behavioral contingencies are adjusted to their final parameter values during stage 4 training. Since the goal of this stage is to produce a subject that listens carefully to tone presentations and works consistently for large numbers of trials, the essential parameter changes involve further increases in the variable hold interval and catch trial probabilities. As shown in Fig. 3D, mouse a9xKo99 has achieved the objectives of stage 4 testing procedures. The mouse performs over 200 detection trials in the session and rarely enters the response area before the sequence of tone presentations. As a result of the steady decline in false alarms over each stage of training, less than 15% of catch trial presentations yield response errors. At the same time the mouse maintains an average detection score of 80% for tone trials and increases the total number of trials completed during each session.

5. Measuring tone detection thresholds

Preliminary stages of training create a well-reinforced introduction to the tone detection task by posing parameter changes in the context of clearly audible stimuli. The final stages of training pursue the effects of stimulus level on a subject's barrier crossing behaviors, and generally do not require major adjustments of behavioral parameters.

Stage 5 training exposes the mouse to gradually decreasing tone presentation levels until the limits of auditory sensitivity are revealed by declining detection scores. To maintain the subject's behavior over prolonged testing with difficult stimulus conditions, sessions are conducted with six to seven tone levels that are spaced at intervals of 5 dB. The stimulus set is arranged to elicit high hit rates for the loudest tone levels and sub-threshold performance for the quietest levels. Each trial presents a tone level that is selected at random from the stimulus set (method of constant stimuli, as reviewed by Niemiec and Moody (1995)). Only the loudest stimulus is presented on the first five trials of each session to 'warm up' the subject. Responses to these trials are not considered in the calculation of threshold.

The three psychometric functions in Fig. 4A plot tone detection rates as a function of stimulus level for an $\alpha 9$ control mouse (a9Bo99). These results reflect changes in the subject's performance at approximately 1-week intervals during stage 5 training; numerical labels indicate the session number of each function which can be used to identify the data on the plots of daily false alarms and thresholds in Fig. 4C. As the subject's detection scores improve over sessions 5–16, the stimulus set is shifted to progressively lower sound pressure levels. Notice how the hit rate for a 42-dB SPL tone climbs from 50% on session number 5 (circles) to over 80% on session 9 (squares). By session number 16 (diamonds), the subject detects over 50% of the presentations of a much quieter 17-dB SPL tone. Responses to catch trials (XT) remain near 25% while this performance enhancement is in progress.

Fig. 4B shows the performance of mouse a9Bo99 toward the end of stage 5 training. By this time major stimulus adjustments have been completed and the investigator's goal is to prolong behavioral testing until the subject achieves stable performance. The subject has failed to attain an acceptable level of stability before session number 34 because of the large variation between psychometric functions and high false alarm rates.

The progression of mouse a9Bo99 toward a stable behavioral threshold becomes apparent when the subject's false alarm rates and detection thresholds are tracked over many behavioral sessions. As shown in Fig. 4C, initial sessions 1–16 indicate a period of learning in which ongoing changes in the stimulus set produce a steady decrease in the daily threshold. Intermediate sessions 17–35 are marked by a lack of stability even though the stimulus set remains essentially unchanged after session 16. False alarm rates exhibit an advancing trend with some sessions reaching rates above 50%. Thresholds shift continuously over a 30-dB range of tone levels. By contrast sessions 36–42 suggest a period of behavioral stability. False alarm rates fall to consistent values near 25%. Daily thresholds remain near 20 dB SPL and display no obvious trends toward improved performance. These final sessions yield a detection threshold of 19.4 dB SPL for mouse a9Bo99.

The goal of stage 6 training is to maintain a subject under threshold conditions until a minimum of two weeks of stable performance is achieved. Fig. 5 illustrates this procedure using data from an $\alpha 9$ knock-out mouse (a9xAn99). Notice how the repeated testing of this subject produces similarly shaped psychometric functions (Fig. 5A), low false alarm rates, and remark-

ably consistent thresholds (Fig. 5C). Formal criteria for stability are likely to be influenced by subject, stimulus, and procedural factors. In the experimental design of this report, responses are considered stable when thresholds fall within ± 5 dB for seven out of ten consecutive sessions and daily false alarm rates remain less than 35%. Clearly, the behavioral performances of mouse a9xAn99 have met these criteria and can be used to estimate the threshold.

Tone detection thresholds are calculated by pooling the results of stable behavioral sessions into one summary psychometric function. In Fig. 5B the combined data of the knock-out mouse a9xAn99 reflect more than 2100 tone trials and 500 catch trials from 19 sessions. The subject's summary detection scores are presented in terms of d' values (error bars indicate the S.D. of d' for the daily psychometric functions that contributed to the summary). The d' statistic is a standard signal detection measure of sensitivity (Green and Swets, 1966) that is calculated by:

$$d' = z(P_{\text{hit}}) - z(P_{\text{false alarm}}),$$

where $z(P_{\text{hit}})$ is the z score for the percentage of hits at a particular tone level and $z(P_{\text{false alarm}})$ is the z score for the percentage of false alarms. This signal detection analysis is a preferred measure of response accuracy because it takes into account not only the subject's correct detections but also false alarm rates. Absolute threshold is defined by psychophysical convention as the sound pressure level that elicits a d' value of 1 (horizontal line). Through the process of interpolation, the summary psychometric function of mouse a9xAn99 yields a threshold of 32.4 dB SPL (arrow).

6. Interpreting tone detection thresholds

An assessment of the role of $\alpha 9$ receptors in hearing sensitivity can be gained by measuring threshold differences between groups of transgenic mice and normal controls. Fig. 6 presents this comparison in the form of statistical box plots. The lower and upper bounds of each box mark the first and third quartile of the distribution of tone detection thresholds for six knock-out mice and six normal controls (this interquartile range encompasses the middle half of each distribution). The box is divided at the median of the distribution. Error bars are drawn to the highest and lowest

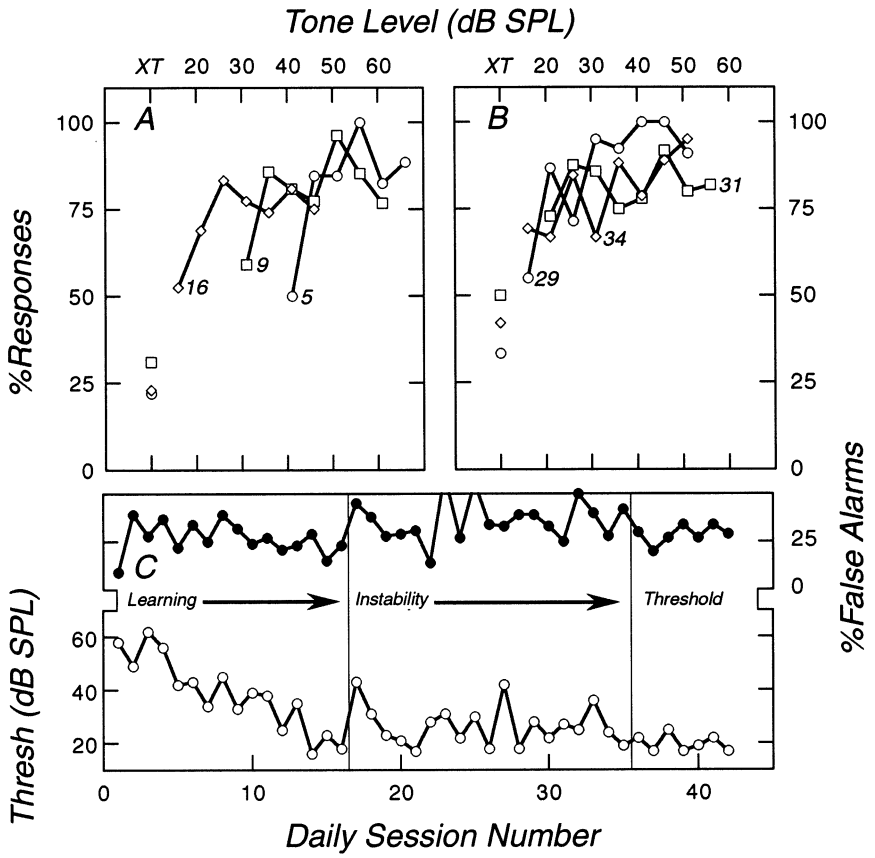

Fig. 4. Behavioral performance of a control mouse (a9Bo99) during stage 5 training. Psychometric functions illustrate typical response patterns for early (A) and late (B) stages of the training sequence. Numerical labels indicate the session number of each function in relation to the accompanying plots of daily false alarm rates and threshold (C).

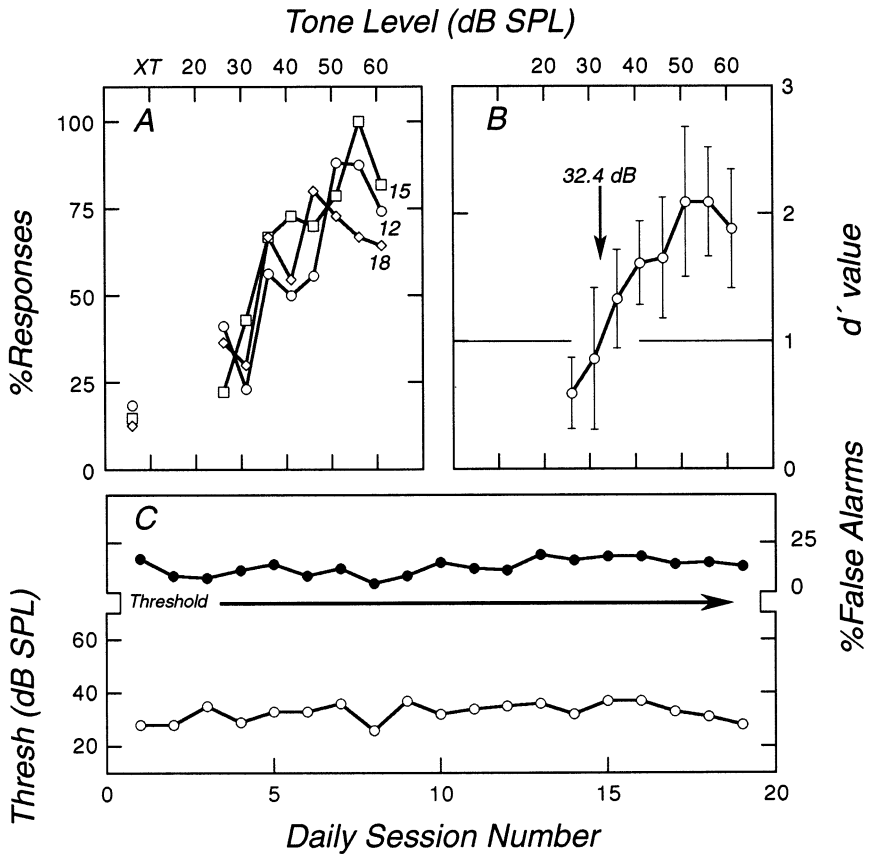

Fig. 5. Behavioral performance of an $\alpha 9$ knock-out mouse (a9xAn99) during stage 6 training. The summary psychometric function (B) shows pooled results from 18 daily sessions. The subject's detection threshold (arrow) is estimated from these data by interpolating the stimulus level at $d' = 1$ (horizontal line). Additional plotting conventions are described in Fig. 4.

thresholds that fall within the 1.5 interquartile range. Both distributions display a high degree of overlap and fail to show significant differences between subject groups when analyzed statistically (two-tailed t-test, $P = 0.95$). These results support our hypothesis that $\alpha 9$ knock-out mice possess the same auditory sensitivity as normal controls. It is interesting to note that the threshold of a9xAn99, whose data are depicted in Fig. 5, shifted at 16-kHz from 32.4 to 21.0 dB SPL after being asked to detect the 16-kHz stimulus embedded in a white-noise masker for several weeks. This is a common finding amongst subjects that report thresholds in quiet twice — once before and once after being challenged by a noise masker. This phenomenon emphasizes the need to monitor hearing for several weeks after stable data are obtained to ensure that the subject reports detection of stimuli at minimal SPLs.

7. Conclusion

This report has illustrated general principles for assessing auditory sensitivity in transgenic mice by presenting a detailed account of the training and testing procedures that are required to measure 16-kHz tone detection thresholds in $\alpha 9$ knock-out mice. With only minor modifications of stimulation paradigms, these guidelines can be used to investigate different aspects of

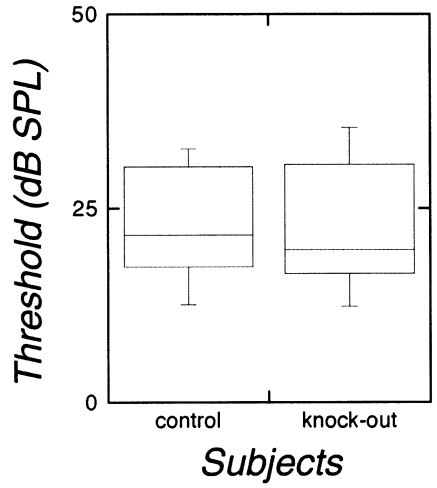

Fig. 6. Detection thresholds of $\alpha 9$ knock-out mice and normal controls. These box plots show the range of 16-kHz thresholds for $\alpha 9$ knock-out ($n = 6$) and normal control ($n = 6$) mice. See text for plot conventions.

auditory function in other mice strains. For example, the C57BL/6 mouse has served as the primary animal model of age-related hearing loss for many years (Mikaelian, 1979; Henry and Chole, 1980). The time course and frequency progression of deafness in this mouse strain can be characterized simply by conducting the detection task with a complete range of tone frequencies. The precise delineation of auditory deficits revealed by these behavioral measures can be related to ongoing changes in cochlear anatomy and physiology to identify the metabolic and genetic factors that contribute to human presbycusis (Li and Borg, 1991; Mizuta et al., 1993; Parham, 1997).

Although the transgenic mice in the current study showed normal thresholds when hearing sensitivity was measured under quiet conditions, the results of previous experiments in humans and other animal species (Williams et al., 1993; May and McQuone, 1995; Micheyl and Collet, 1996; Giraud et al., 1997; Micheyl et al., 1997, but see Sharf and colleagues (Sharf et al., 1994, 1997) for contrary data following olivocochlear lesions in humans) predict deficits in the ability of $\alpha 9$ knock-out mice to detect, locate or discriminate sounds in the presence of background noise. Such disorders are presumed to arise because the olivocochlear systems that enhance auditory processing in noise (Winslow and Sachs, 1987) are rendered ineffective by genetic knockouts of the $\alpha 9$ receptor subunits that subserve the cholinergic function of cochlear hair cells. The perceptual impact of such processing deficits can be investigated by adding masking noise to the tone detection paradigm (Ehret, 1975a), or adapting behavioral measures to more difficult tasks like the discrimination of changes in tone frequency or sound pressure level (Ehret, 1975b). As molecular biologists introduce new strains of mice with precisely altered nervous systems, the subject's own behavior will continue to provide the most formal definition of the functional consequences of genetic manipulation.

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References

Blanchet C, Erostegui C, Sugasawa M, Dulon D. Acetylcholine-induced potassium current of guinea pig outer hair cells: its dependence on a calcium influx through nicotinic-like receptors. J Neurosci 1996;16:2574–84.

Crawley JN. Behavioral phenotyping of transgenic and knockout mice: experimental design and evaluation of general health, sensory functions, motor abilities, and specific behavioral tests. Brain Res 1999;835:18–26.

Crawley JN, Paylor R. A proposed test battery and constellation of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic mice and knockout mice. Horm Behav 1997;31:197–211.

Dallos P, He DZZ, Lin X, Sziklai I, Mehta S, Evans BN. Acetylcholine, outer hair cell motility, and the cochlear amplifier. J Neurosci 1997;17:2212–26.

Deol MS, Green MC. Snell's waltzer, a new mutation affecting behaviour and the inner ear in the mouse. Genet Res 1966;8:339–45.

Dewson JH. Efferent olivocochlear bundle: some relationships to stimulus discrimination in noise. J Neurophysiol 1968;31:122–30.

Ehret G. Masked auditory thresholds, critical ratios, and scales of the basilar membrane of the house mouse (*Mus musculus*). J Comp Physiol 1975a;103:329–41.

Ehret G. Frequency and intensity difference limens and nonlinearities in the ear of the house mouse (*Mus musculus*). J Comp Physiol 1975b;102:321–36.

Elgoyhen AB, Johnson DS, Boulter J, Vetter DE, Heinemann S. Alpha 9: an acetylcholine receptor with novel pharmacological properties expressed in rat cochlear hair cells. Cell 1994;79:705–15.

Evans M. Acetylcholine activates two currents in guinea pig outer hair cells. J Physiol 1996;491:563–78.

Fuchs P, Murrow B. Cholinergic inhibition of short (outer) hair cells of the chick's cochlea. J Neurosci 1992;12:800–9.

Giraud AL, Garnier S, Micheyl C, Lina G, Chays A, Chery-Croze S. Auditory efferents involved in speech-in-noise intelligibility. NeuroReport 1997;8:1779–83.

Green DM, Swets JA. Signal Detection Theory and Psychophysics. New York: Wiley, 1966.

Henry KR, Chole RA. Genotypic differences in behavioral, physiological, and anatomical expressions of age-related hearing loss in the laboratory mouse. Audiology 1980;19:369–83.

Heinz RD, Stiles P, May BJ. Effects of bilateral olivocochlear lesions on vowel formant discrimination in cats. Hear Res 1998;116:10–20.

Housley GD, Ashmore JF. Direct measurement of the action of acetylcholine on isolated outer hair cells of the guinea pig cochlea. Proc R Soc Lond B 1991;244:161–7.

Igarashi M, Alford BR, Nakai Y, Gordon WP. Behavioral auditory function after transection of crossed olivo-cochlear bundle in the cat. I. Pure-tone threshold and perceptual signal-to-noise ratio. Acta Otolaryngol 1972;77:311–7.

Li HS, Borg E. Age-related loss of auditory sensitivity in two mouse genotypes. Acta Otolaryngol 1991;111:827–34.

Liberman MC, McGee JD, Vetter DE, Walsh EJ. Functional role of olivocochlear efferents as assessed by surgical lesions vs. genetic knockouts. Assoc Res Otolaryngol Abs 1999;22:693.

May BJ, McQuone SJ. Effects of bilateral olivocochlear lesions on pure-tone intensity discrimination in cats. Aud Neurosci 1995;1:385–400.

Micheyl C, Collet L. Involvement of the olivocochlear bundle in the detection of tones in noise. J Acoust Soc Am 1996;99:1604–10.

Micheyl C, Perrot X, Collet L. Relationship between auditory intensity discrimination in noise and olivocochlear efferent system activity in humans. Behav Neurosci 1997;111:801–7.

Mikaelian DO. Development and degeneration of hearing in the C57BL/b16 mouse: relation of electrophysiologic responses from round window and cochlear nucleus to cochlear anatomy and behavioral responses. Laryngoscope 1979;89:1–15.

Mizuta K, Nozawa D, Morita H, Hoshino T. Scanning electron microscopy of age-related changes in the C57BL/6J mouse cochlea. Scanning Microsc 1993;7:889–96.

Niemiec AJ, Moody DB. Constant stimulus and tracking procedures for measuring sensitivity. In: Klump GM, Dooling RJ, Fay RR, Stebbins WC, editors. Methods in Comparative Psychoacoustics. Basel: Birkhauser, 1995:65–77.

Parham K. Distortion product otoacoustic emissions in the C57BL/6J mouse model of age-related hearing loss. Hear Res 1997;112:216–34.

Park H-J, Niedzielski AS, Wenthold RJ. Expression of the nicotinic acetylcholine receptor subunit, $\alpha 9$, in the guinea pig cochlea. Hear Res 1997;112:95–105.

Sharf B, Magnan J, Collet L, Ulmer E, Chays A. On the role of the olivocochlear bundle in hearing: a case study. Hear Res 1994;75:11–26.

Sharf B, Magnan J, Chays A. On the role of the olivocochlear bundle in hearing: 16 case studies. Hear Res 1997;103:101–22.

Trahoitis C, Elliot DN. Behavioral investigation of some possible effects of sectioning the olivocochlear bundle. J Acoust Soc Am 1968;47:592–6.

Vetter DE, Liberman MC, Mann J, Barhanin J, Boulter J, Brown MC, Saffiote-Kolman J, Heinemann SF, Elgoyhen AB. Role of (9 nicotinic ACh receptor subunits in the development and function of cochlear efferent innervation. Neuron 1999;23:93–103.

Warr WB, Guinan JJ. Efferent innervation of the organ of Corti: two separate systems. Brain Res 1979;173:152–5.

Williams EA, Brookes GB, Prasher DK. Effects of contralateral acoustic stimulation on otoacoustic emissions following vestibular neurectomy. Scand Audiol 1993;22:197–203.

Winslow RL, Sachs MB. Effect of electrical stimulation of the crossed olivocochlear bundle on auditory nerve response to tones in noise. J Neurophysiol 1987;57:1002–21.