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A Procedure to Enhance Auditory Brainstem Response Reliability in Unrestrained Rats

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LEE, J. A., D. W. NIELSEN AND R. F. BERMAN. *A procedure to enhance auditory brainstem response reliability in unrestrained rats.* PHARMACOL BIOCHEM BEHAV 20(2) 261–267, 1984.— We describe a procedure to record auditory evoked potentials in unrestrained rats. A key feature of this procedure is the attachment of a miniature speaker to the rat's head during recording, so that the distance between the speaker and the rat's ear is held constant. A speaker base is permanently attached to the acrylic supporting the electrode connector. This system enables the recording of reliable auditory evoked potentials over long periods of time with minimal concern for the rat's position or posture.

Rat Auditory evoked response procedure Unrestrained rat Auditory brainstem response

MUCH of the work on auditory evoked potentials in species such as rats and mice has been carried out on anesthetized animals. The auditory stimuli are typically delivered to the ear through a polyethylene tube [9,19] or a funnel [7]. With larger animals such as cats [8] and guinea pigs [5], the stimulus has been presented to the anesthetized animal through hollow ear bars. In this way reliable recordings can be made, as there is an exceptionally high degree of control over the stimulus reaching the ear and no movement artifact.

It is often desirable to record auditory evoked potentials in unanesthetized animals, particularly when pharmacological agents are being studied. Use of an unanesthetized animal is also necessary when a behavioral phenomenon such as stimulus discrimination is required, or when behavioral and electrophysiological measures must be obtained simultaneously.

One solution to the problem of stimulus control for evoked potential recording in unrestrained animals has been to present the stimulus free field through an overhead speaker. Such a procedure, however, offers limited control over the stimulus and requires monitoring of the subject's position and posture. While pressure levels throughout an empty cage may not vary appreciably, sound pressure levels at the ear do vary as a function of body position. Waiting for the animal to assume a certain position may not be practical, possible, or effective.

Squires, Chu, Starr [18] and Chu, Squires, and Starr [4] used an overhead speaker when they recorded the auditory brainstem response from rats. They compensated for changing speaker-ear distances by employing relative wave latency measures, so that waves were described with regard to distance from the first wave. Although this procedure is satisfactory, information on the absolute latency of the first wave is lost. Another of their strategies for data analysis was to align the evoked potentials according to the cochlear microphonic [4]. However, this is not always possible for every recording and could result in loss of resolution of wave latencies.

Procedures that have increased auditory stimulus control for unanesthetized cats have included restraining the animal in a bag with miniature headphones attached to a headpiece [2]. However, restraint is a stressful procedure that could interfere with any pharmacological agents being studied. An elegant procedure developed for unrestrained cats [20] requires a custom-made cap with attached speakers, which fit into each external ear. Such a procedure provides excellent stimulus control, but would not be practical for rats, due to their smaller cranium and ear canal size. A procedure most similar to the one we describe in this paper was developed by Aran and Erre [1] to record the cochlear microphonic, summating potential, and VIIIth nerve action potential in the guinea pig. A small speaker is attached to the guinea pig's head, so that the speaker-ear distance remains constant. A disadvantage of their technique is that the electrical transducer employed does not provide a stimulus of sufficient intensity; so a tube directing the sound to the ear must be glued to the skin of the external meatus prior to each recording session. It is unlikely that the rat would tolerate this procedure as well as the guinea pig. In addition, calibration of their closed sound system requires that the animal be anesthetized, a condition we were trying to avoid.

In general, previous procedures for electrophysiological recording in unanesthetized animals may be effective for short periods of time. However, they may not be satisfactory for longer recording periods, for smaller animals such as rats, or when musculoskeletal responses are required.

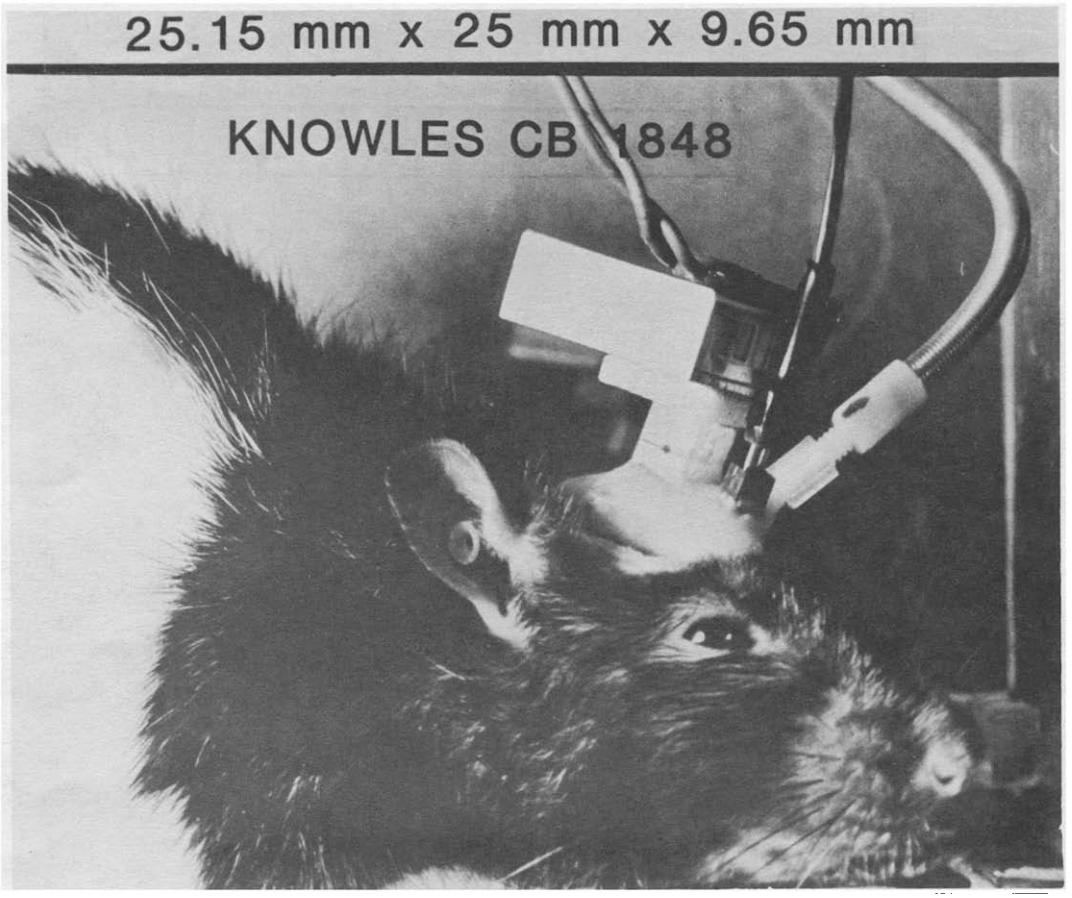

FIG. 1. Knowles speaker (CB 1848) attached to the rat's cranial pedestal. The thermocouple connector (black) and the skull electrode connector (white) are shown.

Because of our concern to present a constant auditory stimulus regardless of an unrestrained rat's position, we have developed a procedure that involves attaching a high output speaker directly to the rat's head during evoked potential recording. We have found the technique to be quite satisfactory, because it allows us to record ABR's over long sessions for several months. Some information on the procedure has been presented previously [14].

In this paper we describe the speaker, the design of the speaker base, a probe tube for measuring stimulus features at the ear entrance, and the surgical procedure for electrode and speaker base placement. Because of a relationship between the organism's temperature and ABR [11,16], we have included a thermocouple for measuring brain temperature in our preparation. Implantation of the thermocouple will be briefly described with the surgical techniques.

MINIATURE SPEAKER AND BASE

The speaker is a Knowles CB 1848 (Knowles Electronics, Franklin Park, IL) encased in stainless steel. It measures 25.15×25×9.65 mm and weighs 14 g (Figs. 1 and 5). A 15 mm length of "$^1/_{16}$ in" square brass tubing (K & S Engineering Co., Chicago, IL) is epoxyed to the bottom of the speaker. This tubing readily fits into a 5 mm long segment of mated "$^1/_8$ in" square brass tubing embedded in the center of a handcrafted Plexiglas base. The Plexiglas base, 15×6×6 mm (Fig. 2), is permanently fixed to the skull by securing it within the acrylic cement that supports the cranial electrodes. Because the speaker attachment and base tubes are square, the speaker will not rotate or shift horizontally during recording. The speaker is prevented from moving vertically by inserting a wire horizontally through small holes aligned in both tubes. The speaker can easily be removed and replaced in exactly the same position.

The Knowles CB 1848 miniature speaker was selected because of its time waveform and spectral characteristics (Fig. 3). The speaker is driven by a 0.1 msec pulse. The initial wave of the time waveform has the highest peak, and subsequent ringing is brief (completed after about 2.5 msec) so that the speaker can be used with high click rates. The frequency spectrum ranges from approximately 500 Hz to 8000 Hz with a pronounced peak at 4000 Hz. These spectral characteristics are similar to those of the TDH-49 earphones used for human auditory evoked potentials. Although the rat's hearing sensitivity, with maximal hearing at 35,000 to 40,000 Hz [6], is much higher than the human's, we have found that this speaker evokes satisfactory auditory brainstem responses.

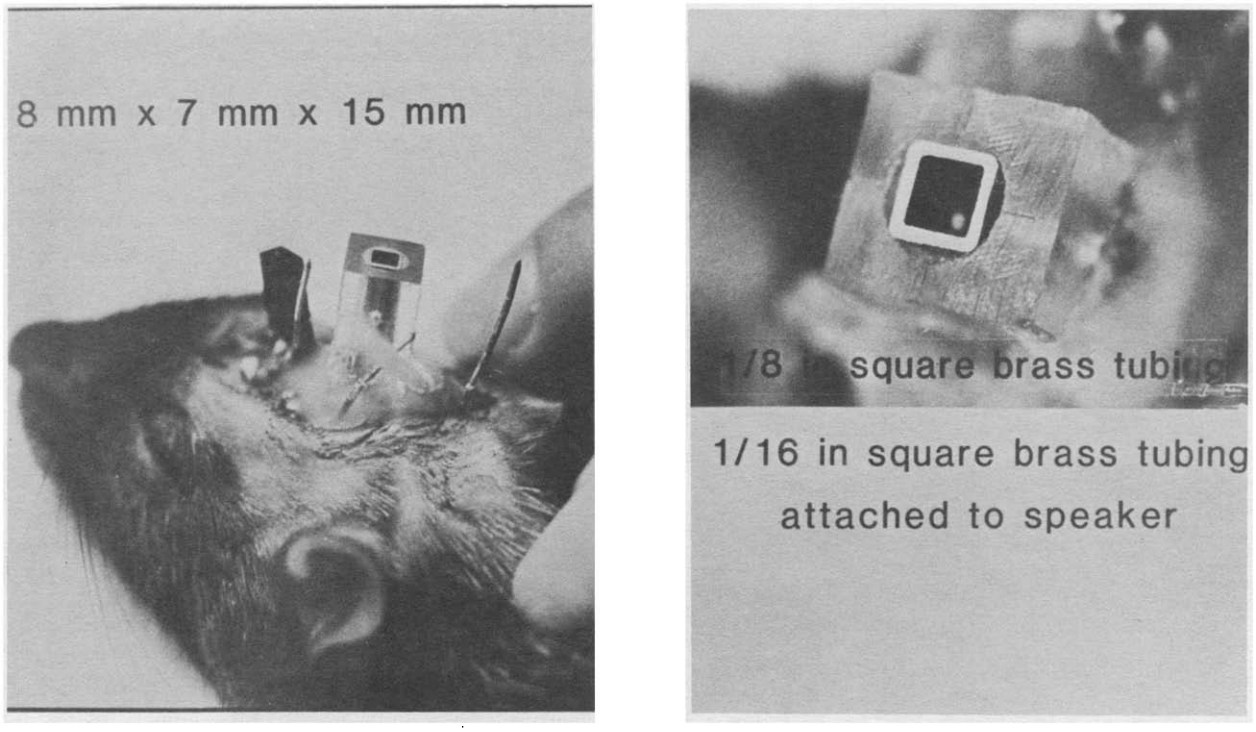

FIG. 2. Plexiglas base for the speaker. On the left it is secured within the acrylic supporting the cranial electrodes. Firm wires attached to the screw electrodes can also be seen. The right photograph is a view of the top of the Plexiglas base with the embedded "1/8 in." square brass tubing.

SURGERY

Surgery is required to mount skull electrodes, implant the thermocouple, and secure the speaker pedestal on the rat's skull. General descriptions of surgery to implant skull screw electrodes can be found in various sources [10]. A procedure for long-term EEG and brain temperature recording with a thermistor is also described by Kleinlogel and Hausammann [13].

In the present procedure, surgery is carried out under general anesthesia (45 mg/kg sodium pentobarbital, IP). After a scalp incision, the skull is cleaned and holes are drilled to implant three screw electrodes in the following locations (approximate): active negative, 8 mm anterior from the bregma on the central suture; active positive, 3.5 mm lateral on the right from the lambda; and ground, 3.0 mm lateral on the left from the bregma. An alternate ground electrode or support screw position is 3.0 mm lateral on the left from bregma. The hole for the thermocouple is drilled 4 mm anterior and 2 mm lateral on the right from bregma.

Prior to electrode implantation, firm wires are soldered to the skull-screw electrodes. After the recording electrodes are in place, the thermocouple is lowered into the forebrain; then recording electrodes and the thermocouple are covered partially with acrylic cement. After a thin layer of acrylic is applied to the area in the center of the skull, the speaker base is immediately positioned and secured with additional acrylic cement (Fig. 2). Next, the skull-screw wires are connected to the wires of a tripolar electrode (Plastic Products, Inc., MS-333), which serves as an electrode connector (Figs. 1 and 5). A mechanical connection is made by wrapping the connector wires around the electrode wires, which are then trimmed. Finally, more acrylic is applied to support the connector. The skin around the acrylic is flushed with saline, and the incision sutured as necessary.

When recordings are not being made, the speaker base and the electrode connector are covered with dust covers (Fig. 4). The dust cover for the electrode connector (Plastic Products) screws on. The speaker base dust cover is constructed of a square brass tube ($^1/_8$ "in") cemented to a square piece of Plexiglas. The cover is kept in place by a wire placed through concentric holes in the base and in the cover square tubing.

PROBE TUBE AND STIMULUS FEATURES

In addition to our concern for stimulus intensity, which led us to fasten the speaker to the rat's head, we were also concerned about the stability of other stimulus features such as time waveform, polarity [17], and frequency spectrum [12], which also affect the ABR. Another issue was the stability of the speaker over time. Several speakers were tested and rejected for various reasons. One speaker showed increased "ringing" after prolonged use, and other speakers, which had good time waveforms and frequency spectra, showed diminished output intensity after being driven for long periods of time.

TABLE 1

WAVE I-VII LATENCIES IN MSEC FOR ABR'S RECORDED ON TWO CONSECUTIVE DAYS AT TWO STIMULUS INTENSITIES (80 AND 60 dB pe SPL)

	Wave Latencies in msec						
	I	II	III	IV	V	VI	VII
80 dB pe SPL							
Day 1	1.28	2.08	2.56	3.36	4.04	5.20	6.32
	1.28	2.08	2.56	3.36	4.08	5.16	6.28
Day 2	1.28	2.08	2.60	3.40	4.04	5.28	6.36
	1.28	2.08	2.56	3.36	4.08	5.28	6.36
65 dB pe SPL							
Day 1	1.44	2.28	2.56	3.48	4.12	5.20	
	1.40	2.28	2.57	3.44	4.16	5.36	
Day 2	1.44	2.32	2.64	3.52	4.20	5.36	
	1.44	2.28	2.60	3.52	4.20	5.36	

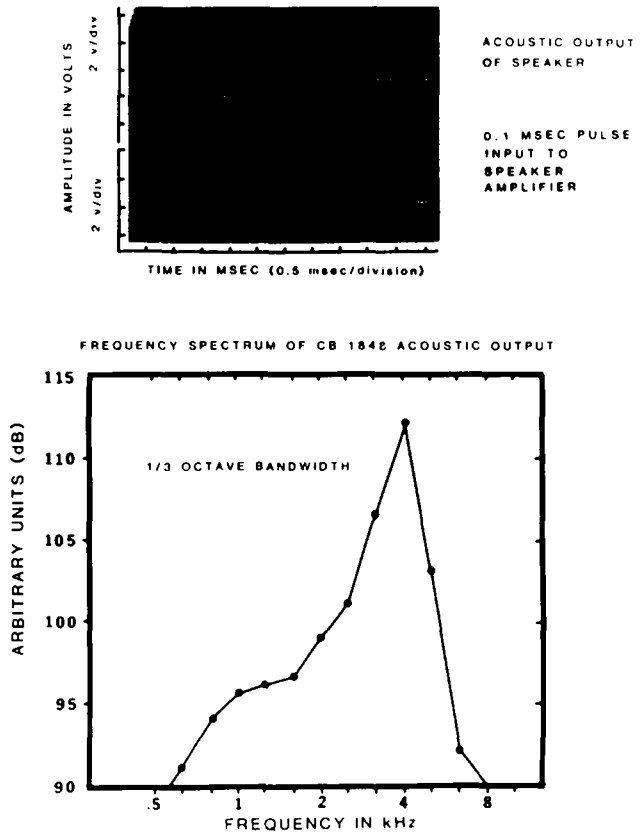

FIG. 3. Time waveform (upper) and frequency spectrum (lower) of the CB 1848 speaker. The speaker is driven by a 0.1 msec pulse, which is displayed below the time waveform.

Because of our concerns for stability of all stimulus features, we developed a procedure to measure stimulus characteristics directly at the ear. To make such measurements, we inserted a probe tube under general anesthesia after the skull screw electrodes were in place. The tube (PE No. 4541) was inserted subcutaneously between the top of the head and the entrance to the ear canal. The end at the ear canal entrance was flared to prevent the pinna skin from growing

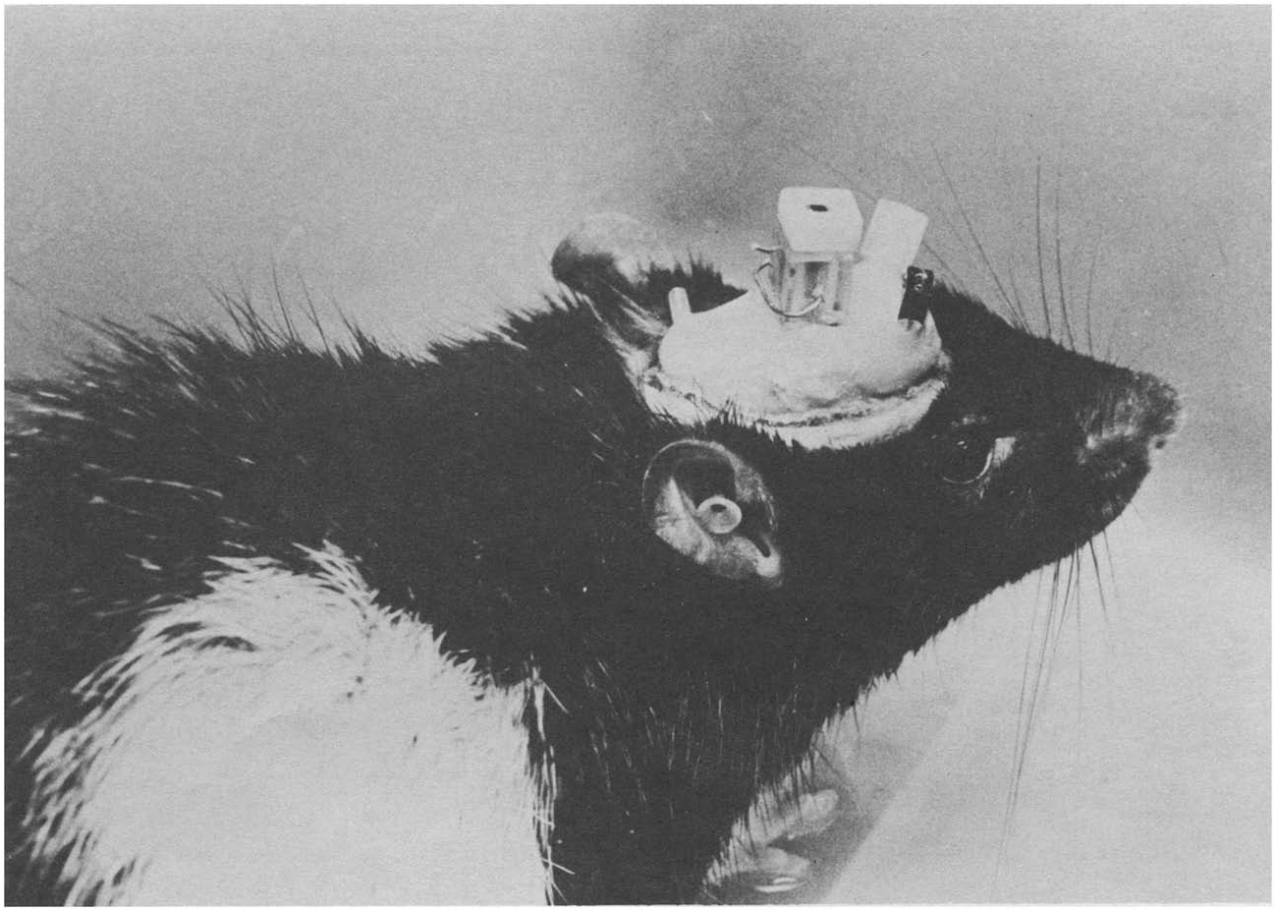

FIG. 4. Dust covers for the speaker base and the electrode connector. Both ends of the probe tube (PE #4541), which goes from the ear canal entrance to the top of the head, are also seen.

over the end of the tube (Figs. 1 and 4). The other end protruded from one side of the cranial pedestal. At this end a calibrated, probe microphone (1.4 mm diameter, Knowles XL-9073) was inserted to measure the stimulus features (Fig. 5).

RESULTS OF RELIABILITY TEST FOR STIMULUS AND ABR

With this calibrated probe microphone we measured the time waveform and the frequency spectrum over time and found them to be satisfactorily constant. We were also satisfied with the reliability of the ABR recordings. The ABR tracings for two consecutive days, recorded with two stimulus intensities (80 dB and 60 dB pe SPL), are shown in Fig. 6. Two ABR's recorded each day are shown. Latencies for ABR waves I-VII are listed in Table 1. Since Wave VII for the ABR's recorded with the lower stimulus intensity was not readily identifiable, Wave VII latencies are given only for those ABR's recorded at the higher stimulus intensity.

After measuring stimulus features directly at the ear (through the probe tube), and determining that the Knowles CB 1848 speaker was reliable, we stopped using the probe tube. Rather, we set the intensity of the speaker before each use by recording the speaker's output with a microphone placed a constant distance (3 cm) from the speaker. In this way we can monitor the time waveform and set the stimulus intensity by setting the height of the largest peak equivalent to that of a 1000 Hz tone. We also monitor consistency of the pulse input to the speaker by displaying it on an oscilloscope with the time waveform.

DISCUSSION

This technique for direct cranial mounting of an electroacoustic transducer is relatively simple to use in rats. The animals adapt to the procedure rapidly, and excellent recordings can be obtained during the first session. It does not appear to interfere with the rat's characteristic behaviors, such as grooming, rearing, or sleeping. Moreover, the technique does indeed provide high reliability, particularly within sessions.

Because high reliability can be obtained in unanesthetized animals, the procedure is particularly suited to the study of electrophysiological effects of pharmacological agents. We have used this procedure to measure acute effects of alcohol over time [15]. Without the complications of anesthesia it is possible to study drug interactions. Drug-behavior interactions can also be investigated with this procedure, since the

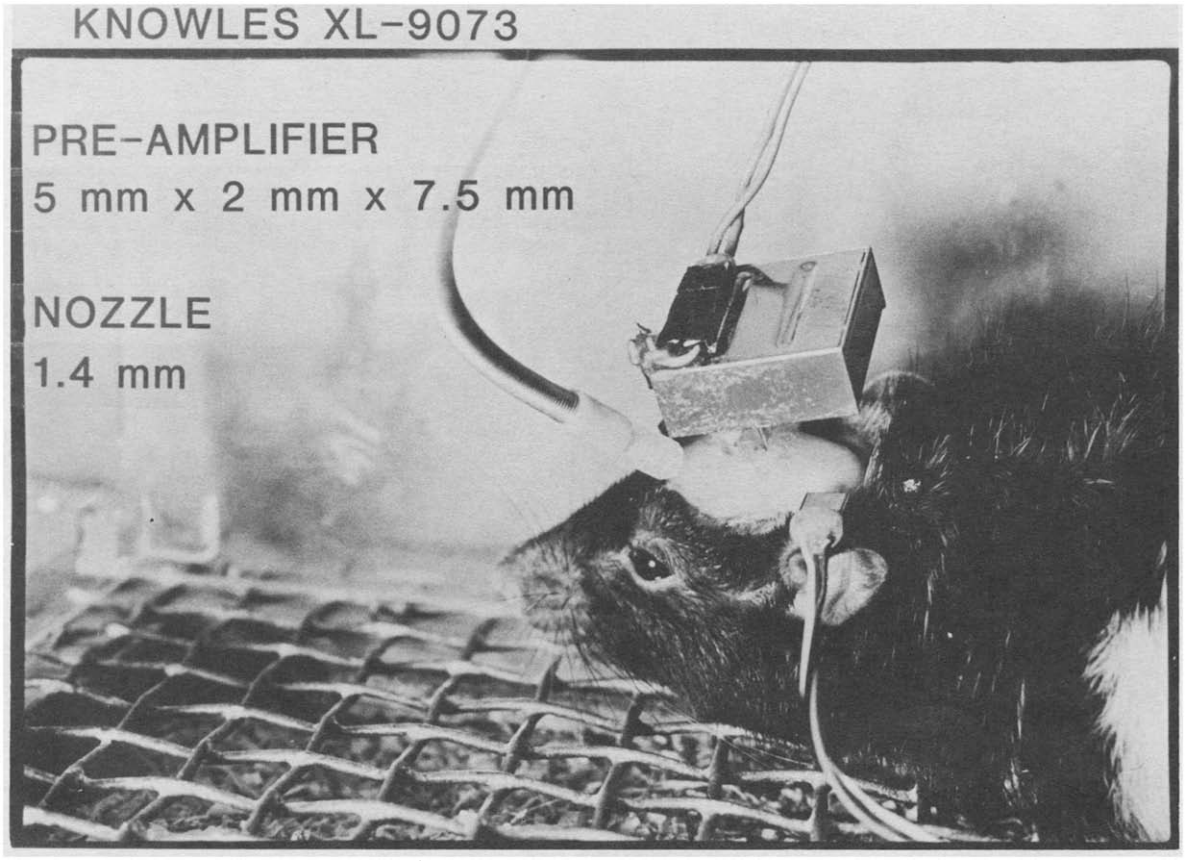

FIG. 5. Probe microphone (Knowles XL-9073) inserted at the top end of the probe tube. The speaker (Knowles CB 1848) and the electrode connector are also shown.

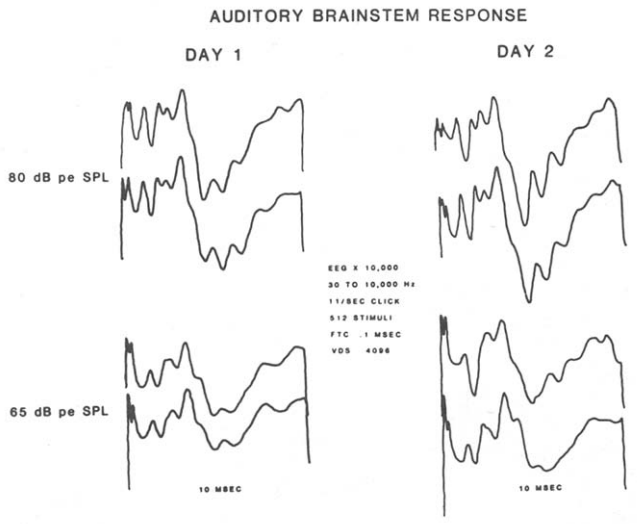

FIG. 6. ABR recordings from two consecutive days, at two different intensities, 80 and 60 dB peak equivalent (pe) SPL. On each day, two ABR's were recorded at each intensity. Each ABR was recorded with 512 stimulus presentations.

rat is free to emit responses such as bar pressing. Behavioral observations can also be carried out by a closed circuit tv monitor, which is already an aspect of our procedure.

To date, we have prepared approximately 17 rats and have found that recordings can be made repeatedly in the same animal over as long as eight months. We have encountered only minor limitations of the procedure. The speaker pedestal and electrode assembly, although kept as small as possible, may be dislodged by repeated handling or other jarring. Therefore, care is required when attaching the electrodes and speaker. Furthermore, animals should be housed in plastic cages devoid of wire mesh or other protrusions that may dislodge the cranial assembly. Because the best recordings are still obtained from quiescent rats, the rats are handled and adapted to the recording chamber and procedures prior to data collection. Monitoring the EEG on an oscilloscope and the rat on a video screen is recommended in order to select optimal recording times. Also, in more active rats we recommend that the speaker be secured with a horizontal wire through the base and the square tube attached to the speaker, referred to above.

The speaker pedestal is cemented close to the skull surface. As a result, electrode placement is determined to some extent by the pedestal position on the skull. If more central electrode placement is desired, cortical electrodes rather

than the skull-screw type described above can be used.

We developed this system to record the auditory brainstem response in rats. It could be adapted for use in species of similar size and for other auditory electric responses. The technique might also be considered for psychoacoustic procedures, such as auditory discrimination and threshold measures, where precise control over stimulus presentation is essential, and a behavioral response from an awake animal is required.

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REFERENCES

1. Aran, J.-M. and J.-P. Erre. Long-term recording of cochleoneural potentials in the guinea pig. In: *Technical Basis of Auditory Investigations, Engineering in Medicine*, edited by H. A. Beagley. Londres: Oxford University Press, 1979, pp. 233–261.
2. Buchwald, J. S., S. B. Holstein and D. S. Weber. Multiple unit recording: technique, interpretation, and experimental applications. In: *Methods in Physiological Psychology Vol I. Bioelectric Recording Techniques*, edited by R. F. Thompson and M. M. Patterson. New York: Academic Press, 1973, pp. 201–242.
3. Buchwald, J. S. and C.-M. Huang. Far field acoustic response: origins in the cat. *Science* **189**: 382–384, 1975.
4. Chu, N.-C., K. C. Squires and A. Starr. Auditory brain stem potentials in chronic alcohol intoxication and alcohol withdrawal. *Arch Neurol* **35**: 596–602, 1978.
5. Gardi, J. N. and C. I. Berlin. Binaural interaction components: their possible origins in guinea pig auditory brainstem response. *Arch Otolaryngol* **107**: 164–168, 1981.
6. Gourevitch, G. Detectability of tones in quiet and in noise by rats and monkeys. In: *Animal Psychophysics: The Design and conduct of Sensory Experiments*, edited by W. C. Stebbins. New York: Plenum Press, 1970, pp. 67–97.
7. Henry, K. R. Auditory nerve and brain stem volume-conducted potentials evoked by pure-tone pips in the CBA/J laboratory mouse. *Audiology* **18**: 93–108, 1979.
8. Huang, C.-M. and J. S. Buchwald. Factors that affect the amplitudes and latencies of the vertex short latency acoustic responses in the cat. *Electroencephalogr Clin Neurophysiol* **44**: 179–186, 1978.
9. Jewett, D. L. and M. N. Romano. Neonatal development of auditory system potentials averaged from the scalp of rat and cat. *Brain Res* **36**: 101–115, 1972.
10. John, E. R. Brain evoked potentials: acquisition and analysis. In: *Methods in Physiological Psychology Vol I. Bioelectric Recording Techniques*, edited by R. F. Thompson and M. M. Patterson. New York: Academic Press, 1973, pp. 317–356.
11. Jones, T. A., J. J. Stockard and W. J. Weidner. The effects of temperature and acute alcohol intoxication on brain stem auditory evoked potentials in the cat. *Electroencephalogr Clin Neurophysiol* **49**: 23–30, 1980.
12. Klein, A. J. and D. C. Teas. Acoustically dependent latency shifts of BSER (wave V) in man. *J Acoust Soc Am* **63**: 1887–1895, 1978.
13. Kleinlogel, H. and K. F. Hausammann. A method of continuous 24 hour cable EEG- and brain temperature-recording in the rat. *Waking Sleeping* **4**: 77–85, 1980.
14. Lee, J. A., R. A. Abbott, D. W. Nielsen and R. F. Berman. A procedure to enhance auditory brainstem response reliability in unrestrained, unanesthetized rats. *Neurosci Abstr* **7**: 51, 1981.
15. Lee, J. A., R. F. Berman, D. W. Nielsen and E. P. Schoener. Alcohol and the auditory brainstem response: dose effects. *Soc Neurosci Abstr* **8**: 595, 1982.
16. Marshall, N. K. and E. Donchin. Circadian variation in the latency of brainstem responses and its relation to body temperature. *Science* **212**: 356–358, 1981.
17. Ornitz, E. M., A. Mo, S. T. Olson and D. O. Walter. Influence of click sound pressure direction on brain stem responses in children. *Audiology* **19**: 245–254, 1980.
18. Squires, K. C., N.-S. Chu and A. Starr. Auditory brain stem potentials with alcohol. *Electroencephalogr Clin Neurophysiol* **45**: 577–584, 1978.
19. Plantz, R. G., J. S. Williston and D. L. Jewett. Spatio-temporal distribution of auditory-evoked far field potentials in rat and cat. *Brain Res* **68**: 55–71, 1974.
20. Worden, F. G., J. T. Marsh. R. D. Abraham and J. R. B. Whittlesey. Variability of evoked auditory potentials and acoustic input control. *Electroencephalogr Clin Neurophysiol* **17**: 524–530, 1964.