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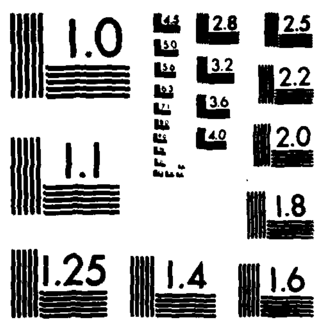

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DEVELOPMENT OF THE SWINE AS A LARGE ANIMAL MODEL FOR NOISE RESEARCH

By
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SENSORY RESEARCH DIVISION

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This report describes an attempt to develop the swine as a large animal model to be used in research on noise induced hearing loss. Animals were trained to perform in a "yes-no" signal detection paradigm for heat as a positive reinforcement. Results indicate that the animals can learn this task; however, the method failed to produce an audiogram. This was attributed to a failure to induce an adequate motivational level in the subjects.

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INTRODUCTION

This report is a summary of an exploratory effort to identify a large animal suitable for studies of noise induced hearing loss. Animal models are used extensively in studies of the effects of both continuous and impulsive noise to establish a data base relating noise parameters to measures of auditory injury. At present, the chinchilla is the primary animal model used at the United States Army Aeromedical Research Laboratory (USAARL). A large data base relating parameters of impulse noise to threshold shift and sensory cell destruction is accumulating under the blast overpressure research project. To facilitate the eventual extrapolation to humans, the data being produced using chinchillas must be augmented by data from other species. In addition, the chinchilla has several shortcomings as the only animal model for this project. It is not suitable for field studies since it is intolerant of heat and high intensity blast. For impulse noise at levels above 160 dB, chinchillas suffer perforations of the tympanic membrane and middle ear disruptions (Eames, et. al., 1975).

The establishment of an animal model for noise research requires the development of procedures for determining the audiogram of the animal and controlling the animal during the exposures. Additional procedures must be developed to determine the transmission properties of the outer and middle ear, surgical destruction of one inner ear, and extraction of the inner ears for histological examination. Further, the animal selected should have physiological similarities to the human auditory system, and have ears for which hearing protection can be adapted.

The first animal selected for evaluation as a potential large animal model was the swine. This choice was made because swine are commonly used medical models for nonauditory physiology, having many similarities to man (Mount and Ingram, 1971; Bustad and McClellan, 1966). Swine also are durable creatures and should be able to withstand environmental extremes encountered in field testing (e.g., exposure to artillery blast waves on a firing range). In addition, swine are relatively inexpensive and easily maintained, which would permit the use of the large number of subjects required in noise research. While no audiogram has been published, swine are regarded to be "auditory animals" relying heavily on auditory cues in their social behavior (Hafez and Signoret, 1969). These factors suggest that swine would make a reasonable candidate for a large animal model.

The development of an audiometric procedure for use with animals involves training the animal to make an observable response to acoustic stimuli. Swine are reported to be easily trained in a variety of learning paradigms (Hafez and Signoret, 1969). They have been successfully trained with both classical and instrumental conditioning (Kratzer, 1971).

Pavlov (from Marcuse and Moore, 1944) attempted to use pigs as research subjects but found them uncooperative and disruptive and concluded that "all pigs are hysterical." Moore and Marcuse (1945) were able to train pigs in a Pavlovian-type paradigm to elicit salivary, cardiac, and motor responses with little difficulty. Liddell and Anderson (1931) found that pigs developed conditioned foreleg reflexes earlier than goats, sheep, or rabbits. The behavioral problems encountered by Pavlov have been experienced by many experimenters, although they may have been of a lesser extent. Marcuse and Moore (1944) studied this behavior and labeled it "tantrum behavior." Restraining the animals in a Pavlovian frame led them to the conclusion that restriction plays an important part in producing excitatory behavior. Because of its restrictive nature and the subsequent behavioral problems associated with its use on pigs, classical conditioning was deemed unsuitable for use in an audiometric procedure.

Instrumental learning procedures have been used by a number of investigators. Pigs have been taught to avoid shock by jumping a barrier in response to a tone (Karas, Willham and Cox, 1962; Baldwin and Stephens, 1973). Marcuse and Moore (1944) trained two sows to lift a box lid to obtain a food reward in an auditory frequency discrimination task. In a series of studies, pigs were conditioned to press a panel with their snouts in order to receive a short burst of radiant heat in a cold environment (Baldwin and Ingram, 1967; Baldwin and Ingram, 1968a; Ingram, Walters and Legge, 1975; Baldwin and Ingram, 1968b).

Jenkins (1979) attempted to determine an audiogram for miniature swine using a panel press for food reinforcement. He used a two-choice paradigm in which the animal was trained to press one response panel when a "tone" trial was presented and to press a second panel when a "no tone" trial was presented. The animal initiated a trial by making an observing response. A pellet of dry food was given for correct responses. The animal quickly learned this task. However, the thresholds obtained by Jenkins appeared to be elevated. He discussed several possible causes for the high thresholds. The two paramount problems were: noise generated by the animal, especially mastication noises associated with the food reinforcement, and nonuniformity of the sound field produced by the test apparatus. Using food reinforcement appears to be inconsistent with threshold determination since the mastication noise will tend to mask threshold level signals.

The use of heat appears to be a promising alternative as a reinforcer for developing a behavioral procedure for determining the audiogram of a pig. Young swine will readily learn to operate a switch for heat reward (Baldwin and Ingram, 1967). Only young animals (preferably 2-4 months) can be used for these studies because of the increased tissue insulation associated with their rapid growth and the subsequent diminishing of cold sensitivity (Mount, 1968). The rate of response is affected by such factors as ambient temperature, amount of heat reinforcement, and level of food intake. Swine have been found to emit high rates of panel pressing in the temperature range of -10°C to 15°C (Baldwin and Ingram, 1967). The amount of heat delivered may be controlled by the distance of the animal from the source and the duration of reinforcement. By altering the height of a bank of lamps suspended above a pig's back, Ingram (1975) found that distance was an important determinant of response rate. Baldwin and Ingram (1968a) studied magnitude of reinforcement by comparing 6- and 12-sec reinforcements with 6 or 12 250w-lamp arrays. They concluded the duration of reinforcement influenced the response rates while the number of lamps did not. It also has been found that pigs on a lower level of food intake (400 g/day) respond more often than animals fed on a higher level (900 g/day) (Baldwin and Ingram, 1968b). Pigs will work steadily for long periods when these factors are arranged properly.

The objectives of the present study were to develop an audiometric procedure for swine based on heat reinforcement and to explore surgical procedures for monauralization and extraction of the inner ears.

METHOD

SUBJECTS

Two male pigs of the species *Sus scrofa* were used in the experiment. They were of mixed breed, 6-9 weeks old, and approximately 30 pounds at the beginning of the study. Both animals had been examined by the laboratory veterinarian and were in excellent physical condition. The pigs were housed singly and fed 600 g/day of Pig Starter Pellets in two rations, one before testing and another immediately afterwards. Water was available to the animals except during testing.

APPARATUS

All testing was conducted in a double-walled sound chamber (IAC, Model 1200 Series). Located in the center of the room was a test cage constructed of heavy hardware cloth siding with steel framework (see Figure 1, page 7). The cage measured 122 cm long, 91 cm high, 61 cm wide, and was elevated 20 cm above the sound room floor by a wooden stand. Rubber matting covered the floor of the test cage to muffle the animal's movements. The response panel at the front of the cage was made of steel grid, with three circular holes situated on a horizontal plane 23 cm above the cage floor. Figure 2 shows this response panel. The holes were 8 cm in diameter and 15 cm between centers. A miniature lamp (General Electric (GE) No. 222) and photocell were mounted on opposite sides of each hole for detecting the animal's responses. Stimulus lights (GE No. 1819) with plastic diffusers were placed 10 cm above the outer two holes.

Two banks of four 250w GE infrared heat lamps, both mounted in 33 cm x 33 cm arrays, were positioned 10 cm from each side of the cage. Pure tone signals were presented by a cabinet-mounted, 15-inch coaxial speaker (Altec 418B) directly facing the front of the cage. The speaker was 91 cm from

FIGURE 1. Interior of test room showing test cage, reinforcement lights, and speaker.

the .esponse panel and had a stimulus light (GE No. 1819) suspended 36 cm from the top of the speaker. A 9-inch (23 cm) fan (IMC, Model No. 12) was placed 15 cm from the rear ot the cage and raised to cage floor level with a stand. An Altec microphone (D60L) was hung 20 cm from the top of the response panel and was wired to an Altec Model 1598A monitor amplifier outside the sound chamber. All experimentation was observed on closed circuit television.

Both trial sequencing and data acquisition were controlled by Coulbourn Instruments (CI) solid state logic modules. Acoustic signals were generated by a Fluke Oscillator (Model 6010A) and gated with an audio gate (CI, S84-04). Signal level was adjusted with a programmable attenuator (CI, S85-08) and a Hewlett-Packard attenuator (Model 350D). The signal then was sent through an Altec amplifier (Model 1594B) and a final level adjustment made with a Grason Stadler Attenuator (Model 1293). A Hewlett-Packard Voltmeter (Model 3400A) was used for calibrating voltages during testing.

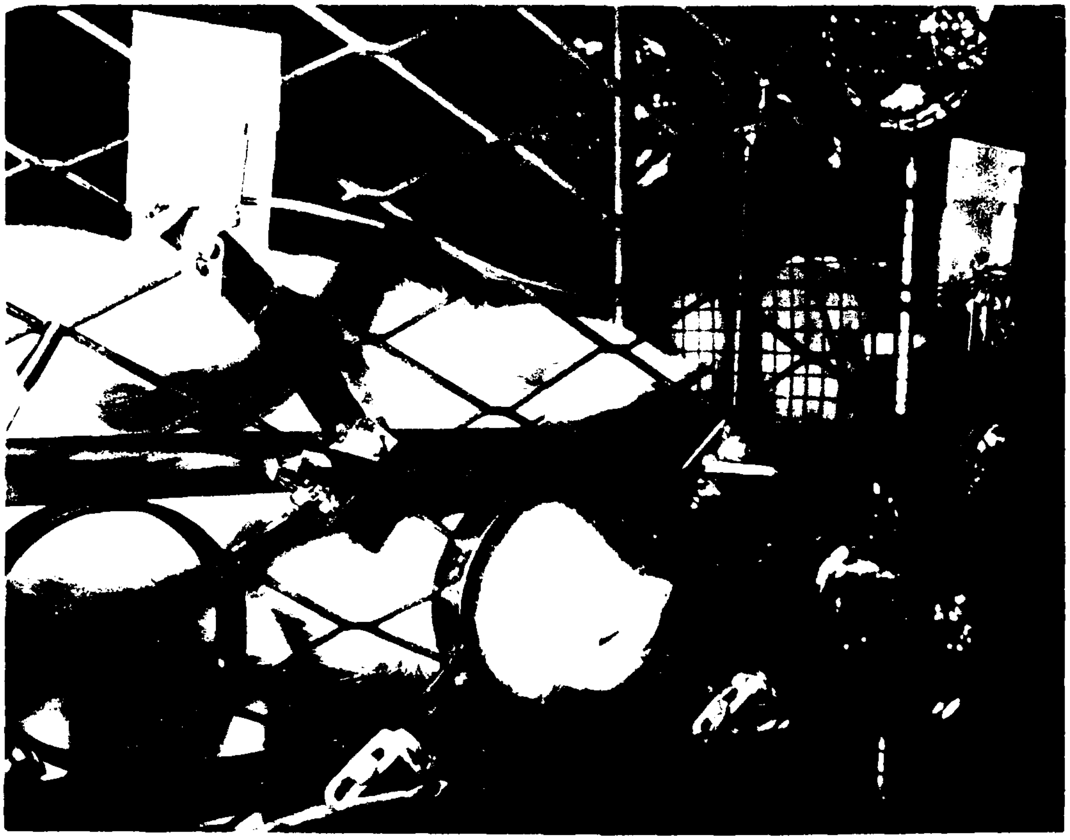

FIGURE 2. Response panel showing animal during observing response.

Sound field calibration was done with a Brüel and Kjaer (B&K) ¼-inch condenser microphone (Type 4133) powered by a Microphone Power Supply (B&K, Type 2804). A measuring amplifier (B&K, Type 26/6) was used for reading sound levels, and a Nicolet 440A spectrum analyzer determined distortion products. The sound field was calibrated by measuring the sound pressure level of pure tones at each test frequency over a region inside the test cage which approximated the animal's head position. This region contained three vertical planes measuring 22.8 cm by 22.8 cm and located from 7.6 cm to 22.8 cm from the response panel into the cage. Each plane consisted of 16 measurement points (4x4) with 7.6 cm between points. The planes were laterally centered upon the center response hole and ranged from the bottom of the response holes to 15.2 cm above them. Table 1 (page 10) shows the mean, median, and range for the 48 values of each frequency for maximum signal level achievable with the audio circuit as described.

The sound chamber was cooled by blowing cold air from an 18,000 BTU window air conditioner through the ventilation system of the room. Custom-built duct work joined the air conditioning unit and ventilation ports.

TABLE 1

MEAN, MEDIAN AND RANGE IN dB SPL FOR THE 48 CALIBRATION VALUES OF EACH FREQUENCY

FREQ (in kHz)	$\bar{X}$	M	R
1.4	100.70	100.55	9.5
1.0	96.70	97.50	9.7
.500	94.93	95.40	11.1
2.0	106.83	106.70	6.0
.125	98.97	99.20	2.2
4.0	91.00	91.75	12.6
8.0	76.17	77.10	15.4
.250	95.70	95.80	4.6
5.7	87.47	87.35	14.2
2.8	100.67	101.05	6.6

The temperature in the test chamber was monitored with a mercury thermometer suspended near the front of the speaker. Ambient noise levels in the test chamber with the air conditioner and all test equipment running are given in Table 2 (page 11).

PROCEDURE

The training procedure was designed to bring the animal's behavior under the control of an auditory stimulus, a 500-msec sinusoidal signal (tone). The final paradigm was patterned after a "yes-no" signal detection task (Green and Swets, 1966). Figure 3 (page 12) shows a schematic diagram of the major events within a trial. In each trial the animal was required to make one response if a signal was presented and a different response if no signal was presented. A trial was initiated by lighting the stimulus light located on the front of the speaker. After the appearance of the stimulus light, the subject was required to emit an observing response. The response was the insertion of the snout into the center response hole. After the observing response was held for 500 msec the stimulus light was turned off and a 500-msec observation interval occurred. During the

TABLE 2

AMBIENT NOISE LEVELS IN THE TEST CHAMBER
WITH THE AIR CONDITIONER AND ALL TEST EQUIPMENT RUNNING

	Test Frequencies in Hertz								
	31.5	63	125	250	500	1K	2K	4K	8K
dB	44.2	35.0	27.8	19.8	13.2	11.0	10.0	10.5	10.5

observation interval the signal was presented or not presented at random. The signal occurred on approximately 50% of the trials. After the observation interval, the subject had to make a response before the trial sequence would proceed. A correct response on a signal trial was defined to be the insertion of the animal's snout in the left response hole. A correct response on a no signal trial was the insertion of the snout in the right response hole. After the response was made, the feedback interval was initiated. If a correct response was made, the heat lamps were turned on for 2.5 seconds. If an error occurred, a nonheat time-out of 2.5 seconds was given. After the feedback interval, a 3-second time-out was given as minimum inter-trial interval. Any response during the feedback and inter-trial intervals was ignored. The next trial was then initiated by the reappearance of the stimulus light.

Three distinct stages of training were used to teach this paradigm to the subjects. In the first stage, the animal was trained to emit the observing response. The pig was placed in the test cage at an ambient room temperature of $10^{\circ}C \pm 2^{\circ}$. During this stage of training, the subject was only required to hold the observing response for 500 msec to receive the heat reinforcement. A 3-second time-out followed the reinforcement

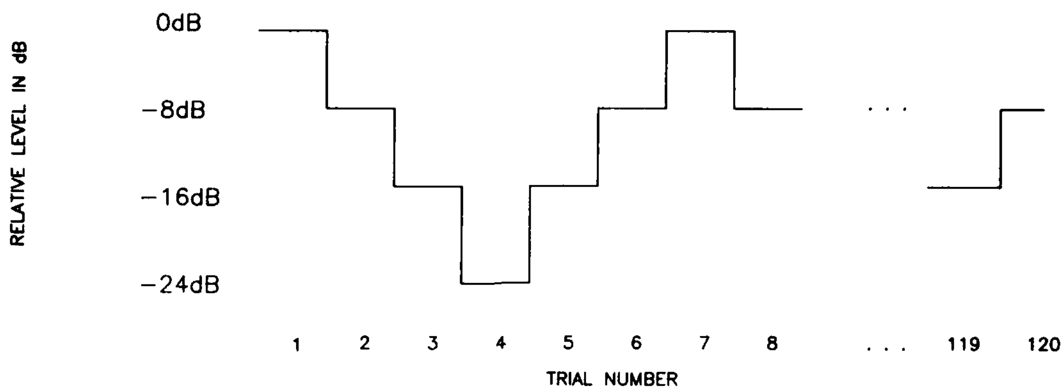

FIGURE 3. Schematic Diagram of Relative Signal Levels Across Trials

and a new trial started with the onset of the stimulus light. Responses made when the heat lamps were on or during the time-out were not reinforced. Similarly, any response that carried over to the next trial interval would keep the stimulus light off, thus delaying a new trial until the pig pulled its snout out of the response hole. During the first few training sessions, the subject's attention was drawn to the response hole with food and vocal encouragement by the experimenter. The observing response was considered to be learned when the number of reinforcements were greater than 120 for a 45-minute session. Except where noted, sessions were given once per day at the same time of day ± 1 hour.

In the second stage, the full trial sequence was implemented. The observing response was no longer reinforced. The reinforcement was contingent on the response to the observation interval. During the response interval a correlated visual cue, consisting of a light panel being illuminated above the correct response hole, was used. This correlated visual cue was the only difference between the second and the third stages. The transition to the third stage was accomplished by reducing the illumination of the cue lights in four steps. The fourth step eliminated the visual cue.

During the last two training stages a block of 120 trials was presented, 12 trials at each of 10 frequencies in stage two and 120 trials at 1000 Hz in stage three. The signal level was controlled by the programmable attenuator to produce descending-ascending staircases of four levels. On each trial the signal level changed by 8 dB. Figure 3 (page 12) shows a schematic diagram of signal levels across trials.

During the second and third stages of training the signal levels were selected so that the lowest level in the staircase at each frequency was clearly audible to the experimenter. Late in the third stage training these levels were reduced by 16 dB.

On each trial the subject's response was recorded in one of four categories: (1) signal presented - left response (hit); (2) signal presented - right response (miss); (3) no signal presented - left response (false alarm); and (4) no signal presented - right response (correct rejection). Four counters were used to record these data for each signal level. This form of data recording permits the calculation of percent correct responding as well as the signal detection index, d' (Elliott, 1964; Green and Swets, 1966).

RESULTS AND DISCUSSION

The first stage training (observing response) for the first subject, "Joe," proceeded rapidly. During the first two sessions the experimenter was present in the room and the total number of reinforced responses was less than 120 in 45 minutes. In the third session the subject emitted 187 reinforced observing responses with no assistance from the experimenter. Table 3 (page 14) gives the number of reinforced responses for sessions 3 through 7. Data for sessions 1 and 2 are omitted since they were contaminted with experimenter-induced responses.

While the response rate for session 4 appears low, most of the responses were made during the last 35 minutes. This suggested that an adaptation period during which the subject would cool down might facilitate the responding. Therefore, the procedure was modified to include an initial adaptation period of 15 minutes for all sessions throughout the remainder of the experiment. During the adaptation period, the subject was kept in the transport cage in the cold environment and was moved to the test cage to begin the test session.

After seven sessions of training on the observing response, Joe was started on the second stage of training using tones and visual cues. During the first seven sessions of the tone training, response rates were low resulting in incomplete blocks of data. In addition, experimenter-assisted responses contaminated the percentage of correct responses during

TABLE 3

NUMBER OF REINFORCED RESPONSES FOR SESSIONS THREE THROUGH SEVEN

Session No.	Time	Session Time/Minutes	Reinforcements
3	A.M.	45	187
4	P.M.	70	95
5	A.M.	45	195
6	P.M.	45	129
7	P.M.	45	172

TABLE 4

PERCENT CORRECT RESPONDING DURING LAST EIGHT SESSIONS OF SECOND STAGE TRAINING

Session No.	P(C)	No. of Trials	Cue Light Intensity
8	89.1	120	4
9	84.9	73	4
10	83.9	118	4
11	94.1	120	4
12	90.3	62	3
13	82.0	89	2
14	63.8	47	2
15	70.8	120	1

these sessions. By the eighth session of tone training, the subject completed a 120-trial block without experimenter intervention. During this phase of training signals at all levels and all frequencies were clearly audible. Therefore, all of the trials on each session were pooled to give an overall percent correct as a measure of how well Joe had learned the task. Table 4 (page 14) contains a summary of the percent correct responses for the last eight sessions of the second stage training. By session 12, the subject was showing signs of low motivation. He spent inordinate amounts of time at the back of the test cage or lying down. In session 13, the blower fan was employed to induce a "wind chill factor" in an effort to increase motivation. This proved to be disruptive at first as the subject would show signs of agitation. After a while, the subject settled down and the fan could be used to reduce the amount of time spent in competing behaviors. By sessions 11 and 12 a fairly high level of performance had been achieved. The introduction of the fan and the reduction of the light cues contributed to the reduction in performance after session 12. It is possible the use of cue lights was counterproductive in that relearning the auditory task may have occurred after they were phased out.

By the time the third stage of training was started, Joe was approximately 13 weeks old. Since training was taking longer than anticipated, it was decided to abandon the attempt to obtain a full audiogram and concentrate on obtaining a threshold at one frequency at a time. The first frequency to be tested in isolation was 1000 Hz. Throughout the third stage training all trials in a session used the same frequency signal.

Table 5 (page 16) contains a summary of the percent correct for the first 12 sessions in the third stage training. In an effort to run two sessions on the same day, sessions 5 and 6 were given without removing the subject from the test cage. Similarly, sessions 8 and 9 were given without interruption. As shown by the low number of trials in sessions 6 and 9, this procedure was not successful.

For those sessions on which complete blocks of trials were obtained, the data were analyzed to produce d' values at each signal level. These results are shown in Table 6 (page 16). Examination of Tables 5 and 6 reveals that over sessions the performance shows an unacceptably high amount of variability and the relationship between performance and signal level does not display the rapid reduction characteristic of "threshold." These results are probably due to inadequate motivation. In session 13 the subject rooted up the floor mat and ceased responding after 59 trials. During session 14 the subject rooted up the floor mat and continued to push it around the cage without completing any trials. As a result no more training sessions were attempted.

The second subject, "Steve," started training after all sessions with Joe were complete. An adaptation period was utilized starting with the first session. In all other respects, Steve was treated the same as Joe. However, after four sessions Steve had failed to meet the 120 reinforced response criterion for rate of emitting the observing response. Observation of the

TABLE 5

SUMMARY OF PERCENT CORRECT FOR THE FIRST 12 SESSIONS IN THE THIRD STAGE TRAINING

Session No.	Percent Correct For 70 dB	62 dB	54 dB	46 dB	38 dB	30 dB	Total No. of Trials
1	90.0	68.4	73.6	44.4			57
2	95.0	75.0	72.5	80.0			120
3	58.0	72.0	70.8	75.0			73
4	70.0	80.0	82.5	80.0			120
5	85.0	77.5	67.5	60.0			120
6			85.7	76.9	57.1	75.0	42
7	85.0	75.0	72.5	50.0			120
8	85.0	90.0	80.0	90.0			120
9			80.0	62.5	37.5	50.0	25
10	90.0	80.0	80.0	70.0			120
11	85.0	80.0	77.5	75.0			120
12	85.0	87.5	90.0	85.0			120
Average *							

* Average was calculated for 8 complete sessions only.

TABLE 6

RESULTS FROM ANALYZED DATA TO PRODUCE D' VALUES

Session No.	d' Values for 70 dB	62 dB	54 dB	46 dB
2	3.55+	1.24	1.30	1.68
4	.86	1.84	1.94	1.68
5	2.65+	1.52	.78	.51
7	2.76+	1.32	1.24	.28
8	2.12	2.72	1.68	3.16+
10	3.09+	1.68	2.02	1.88+
11	2.11	1.68	1.62	1.26
12	2.08	2.36	3.02+	2.84+

+ d' are indeterminate due to either zero misses or zero false alarms.

animal indicated that he had little interest in heat as a reinforcer. Steve could be easily enticed to make an observing response for a food pellet (also producing heat), but he did not show the subtle signs of reinforcement from the heat as Joe had shown. Steve would disregard the onset of the heat lamps and return to competing behaviors such as rooting, teething, and escape attempts from the test cage. During the later sessions he would emit occasional observing responses which were interspersed with long periods of these competing behaviors. Steve's overall behavior indicated that he had learned how to produce heat but simply was not motivated to do so.

This difference between the two subjects may be partially attributable to differences in the home cage temperatures. The home cages were outdoors where temperatures depended upon prevailing weather patterns. When training with Joe was initiated, the home cage temperatures would drop to 5°C at night and were usually below 12°C at the time he was brought into the test room. The training of Steve was begun later in the spring when outside ambient temperatures did not go below 20°C and were typically above 25°C when Steve was brought into the test room. It was believed that Steve's transfer from a warm environment (above 25°C) to a cold environment (10°C) resulted in an insufficient motivational level for producing high response rates (over 120) for heat reinforcement. Because of his failure to meet the required rates of responding in the initial training, audiometric training with Steve was discontinued.

After audiometric training had proceeded as far as possible, both subjects were used in an attempt to develop a surgical monauralization procedure. In both cases, the attempt at monauralization was unsuccessful. The external ear canal was found to be small, 2-4 mm in diameter, and long, 25 to 50 mm. This made the middle ear very difficult to open surgically.

CONCLUSIONS

A positive reinforcement paradigm based on heat as the reinforcer can be used to train swine in a yes-no signal detection task. The motivation level of the subject as influenced by the home cage ambient temperature and the test cage ambient temperature may be critical to the success of this procedure. Monauralization of the swine cannot be easily accomplished using standard surgical procedures. The development of the swine as a large animal model for noise research remains incomplete due to the failure to determine a valid audiogram and the failure to develop an acceptable monauralization procedure.

RECOMMENDATIONS

A procedure for monauralization should be developed before additional efforts to develop an audiometric procedure are undertaken. Alternatives to the positive reinforcement procedure using heat should be considered. If heat reinforcement is used, the test chamber should be equipped with low temperature refrigeration to permit testing in temperatures as low as -10°C. Consideration should be given to controlling the ambient temperature in the home cage.

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