J. Pharm. Pharmac., 1975, **27**, 907–913 *Received February* 2, 1975

The quantification of kanamycin ototoxicity in the rat using conditioned tone discrimination*

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Twelve male Lister hooded rats were conditioned to discriminate an 8 kHz tone (56·5 ± 0·5 dB re 0·0002 dyne cm^{-2}) and were subsequently injected subcutaneously with kanamycin (400 mg kg^{-1} day^{-1}) for 28 days; during this time and for five weeks after dosage was stopped, the nature and extent of the resultant hearing deficits were studied. The animals' Preyer reflex thresholds were determined at intervals during the study. Only one rat was unaffected by the kanamycin dosage. The onset of hearing impairment (reduced discrimination performance), which was gradual in some rats and sudden in others, generally occurred during the fourth week of kanamycin dosage although the earliest onset was towards the end of the second week. In most animals the hearing impairment progressed after kanamycin was stopped and in one rat there was a latency between the end of drug dosage and onset of hearing impairment. Hearing impairment was irreversible in five rats. One rat, whose impairment was slight, recovered normal hearing. Some rats showed a reduced discrimination performance at a time when their Preyer reflex threshold showed no elevation suggesting that kanamycin, at least initially, caused a threshold elevation rather than reduced sensitivity to intense sounds.

Of the many experimental animal studies on drug-induced auditory toxicity, few have attempted to determine directly the deleterious effects of the drug on hearing. Most workers have preferred to extrapolate information obtained by electrophysiological measurements of auditory function, for example, measurement of the various cochlear potentials.

The effect of ototoxic drugs on some indices of hearing, such as acoustic startle response (Harpur, 1974) or the Preyer reflex (Vernier & Alleva, 1968), have been studied with limited success. However, such studies have provided only qualitative information on hearing impairment.

Reliable quantitative information on the effect of ototoxic drugs on hearing can be obtained only by behavioural studies. Although such studies have been conducted on several different species of animal, for example, monkeys (Stebbins, Clark & others, 1973), guinea-pigs (Ernstson, 1972) or rats (Gourevitch, Hack & Hawkins, 1960), the total number of these studies is small, probably due to the time and difficulty involved in training the animals. However, the operant conditioning of rats appears to offer the least disadvantages in this respect.

Experience has shown that the ototoxic effects of drugs can be demonstrated in healthy laboratory animals only when dosages much larger than the normal therapeu-

*An abstract of this paper was presented at the British Pharmaceutical Conference, Norwich 1975.

tic dose regimens are used. Hawkins (1959) reported that it was necessary to administer kanamycin at a dose of 400 mg kg^{-1} day^{-1} in order to produce permanent deafness in rats.

In this present investigation rats were conditioned to discriminate a low intensity acoustic signal (an 8 kHz tone). The rats subsequently received daily subcutaneous injections of kanamycin (400 mg kg^{-1}) and a study was made of the nature and extent of the resultant hearing deficits.

METHODS

Animals

Twelve experimentally naive male Lister hooded rats, four months old at the beginning of the study, were used. They were housed individually with drinking water available at all times, and were reduced, by means of food deprivation, to 85% of their free-feeding weights. They were maintained at this level by provision of a daily weighed food ration.

Apparatus

Two operant test chambers were constructed from basic components and incorporated commercial response levers, pellet dispensers and food troughs (Campden Instruments Limited). The programming equipment for each box was constructed in module form (Fig. 1). High Threshold Logic (HTL) and Transistor–Transistor Logic (TTL) integrated circuits were used to construct the pulse shapers, timers and counters; six-digit electromechanical count displays were used. The tone and no-tone timers were calibrated at 1 s intervals from 1 to 100 s and the pre-determined counters could be set to operate the pellet dispensers after any number of responses in the range 1 to 99. The power supplies provided 24 V d.c. for operation of the pellet dispensers and electromechanical counters, and 15 V and 5 V regulated d.c. for the HTL and TTL circuits.

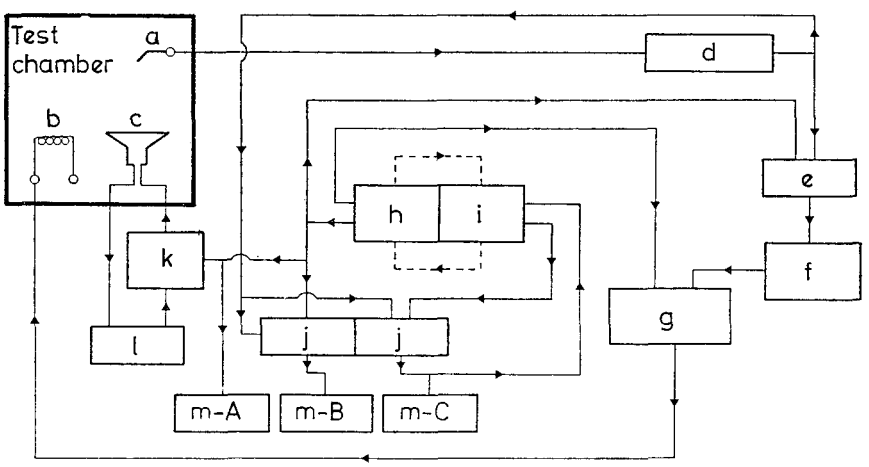

FIG. 1. Line diagram of one operant test chamber and its programming circuitry. a—lever, b—pellet dispenser, c—speaker, d—pulse shaper, e—nand gate, f—level translator, g—reinforcement counter, h—tone timer, i—no-tone timer, j—nand gate, k—tone switch, l—signal generator, m—counter displays, A—tone periods, B—lever ops (tone), C—lever ops (no-tone).

Each test chamber was enclosed within a sound resistant housing, ventilated by a small centrifugal fan which provided a steady background noise level of 70·5 ± 0·5 dB (re 0·0002 dyne cm^{-2}). An 8 ohm speaker was mounted centrally on the lid.

The acoustic signal, an 8 kHz tone, was generated by an audio frequency signal generator (Advance Instruments Limited, Type HIE) and amplified, where appropriate, by a 15 W amplifier (North Court). The signal intensity was 56·5 + 0·5 dB (re 0·0002 dyne cm^{-2}), measured with the fan switched off.

Training

Following reduction of their body weight the animals were shaped to press a lever to gain a food reward (45 mg pellet). During this initial training the operant chambers were under manual control. The rats were then given one or two (1 h) sessions during which each lever press was automatically reinforced with a food pellet. Following these sessions of continuous reinforcement, a schedule of 30 s tone and a no-tone interval of 10 s was introduced. During tone each response (lever press) was reinforced while responses during the no-tone period were suppressed by the provision that each response postponed the onset of tone for a further 10 s. Subsequently, the tone period was reduced to 10 s and, in successive sessions, the number of responses during tone required for reinforcement was increased—eventually to 15. At this stage the program shown in Fig. 2 was in use. The total number of lever presses, during a session, in both the tone and no-tone periods was recorded and discrimination of tone was indicated by a higher rate of lever pressing in the tone period than in the no-tone period; the extent of this differential was taken as a measure of discrimination performance (D.P.). The animals were trained, in hourly sessions, until their D.P. stabilized, and this served as a baseline for subsequent comparisons. They were then submitted to 1 h trials at intervals of one, two, three or four days throughout the course of the experiment.

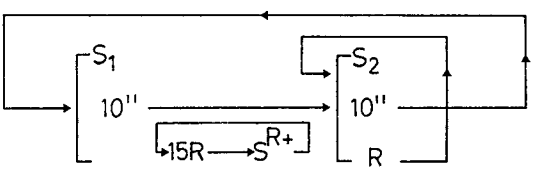

FIG. 2. Reinforcement schedule used to promote a differential between rates of lever pressing in the tone and no-tone periods. S_1–tone, S_2—no-tone, R–response, S^{R+}—reinforcement. S_1 is a fixed interval of 10 s. In the presence of S_1 every fifteenth response is reinforced with a food pellet. S_2 is also a fixed interval of 10 s in the absence of any response. S_2 is replaced by S_1 at the end of this 10 s, although each response during S_2 delays the onset of tone (S_1) by a further 10 s.

Preyer reflex threshold measurements

At intervals during the study, the Preyer (pinna) reflex of the rats was assessed to 8 kHz tones, presented at intensities of 70, 80 and 90 dB. This approach gave an estimate of each animal's Preyer reflex threshold (P.R.T.). Each rat remained in its home cage and tones were presented through a speaker held six inches from the animal. The presence or absence of the pinna reflex in response to each tone was assessed by visual observation, and the minimum intensity required to evoke the response was recorded for each animal.

Kanamycin administration

When baseline D.P.'s were established (after about three weeks), daily subcutaneous injections of kanamycin (400 mg kg^{-1}) were begun; these injections were continued for 28 days and the D.P. of the rats was monitored for a further five weeks after completion of the drug course. With some rats, whose D.P. had clearly been impaired by the drug, the intensity of the signal tone was increased during the last three trials.

RESULTS AND DISCUSSION

Tone discrimination

In Fig. 3 the total number of lever presses during both the tone and no-tone periods of a trial have been plotted for each of four rats. Performances during training are not shown.

Upon starting drug administration, there was an immediate drop in D.P. in about 50% of the animals. This fall was transient in each animal except in Rat No. 5 (Fig. 3,C) whose performance stabilized at a slightly lower level. Recovery was generally rapid (Fig. 3,D) and was always complete after one week. This drop in performance was thought to be due to the stress of drug administration and in particular to its effect on the animals' hunger drive. This was apparent from the fact that the effect was confined to a reduction in the number of lever presses during tone—consistent with a reduced food requirement.

Some D.P.'s were unaffected, or only slightly affected by kanamycin administration. In those rats whose performance was most impaired by kanamycin the onset of the effect was generally during the last (fourth) week of drug administration (Fig. 3, A, B), and the effect progressed despite termination of dosage. However, with two rats there was no detectable effect on performance until after the drug had been stopped and with one of these (Fig. 3, D) the performance was not seriously impaired until two weeks after drug administration was completed.

In almost all cases the onset of any effect on D.P. was detected by a simultaneous decrease in the number of lever presses during tone and an increase in the number of lever presses during the no-tone period (Fig. 3). This equalization of the number of lever presses during the tone and no-tone periods could be gradual (Fig. 3, A) or sudden (Fig. 3, B).

The impairment of D.P. was either irreversible (Fig. 3, B) or partially reversible. In Fig. 3, D it is evident that, although this animal's performance was almost totally extinguished, it began to recover almost immediately; recovery, however, was incomplete. Similarly, some animals whose performance was severely impaired, even for a period of time, did show varying degrees of spontaneous recovery (e.g. Fig. 3, A). However, recovery was complete (i.e. the effect was transient) only where the impairment was slight (Fig. 3, C).

The signal was presented to eight of the rats at an increased intensity during the final three trials. With three rats this had no effect, although the stimulus intensity was raised from 56·5 dB to 80 dB. One rat showed no improvement in D.P. but there was a rise in the number of responses in the no-tone period (Fig. 3, B). This suggested that although the animal could now hear the signal, its response training had long been extinguished. With four other rats, an increase in the intensity of the signal to 70 dB produced an improvement in D.P. (e.g. Fig. 3, A), and this improvement was

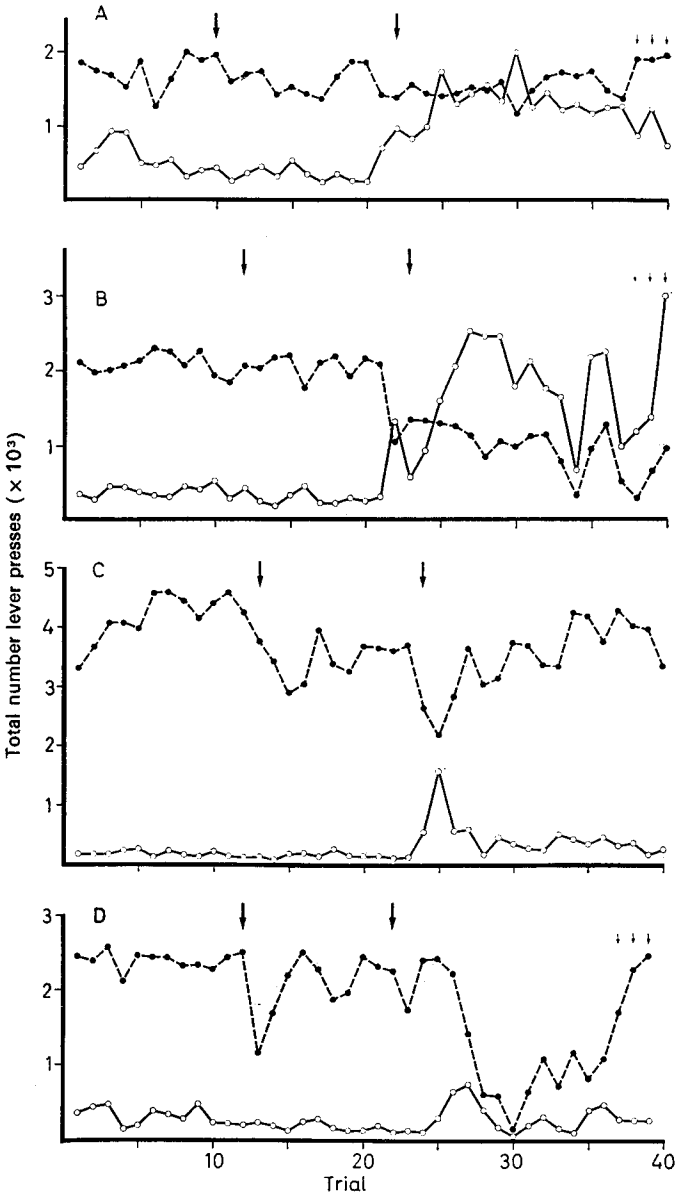

FIG. 3,A–D. Tone discrimination performance of Rats No. 3 to 6 showing lever presses during tone (●— — — —●) and no-tone (○———○). An increased signal intensity (70 dB) during the final three trials is indicated by the small arrows ↓↓↓. Large arrows denote commencement and termination of administration of kanamycin (400 mg kg^{-1} day^{-1}).

marked in one animal (Fig. 3, D). However, in all of these rats, whether D.P. had originally been abolished (Fig. 3, A), severely impaired (Fig. 3, D) or just reduced, there had been some spontaneous recovery before the signal intensity was increased.

Preyer (pinna) reflex threshold (P.R.T.)

The P.R.T.'s of the 12 rats are in Table 1. Since the tests of Preyer reflex were made at only three intensities, the accuracy of the recorded thresholds was limited.

For example, a recorded P.R.T. of 80 dB implies that the reflex was absent at 70 but present at 80 dB. Thus the actual threshold could lie between 71 and 80 dB inclusive. Therefore, only rises of 20 dB in recorded threshold were regarded as being of consequence.

The first rises in P.R.T. occurred two weeks after the termination of kanamycin administration. However, from D.P. studies it was evident that the hearing of some animals was affected during the second week of drug administration i.e. four weeks earlier. For example, the D.P.'s of rat No. 9 and rat No. 12 were abolished before

Table 1. *The Preyer reflex threshold (dB) to an 8 kHz tone of the twelve Lister hooded rats.* They were administered kanamycin (400 mg kg^{-1} day^{-1}, s.c.) from day 5 to day 32.

Rat No.	Day of Test					
	1	25	32	46	60	67
1	70	70	80	70	80/70	70
2	70	70	70	70	70	80/70
3	70	70	70	70	80/90	70/80
4	70	70	80	90+	90+	90+
5	70	70	70	70	70	70
6	70	70	70	70	70	70
7	80	70	70	70	90	70/80
8	70	70	70	80	90	80/90
9	70	70	70	90	90+	90+
10	70	70	70	70	80/90	70
11	70	70	70	70	90/80	80/70
12	80	80	70	90+	90+	90

90+ indicates that the reflex was absent at this, the highest test intensity. Two threshold intensities were recorded where differences were detected between the left and right ears.

kanamycin was stopped, but at this stage both animals had a normal P.R.T. Similarly, two weeks after completion of the injections of kanamycin several rats showed complete loss of D.P. but no rise in P.R.T. However, except where the effect on D.P. was very brief (rat No. 5, Fig. 3, C and rat No. 6, Fig. 3, D) the reduced performance was always followed, after a time lapse, by a rise in P.R.T. Furthermore, in animals whose D.P. showed partial recovery, the P.R.T. also tended to return to normal, for example, rat No. 3 (Fig. 3, A). Equally, animals whose D.P. was irreversibly impaired, showed a persistently elevated P.R.T., for example, rat No. 4 (Fig. 3, B).

From the final two measurements of P.R.T. it is evident that, although the effects of kanamycin on threshold were bilateral, they were not necessarily uniformly so. However, there was no evidence of unilateral effects and the recorded differences between ears were probably not significant in view of the limitations to the accuracy of the method. Certainly, had the method been highly accurate, it is unlikely that the initial P.R.T. would have shown bilateral symmetry.

Summary of findings

The use of conditioned tone discrimination in kanamycin-injected rats permitted a quantitative study of the gradual development of the resulting hearing impairment. The results also provided information about the nature and properties of kanamycin induced deafness.

There was considerable variation in the extent to which kanamycin impaired the animals' hearing and also in how this affected their D.P. However, because each animal was assessed against its own steady baseline performance, advantage could be taken of such individuality to extract more information from the study. Thus the following observations may be made:—

(1) The onset of any hearing impairment was generally during the fourth week of kanamycin administration when the total dose was 3 to 4 g.

(2) The earliest onset occurred towards the end of the second week when the dose was less than 2 g.

(3) The onset of hearing impairment could be gradual or sudden.

(4) In most rats there was evidence of progression of the hearing impairment after kanamycin administration was stopped.

(5) A latency between the end of kanamycin administration and the onset of hearing impairment was observed in one rat.

(6) The hearing impairment was severe and irreversible in four rats out of 11 affected.

(7) Partial recovery of hearing occurred in five rats.

(8) Only in one rat, where the hearing impairment had been slight, was there complete recovery.

(9) Only one rat was unaffected by the four week period of kanamycin administration.

Throughout the preceding observations, the term "hearing impairment" has been substituted for "reduced discrimination performance". This assumed that any reduction in D.P. had resulted solely from a hearing decrement. Evidence that it was not caused by decreased hunger drive, nor by generalized debility of the animals, was seen in that although D.P. was reduced there was normally no fall, and often an increase, in the overall rate of lever pressing. Furthermore, there was no increase in the latency before the animals commenced eating their daily food ration, and no food remained uneaten. That the animals' inability to detect the signal was the only cause of reduced D.P. was confirmed when those animals, whose conditioned responding had not been extinguished, showed an improved D.P. to the signal of higher intensity.

The observation that some animals showed a reduced D.P. at a time when their P.R.T. showed no elevation is not without significance. Ernstson (1972) who observed a similar effect in a study with guinea-pigs, interpreted this finding as evidence of loudness recruitment. However, it might be less controversial to conclude that the effect of kanamycin on the hearing of rats, at least initially, is to cause a threshold elevation rather than to reduce sensitivity to intense sounds.

Acknowledgements

The authors acknowledge the technical assistance of Mr. R. Wilson and Miss R. Mitchell and the financial support of the Department of Health & Social Services (Northern Ireland) via a Research Fellowship (E.S.H.).

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