Auditory detection behavior in parathion treated squirrel monkeys

Peter Reischl
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Iowa State University, Ph.D., 1973
Health Sciences, pathology

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Auditory detection behavior in parathion treated squirrel monkeys

by

Peter Reischl

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of The Requirements for the Degree of DOCTOR OF PHILOSOPHY

Major: Biomedical Engineering

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Chairman, Program in Biomedical Engineering

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For the Graduate College

Iowa State University
Ames, Iowa

1973
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATION</td>
<td>iv</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Toxicology of Parathion</td>
<td>3</td>
</tr>
<tr>
<td>EEG changes</td>
<td>5</td>
</tr>
<tr>
<td>Subacute cholinesterase changes</td>
<td>7</td>
</tr>
<tr>
<td>Behavioral effects</td>
<td>9</td>
</tr>
<tr>
<td>Further Research Needed</td>
<td>11</td>
</tr>
<tr>
<td>Audiometric Measurements</td>
<td>14</td>
</tr>
<tr>
<td>Threshold testing</td>
<td>15</td>
</tr>
<tr>
<td>Instrumentation</td>
<td>17</td>
</tr>
<tr>
<td>METHODS</td>
<td>21</td>
</tr>
<tr>
<td>Subjects</td>
<td>21</td>
</tr>
<tr>
<td>Caging</td>
<td>22</td>
</tr>
<tr>
<td>Colony Room</td>
<td>23</td>
</tr>
<tr>
<td>Test Environment</td>
<td>23</td>
</tr>
<tr>
<td>Behavioral Task</td>
<td>29</td>
</tr>
<tr>
<td>Control and Data Acquisition</td>
<td>31</td>
</tr>
<tr>
<td>Response Measures</td>
<td>35</td>
</tr>
<tr>
<td>Habituation and Initial Training</td>
<td>36</td>
</tr>
<tr>
<td>Subject Attrition</td>
<td>37</td>
</tr>
<tr>
<td>Auditory Detection Training</td>
<td>37</td>
</tr>
<tr>
<td>Assignment of Treatment and Dosing Procedures</td>
<td>38</td>
</tr>
<tr>
<td>Analysis of Results</td>
<td>40</td>
</tr>
</tbody>
</table>
# RESULTS

- Baseline Hearing Thresholds: 43
- Post-exposure Hearing Thresholds: 46
- Correct Response Scores: 52
- Spontaneous Response Scores: 57
- Body Weight Data: 60

# DISCUSSION

# SUMMARY

# REFERENCES

# ACKNOWLEDGMENTS

# APPENDIX A: BASELINE HEARING THRESHOLD DATA, IN dB RE 0.0002 d/cm²

# APPENDIX B: TONE PARAMETERS
DEDICATION

To my parents with affection, admiration, and respect.
INTRODUCTION

Man's need for food and shelter has inextricably involved him with the use of pesticides. Agricultural pesticides have become a necessity to provide food and fiber for an expanding world population. The welfare of the United States and other economically advanced food-producing countries will depend largely upon their success in expanding food supplies to adequate levels in economically underdeveloped countries. If food supplies do not keep pace with population growth, the peace and security of the entire world may be threatened.

In addition, pesticides are important to our national economy. They are essential tools in agriculture, the nation's biggest industry. Often, agricultural pesticides represent the difference between profitable crop production and no crop production at all. About 70 percent of the agricultural crops produced in the United States cannot be successfully grown without the use of insect control measures (Walker, 1970).

Since some insecticides have become a part of the environment, increased attention has been focused upon possible biologic effects of chronic, low-level exposure to these agents. Many insecticides have a pronounced effect on the central nervous system when they are presented to animals in acutely toxic quantities (DeCandole et al., 1953; Sandler et al., 1969; Van Gelder et al., 1970). Chronic low-level exposure to dieldrin, one of the persistent insecticides, however, is sufficient to adversely affect learning by squirrel monkeys (Smith, 1972).

With the continuing decline in the use of persistent insecticides, especially DDT, the use of parathion has steadily increased. Parathion is
far less resistant to chemical breakdown than the persistent insecticides which results in a considerably smaller residue problem. In spite of this, parathion residues do appear in the American diet (Duggan and Weatherwax, 1967; Duggan and Lipscomb, 1969; Corneliussen, 1970). It is of interest, therefore, to explore the possibility that the central nervous system may be affected by prolonged low-level parathion exposure.

The Behavioral Toxicology Laboratory at Iowa State University has been developing methods for studying the effects of insecticides on brain function in the intact animal. By using behavioral tests, the reaction of the intact animal in a complex "real life" situation such as vigilance, learning, memory, or avoidance can be evaluated before and after exposure to an insecticide. Changes in these areas are of special interest to the behavioral toxicologist (Ruffin, 1963).

Behavioral toxicology is a relatively new area of research (Weiss and Laties, 1969). The study of interactions among neurochemical events, electrophysiologic potentials, and behavior is an outgrowth of several traditional disciplines (Russell, 1969). Success is contingent upon new ideas and solutions to technologic problems in instrumentation, in neurochemistry, neuropharmacology, neurophysiology, and in psychology. It is in keeping with this concept that the Behavioral Toxicology Laboratory not only draws heavily from the disciplines of psychology, neuropharmacology, and physiology but from the Biomedical Engineering Program at Iowa State University as well.
Toxicology of Parathion

Parathion, also known by several trade names,\(^1\) chemically is 0,0'diethyl 0-p-nitrophenyl thiophosphate. Its initial synthesis occurred shortly before and during World War II with the development of a comparatively new class of highly toxic chemicals, the organophosphates. Synthesized chiefly by Schrader, of I. G. Farbenindustrie, organophosphates were employed first as agricultural insecticides and later as potential chemical warfare agents.

Parathion is slightly soluble in water but highly soluble in many organic solvents including ethers, alcohols, and animal and vegetable oils. Its half-life is given as 203,000 hours at pH 8 and approximately 203 hours at pH 11, with faster hydrolysis in amino acid, chlorine, or copper rich environments (O'Brien, 1967). Parathion is easily absorbed from the gastro-intestinal tract, as well as through the skin and mucous membranes (Koelle, 1971).

The extreme toxicity of the new organophosphate chemicals was found to be due to their relatively irreversible inactivation of cholinesterase. However, the anti-cholinesterase activity of parathion is extremely low (Koelle, 1971). \textit{In vivo} parathion is metabolized via microsomally-induced oxidative desulfuration in the liver to the much more active anti-cholinesterase agent 0,0'diethyl 0-p-nitrophenyl phosphate, also known as paraoxon (O'Brien, 1960; O'Brien, 1967). Most of the anti-cholinesterase activity and consequent toxicosis is attributed to paraoxon.

\(^1\)AAT, Alkron, Aphamite, Compound 3422, Corothion, DNTP, DPP, E-604, Genithion, Mackothion, Niran, Penphos, Phos-Kil, Plantthion, Thiophos, Vapophos (Geeason \textit{et al.}, 1968).
The acute oral LD$_{50}$ of parathion for male white rats is reported as 13 mg/kg body weight while for female white rats it is the much lower value of 3.6 mg/kg (Hayes, 1963). In human exposure, as little as 1.2 mg parathion/kg body weight led to the rapid death of a man. Five-year-old children have been reported to be killed by eating 2 mg of parathion, a dosage of about 0.1 mg/kg. Dairy calves under two weeks of age were severely poisoned after several oral doses of 0.5 mg/kg parathion (Radeleff, 1964).

The signs and symptoms of severe poisoning are due to the inhibition of cholinesterase and have been divided into nicotinic and muscarinic effects. Nicotinic actions at the neuromuscular junctions of skeletal muscles usually consist of fatigability, involuntary twitching, and eventually severe weakness and paralysis. The muscarinic effects are salivation, lacrimation, urination, involuntary defecation, sweating, and hypotension.

Central nervous system cholinesterases are inhibited by parathion (Grob et al., 1949; Grob et al., 1950; Locker and Siedek, 1952; Reiff et al., 1971). Consequently, parathion poisoning has a distinct central nervous system component. The central nervous system manifestations of poisoning include tension, anxiety, nervousness, restlessness (Grob and Harvey, 1953), forgetfulness, personality changes (Holmes, 1956), dizziness, emotional lability, inability to perform familiar tasks (Dille and Smith, 1964), impairment of memory, difficulty in concentration (Gershon and Shaw, 1961; Metcalf and Holmes, 1969), disorientation in space and time, and depersonalization (Durham and Hayes, 1962; Durham et al., 1965; Brown, 1971). In more severe cases, these manifestations may be followed by coma, convulsions, and central respiratory paralysis (DeCandole et al., 1953).
The broad spectrum of effects on the central nervous system reflects a combination of excitatory, succeeded by inhibitory, actions at various cortical and deep subcortical levels. These actions are presumably based on the inhibition of acetylcholinesterase and subsequent accumulation of acetylcholine at the terminations of cholinergic tracts. From their electroencephalographic study, Metcalf and Holmes (1969) speculated that the central effects are related to the specific impact of organophosphate compounds on the midbrain's acetylcholinesterase-rich pontine centers. It has been found that the specific sites of action of parathion in the central nervous system are the cerebral cortex and reticular formation (Mitra, 1966).

EEG changes

The electroencephalograms (EEGs) of occupationally exposed subjects showing mild to severe toxicosis have been reported to show changes. Holmes (1956) mentions paroxysms of high voltage, slow waves (3-6 Hz) following acute organophosphate insecticide exposure. Grob and Harvey (1953) describe EEG symptomatology to nerve gas as well as parathion poisoning. They report an irregularity in EEG rhythm, variation, and increase in potential and intermittent bursts of abnormal waves similar to those seen in epilepsy. Agricultural workers who have been chronically exposed to organophosphates show less severe but related symptoms (Gershon and Shaw, 1961; Dille and Smith, 1964; Metcalf and Holmes, 1969). Their EEGs are characterized by medium-voltage, slow activity in the theta range (4-6 Hz) for periods of 2 to 4 seconds. Decreased vigilance performance in sleep-
deprived subjects on an auditory detection task has been correlated with similar medium to high voltage waves (Mirsky and Cardon, 1962).

Rhesus monkeys, following prolonged feeding of parathion (Santolucito and Morrison, 1971), showed reduction in the number of low amplitude fast waves and high amplitude slow waves. The monkeys were fed 0.1 or 0.3 mg/kg/day parathion for 18 months, 5 days a week. The EEGs were obtained from animals treated with phencyclidine hydrochloride and sodium thiamylal. The animals showed no clinical signs of poisoning nor changes in gross behavior during the 18-month study.

At least one investigator (Brown, 1971) speaks of a striking correlation between the electroencephalographic picture in persons mildly poisoned with parathion and the EEG in persons with temporal lobe seizure disorders. Since the temporal lobes are especially important in interpreting visual and auditory experiences, any dysfunction might disrupt hearing or vision. There are several such cases in the literature. Gershon and Shaw (1961) found auditory hallucinations in patients chronically exposed to organophosphate insecticides. Petty (1958) reported a subject with a 36 percent hearing loss following frequent parathion exposures. After several acute poisonings, both vestibular and cochlear components of the eighth cranial nerve were damaged. Metcalf and Holmes (1969) examined visual and auditory evoked responses using a summing computer on chronically organophosphate-exposed agricultural workers. In their subjects, the visual and auditory evoked responses showed trends toward lower amplitudes and longer peak latencies than those of a control group. The suppression of auditory nerve action potentials by excessive central nervous
system acetylcholine (Amaro et al., 1966; Daigneault and Brown, 1966) may account for decrements in the auditory evoked response.

**Subacute cholinesterase changes**

The neuronal interaction in behavior is not yet fully understood, but it is generally believed that biochemical mechanisms are involved, one of which involves acetylcholine and cholinesterase (Rosencrans et al., 1968).

Measurement of brain cholinesterase usually requires destruction of the subject. This destruction may be neither possible nor desirable. Consequently, cholinesterase activity of blood is often measured, although it is not an exact reflection of brain cholinesterase concentration.

Cholinesterase is found in the plasma as pseudocholinesterase which will hydrolyze not only acetylcholine but other related compounds as well. Cholinesterase is also found in the red blood cell as true cholinesterase which specifically hydrolyzes acetylcholine.

Several investigators speak of moderate cholinesterase changes following subacute, symptom-free (0.05-0.9 mg/kg) parathion exposure.

After a single 0.1 mg/kg feeding of parathion to guinea pigs, Locker and Siedek (1952) reported a 10 percent decrease in brain cholinesterase and a 50 percent decrease in liver cholinesterase within 2 days following exposure. Plasma cholinesterase increased within 6 days to 150 percent of the pre-exposed level. Other investigators (Villeneuve et al., 1972) found that a single intravenous dose of 0.1 mg parathion/kg to pregnant, conscious sheep at 90-100 days of gestation decreased plasma cholinesterase by 50 percent in the mother and 25 percent in the fetus. Likewise in primate studies, 0.3 mg parathion/kg administered intravenously to 8 male rhesus
monkeys caused an abrupt decrease in whole blood cholinesterase in the first 30 minutes with a maximum depression after 6 hours (Copeland et al., 1971). Single oral doses of 0.5 and 1.0 mg parathion/kg depressed true blood cholinesterase within 5 to 6 hours by 25 percent and 39 percent, respectively, in rhesus and bonnet monkeys (Reiter et al., 1973a).

In prolonged subacute feeding studies, Frawley et al. (1952) and Casterline and Williams (1971) reported that 5 and 4 ppm parathion (0.3-0.6 mg/kg) caused cumulative inhibition of brain and erythrocyte cholinesterase in the rat. Rat plasma cholinesterase became significantly inhibited in 10 days after daily injecting the animals intraperitoneally with as little as 0.25 mg parathion/kg (Williams, 1970). In a 24-week dog feeding study, 0.047 mg/kg resulted in a 60-70 percent inhibition of plasma cholinesterase (Frawley and Fuyat, 1957).

From a human study, Williams et al. (1958) concluded that ingestion of 0.050 mg parathion/kg for a period of three weeks produces no decrease in human plasma and red cell cholinesterase activity, while lower dosages induce some increase in the plasma cholinesterase level. A dose of 7.5 mg/day (about 0.1 mg/kg) produced a 20-25 percent depression of plasma cholinesterase in man when ingested over a period of several weeks (Rider et al., 1969).

No deleterious signs were exhibited by any of the experimental subjects having subacute cholinesterase changes. Similarly, Nakatsugawa et al. (1969) reported no symptoms after feeding rats a single 0.2 mg/kg or 1 mg/kg dose of parathion. Dairy cows fed 0.11 mg parathion/kg for 81 days showed no harmful effects (Dahm et al., 1950).
Behavioral effects

It is important to know whether mental effects may precede more disabling signs and symptoms of intoxication or whether such neurologic effects may take place in the complete absence of clinical toxicosis. Several investigations reviewed by Medved et al. (1964) have shown that changes in conditioned behavior become evident with a chronic 40-60 percent inhibition of serum and erythrocyte cholinesterases. The chronic lowering of brain cholinesterase concentration below a "critical level" of 60-65 percent of normal has been shown to markedly affect extinction of conditioned avoidance behavior in male white rats (Russell et al., 1961).

Karpov (1960) found that a relatively large oral threshold dose of 1 to 3 mg parathion/kg affected the conditioned secretory reflex of dogs. Earlier evidence indicated that as little as 0.1 mg paraoxon/kg accelerated avoidance conditioning (Lucomskaja, 1957), improved discrimination, and hastened extinction (Savateev, 1957) in mice.

In a recent primate study (Reiter et al., 1973a), a single dose of parathion ranging from 0.5-2.0 mg/kg was administered orally to rhesus and bonnet monkeys. A dose of 0.5 mg/kg had no effect on the performance of a visual discrimination task, but again the larger doses disrupted performance. Inhibition of true cholinesterase activity in the blood occurred 5-6 hours after parathion administration and was 25, 39, and 58 percent for doses of 0.5, 1.0, and 2.0 mg/kg, respectively.

Feeding a single 6 mg/kg dose of parathion to mice 1 hour before a one-trial passive-avoidance test was effective in blocking learning while giving 1-4 mg parathion/kg subcutaneously for 6 days prior to testing had no effect (Reiter et al., 1973b). After the sixth injection, a 2 mg/kg
subcutaneous dose produced the same degree of acetylcholinesterase inhibition in 18 hours which was present 0.5 hour after the 6 mg/kg acute dose.

Several investigators studied the acquisition of shuttle-box avoidance conditioning in parathion-exposed rats. Bignami and Gatti (1967) fed 0.05 and 0.25 mg parathion/kg to rats for 3 days prior to and throughout a 6-day experiment. Casterline and Brodie (1971) fed Osborne-Mendel rats 4 ppm (0.3-0.6 mg/kg body weight) parathion in a protein-deficient diet for 11 weeks. All dosed animals in both experiments acquired the avoidance response more slowly than the controls. A 5-second conditioned stimulus (light and sound) was terminated by an electric shock. Compared to the control group, the treated animals demonstrated a reduced ability to associate lever-pressing with avoidance or termination of the noxious shock stimulus.

The shuttle-box avoidance experiment with pre-trained rats which were later exposed to parathion (Bignami and Gatti, 1967) gave rather discouraging results. Daily doses of parathion were given over more than 16 days to study chronic cumulative effects. Doses were increased up to 5 mg/kg per day without obtaining any modification of conditioned behavior before death.

A mental alertness test conducted on a group of human subjects with varying degrees of chronic exposure to organic phosphorus insecticides, including parathion, showed deleterious effects of these chemicals (Durham et al., 1965). The test was a self-paced vigilance test (Gersoni U test) in which the subjects were instructed to draw a slanted or vertical line through each letter "R" encountered. The exposed group demonstrated a poorer score than the controls. Subjects involved in the test had con-
suited doctors previously with complaints of mild confusion and weakness. Tests revealed their blood cholinesterase activity was not depressed below normal.

Decrement in behavior were also observed in the chronic study of phosdrin, another organophosphate insecticide. These effects were initiated at doses lower than those which precipitated external symptoms of poisoning (Lewis et al., 1973). Squirrel monkeys (Saimiri sciurea) were tested in operant behavior chambers on variable-interval schedules of reinforcement. A LINC-8 computer controlled experimental testing, reinforcement contingency, and data recording. The monkeys' response rates decreased with increasing doses of the pesticide.

Further Research Needed

The presently demonstrated biochemical effects resulting from exposure to the organophosphates include changes in serum and liver β-glucuronidase activity (Williams, 1970), inhibition of liver carboxylesterase activity (Murphy and Cheever, 1972), increase in urine p-nitrophenol concentration (Roan et al., 1969), and the inhibition of cholinesterase enzymes of the blood and tissues. Any toxicosis or behavioral alterations produced, however, are usually explained solely by the reduction in cholinesterase with the concomitant increase in acetylcholine.

One reviewer (Clark, 1971) concluded that little evidence of behavioral deficits can be found with organophosphate exposure, apart from obvious somatic symptoms. The organophosphate compounds may affect mental alertness if absorbed in amounts large enough to produce clinical signs of systemic illness (Durham et al., 1965). Consequently, hazards of parathion
exposure to persons with environmental or incidental contact are generally believed to be negligible (Ganelin et al., 1964; Stoller et al., 1965).

Accumulation of the organophosphorus insecticides in the tissues has not been observed. It is conceivable that repeated inhibition of cholinesterase, however small, and perhaps the interaction of the pesticide with other cellular constituents over a long period of time might eventually lead to alteration in biochemical function and pathological change (Gershon and Shaw, 1961). Therefore, the chronic effects of organophosphorus insecticides depend upon accumulated physiologic effects of frequent exposure to non-acute doses (Tsumuki et al., 1970).

The possibility that behavioral changes free from somatic symptoms may occur after prolonged low-level organophosphate exposure is expressed by Bignami and Gatti (1967), Medved et al. (1964), and Lewis et al. (1973). The increasing use of parathion and other organophosphate insecticides makes further investigation of exact behavioral effects mandatory (Clark, 1971; Lewis et al., 1973). Metcalf and Holmes (1969) likewise called urgently for intensive research. The possibility exists that long-term exposure to organophosphate insecticides may induce irreversible or only slowly reversible brain dysfunction.

Because of the obscurity surrounding these questions, the Behavioral Toxicology Laboratory at Iowa State University has become involved in the study of chronic exposures of laboratory squirrel monkeys to parathion. The use of non-human primates in the research of neurotoxicants facilitates the extrapolation of data obtained to humans. The squirrel monkey is preferable over other primates because of its small size, availability, ease of maintenance, and relatively minor disease problems (Rosenblum and Cooper,
1968) and has been recommended for behavioral and pharmacological research (Kelleher et al., 1963). The squirrel monkey is in many respects similar to man when considered as a model for drug studies. Differences in the metabolism of particular drugs exist as they also exist for rhesus monkeys (Peters, 1971). Such differences are not expected to invalidate an extrapolation from squirrel monkeys to humans with respect to parathion exposure.

In earlier studies, the Behavioral Toxicology Laboratory has demonstrated that the organochlorine insecticide dieldrin and the organophosphate insecticide ruelene both disrupt behavior in an auditory detection task in sheep (Sandler et al., 1969; Van Gelder et al., 1970). Sheep were trained on an operant vigilance task. The sheep earned a reward when they responded to a randomly presented 0.1 second tone. Upon daily ingestion of an acute dose of the insecticide, the ability of the sheep to earn reinforcements was severely disrupted.

In light of the literature reviewed, it was decided to investigate auditory detection behavior in chronically parathion-exposed squirrel monkeys. Experience in this and other laboratories indicated that squirrel monkeys could be trained in an operant situation to respond only in the presence of a discriminative stimulus and withhold responses at other times. The animals were trained until they reliably responded within 2.5 seconds after the cessation of 0.1 second audible tones.

It is hypothesized that a chronic 0.1 mg/kg daily oral dose of parathion will produce a decrement in the squirrel monkey's tone reporting behavior and that upon pure tone threshold testing, this decrement will be reflected in an altered audiogram.
Audiometric Measurements

The audiogram is a record of an animal's hearing thresholds measured with a number of pure tones. Pure tones are relatively rare acoustic events in the normal environment. However, the French mathematician, Fourier, demonstrated that any arbitrarily complex periodic waveshape may be represented by sinusoids of different amplitudes and frequencies. The audiogram usually contains the tested pure tones of 500, 1000, 2000, 4000, and 8000 Hz.

Behavioral audiometric measurements require training of the subjects to report the pure tone stimuli, as well as the analysis of the subject's responses. The measurements should indicate some of the resolving power of the sensory system.

To establish a stable behavioral baseline, the subject is conditioned to respond in an orderly and predictable manner under a specific set of conditions. The subject is conditioned to emit a response only in the presence of the discriminative stimulus, hence the term stimulus control.

In an appetitively maintained procedure, deprivation of food may be necessary before conditioning. Punishment contingencies are generally introduced simultaneously with the schedule of reinforcement to maximize stimulus control. A punishing stimulus has the effect of decreasing the probability of false reports.

In the study of auditory acuity of monkeys, Wendt (1934) cautioned against long-term undesirable emotional and behavioral effects produced by the use of electric shock. A number of investigators (Fujita and Elliott, 1965; Stebbins et al., 1966; Beecher, 1972; Clopton, 1972; Green, 1972) have used a time-out period, an extension of the non-reinforcement inter-
val, for punishment, instead of shock. Fujita and Elliott (1965) and Green (1972) have concluded that the ability to detect a signal appears to be independent of the conditioning technique employed.

**Threshold testing**

There is some evidence that psychophysical measures such as thresholds are relatively invariant with different testing methods (Green, 1960; Stebbins, 1970a). However, additional laboratory work in this area is necessary (Stebbins, 1970b). The psychophysical method of constant stimuli has proven very reliable in monkeys because of strong stimulus control (Stebbins et al., 1966; Stebbins, 1970a; Green, 1972).

The method of constant stimuli as used by Stebbins (1970a) requires prior selection of a set of 4 to 7 stimuli. The stimuli are presented in a random order over a number of trials. The proportion of "yes" responses is plotted against the corresponding stimulus value. The threshold is interpolated from the resulting ogival function and is a statistic defined as that stimulus intensity to which a "yes" response was reported 50 percent of the time.

The principal objections against the method of constant stimuli are the sparseness of data points in the region of interest (Smith, 1961), inefficiency for animal research where satiation is a factor (Stebbins, 1970a), and lengthy sessions. However, the large proportion of suprathreshold stimuli prove beneficial in the maintenance of stimulus control.

In the psychophysical method of limits, stimulus intensity is varied over successive trials in discrete steps, first in a series of descending
order and then one in ascending order. Average thresholds are calculated from the point in each series where the animal's response changes.

Frequently, the ascending series of stimulus presentation will be omitted, since it necessitates sequentially presenting several stimuli below the animal's threshold making it difficult to maintain good stimulus control. Evidence from human subjects indicates that the use of the descending method alone leads to low estimates of the threshold (Stebbins, 1971).

Using the method of limits with an omitted ascending series, Fujita and Elliott (1965) employed a relatively simple technique to obtain auditory thresholds in three species of monkeys, *Saimiri sciureus*, *Macaca mulatta*, and *Macaca irus*. The basic apparatus included a loudspeaker, a cage, and a sound-deadened room. Trial onset was indicated by the occurrence of a tone. If a bar press occurred within 5 seconds, food reinforcement followed. A variable intertrial interval was used, and responses occurring in this period resulted in postponement of stimulus onset.

The hearing threshold audiogram obtained on a limited number of animals indicated that the audible frequency range of the squirrel monkey extended from about 62 to 33 kHz. Sensitivity was maximum at 16 kHz. The mid-frequency point of decreased sensitivity at 2-4 kHz often seen in other primate audiograms was present. Myers and Schuknecht (1965), Myers and Bernstein (1965), and Hunter-Duvar and Elliott (1972), using similar instrumentation and technique but using avoidance conditioning instead of appetitive reward, made analogous observations. On the other hand, squirrel monkeys tested with the psychophysical method of constant stimuli showed higher hearing thresholds (Green, 1972). The monkeys were main-
tained either on an appetitive or aversive reinforcement schedule. The monkeys' hearing sensitivity was maximum at 8 kHz. The mid-frequency point of decreased sensitivity at 2-4 kHz was absent.

**Instrumentation**

A common procedure used for delivery of sound to a test animal is to place the animal in a cage located in a sound-attenuating enclosure containing a speaker source driven by an oscillator (Harris, 1943; Fujita and Elliott, 1965; Myers and Bernstein, 1965; Hunter-Duvar and Elliott, 1972). Walls and any object inside the chamber reflect sound and consequently distort the sound field. A most desirable enclosure would have an anechoic interior. However, evaluating the characteristics of the sound field in relation to the physical position of the subject is difficult to do in any experimental arrangement.

For more accurate specification of the sound entering the external ear, earphones are sometimes preferred. Earphones are advantageous only if they can be maintained in proper position over the entrance of the ear canals for the course of the experiment. Consequently, immobilization of the animal is usually needed which requires vigorous restraint of the tested primate in a monkey chair. But chair restraint elevates already high plasma cortisol levels of the squirrel monkey (Brown et al., 1970) indicating extreme stress, undesirable in behavioral experiments. At the higher frequencies, standing waves are present in the ear canals, making precise calibration of the earphones problematical (Stebbins, 1970a).

Precise measurement of the stimuli in physical units appropriate to the source of energy is essential if any quantitative statements about the
sensory acuity of the animal are to be made. In quantifying a sound field, a preferred measure would be the sound pressure impinging on the eardrum of the subject. Such a measure is almost never achieved, especially in an intact animal. When animals are free to move in a sound field, pressure response measurements are usually made at various locations within the field, and an average is then calculated. These pressure response measurements are usually made via a calibrated microphone, which has both a free-field and a pressure response calibration curve (Peterson and Gross, 1967). The pressure response indicates the sound pressure that impinges on the microphone, while the free field response is a measure of the sound pressure if the microphone were not present.

The free sound field is usually produced by an electroacoustic transducer. There are two general types: the direct radiator and horn type loudspeaker. The diaphragm of the direct radiator is coupled directly to the air while the horn loudspeaker is coupled to the air by means of a horn.

The direct radiator loudspeaker is most commonly used because of its simplicity in construction, small space requirements, and relatively uniform frequency response characteristics. However, reproduction over a wide frequency range is restricted. The two extreme ends of the frequency spectrum from 100 to 15,000 Hz are the most difficult to reproduce with efficiency comparable to that of the mid-frequency range. Inefficiency at the lower frequencies is primarily due to a small radiation mechanical resistance while efficiency at the higher frequencies is limited by the mechanical mass of the vibrating system. For low frequency tones, a large radiation mechanical resistance may be obtained by using a large cone. Higher
tones are best produced by a light voice coil and a small cone or by speakers such as the electrostatic type, especially designed for very high frequency reproduction.

The horn loudspeaker incorporates the ability of the horn to present almost any value of acoustical resistance to the generator. This feature is valuable for obtaining maximum overall efficiency in the design of an acoustical system. By employing a suitable combination of horns, directional characteristics which are independent of the frequency can be achieved (Olson, 1957).

Evaluation of harmonic distortion of the sound system via a harmonic analyzer is important. A tone that is not audible to an animal at its fundamental frequency can have harmonics which can be audible, especially if the harmonics fall in the sensitive portion of the animal's hearing threshold. The problem is discussed by Dalland (1965), who encountered it in his work with the bat.

Harmonics may find their origin either in the speaker system or in any one of the electronic components such as the oscillator or amplifier. Harmonics are manifested by the loudspeaker whenever the amplitude or excursion of the cone is large. Under these conditions, the excursions of the speaker exceed the linear portion of the stress-strain characteristics of the cone material. Further harmonic distortion is added by a non-linear cone suspension system. To minimize harmonic distortion produced within the electrical portion of the sound system, Gourevitch (1970) advised the incorporation of a band-pass filter in the system.

A further effect of non-linearities in a cone suspension system is a jump phenomenon in the output of the speaker whenever it is energized to
produce a tone (Olson, 1957). Consequently, for hearing threshold measurements, it is important to have gradual onset of the tone to prevent transients which could be audible.

Collection of threshold data may be manually executed by the investigator (Harris, 1943). However, automated programming and recording equipment has come into common use (Moody, 1970; Sidowski, 1973). Solid state control is by far the most reliable method of implementing experimental procedures and data collection, especially with the use of an on-line computer. Because of the versatility of an on-line computer and also because of recent advances in computer technology, this approach is rapidly becoming the most economical (Moody, 1970).
METHODS

Subjects

Eight selected male squirrel monkeys (Saimiri sciureus), ranging in weight from 700 to 1000 g, were used in this study. The animals were part of a larger shipment of 28 similar squirrel monkeys obtained from Tarpon Zoo of Tarpon Springs, Florida. Tarpon Zoo had received these animals from South America and kept them for a minimum of 90 days. During this period, they were conditioned to high-protein monkey chow and wormed. The monkeys were shipped 6 to 8 animals per cage to the Behavioral Toxicology Laboratory via air freight in two shipments, 5 weeks apart.

On arrival of the first shipment of 12 monkeys, the animals were anesthetized with carbon dioxide (CO₂) before transfer into laboratory housing. Each cage of 6 animals was submerged for about 30 seconds into a large plastic container filled with CO₂ gas emitted from dry ice.

The animals rapidly became anesthetized, and the cage was withdrawn from the container, quickly opened, and the monkeys transferred into stainless steel primate cages measuring 46 cm wide, 61 cm deep, and 81 cm high. Three to 4 animals were placed in each cage. Recovery of the animals took less than a minute.

With the arrival of the second shipment of animals, no CO₂ was used. The monkeys were transferred into the stainless steel cages by placing a partially opened shipping cage adjacent to the entrance of a stainless steel cage. Three to 4 animals were permitted to enter each cage.

One animal from the second shipment was assigned at random for initial participation in this study, while all 12 CO₂ anesthetized animals were
potential participants. Later, after behavioral training, 8 of the 13 mon­
keys were selected for the final phase of the study. Of this final group, 
Monkey #113 came from the non-CO₂-treated shipment.

Caging

Animals were kept in their community cages for about 3 weeks. Monkey 
chow² was fed ad lib during this period with occasional roasted unsalted 
peanuts,³ orange, and miniature marshmallow⁴ supplement.

Later each monkey was moved to an adjacent colony room. Each animal 
was housed in an individual cage, with side panels constructed of 0.635 cm 
plexiglass, 36 cm deep and 81 cm high. The lower rear of the cage was a 
33 cm wide by 41 cm high plexiglass panel. The lower portion of the cage 
front was a plexiglass guillotine action door, compatible with a commercial 
monkey transfer cage.

The ceiling, upper halves of the front and rear, middle perch, and 
floor were made of 0.476 cm diameter stainless steel rods, spaced in paralle-
lel 3.8 cm center to center. A galvanized metal drop pan, 5 cm high, 32 cm 
wide, and 36 cm long, was sandwiched between the plexiglass side panels. 
The floor of stainless steel bars was 15 cm above the bottom of the drop 
pans containing pine woodchips. An automatic watering fount⁵ was in the

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²Wayne Monkey Diet, by Allied Mills, Inc., Chicago, Ill.
³Roasted Unsalted Peanuts, by Planters, Suffolk, Va.
⁴Miniature Marshmallows, by Kraft Foods, Chicago, Ill.
⁵Water fount type LV-100, by Valentine Equipment Company, 2630 W. 
Arthington St., Chicago, Ill. 60612.
ceiling of the cage. A stainless steel\textsuperscript{6} feeder was hung with aluminum strap hooks onto the front of the cage.

Colony Room

The individual cages were housed in 2 levels within the colony room. The lower cages were 40 cm from the floor while the upper cages were approximately 135 cm from the floor. The colony room was windowless, and ventilation was provided via an exhaust fan to the outside. A timed relay switch was used to control a 12 hour on and 12 hour off incandescent light day-night cycle. Central air conditioning during the summer months and propane gas heat during the winter maintained a temperature near 27\degree C during the day and 24\degree C during the night. During the winter months, the room air was humidified and maintained near 40 percent humidity. During the summer months, no humidity was added to the air which had a usual humidity reading near 60 percent. Laboratory personnel always wore a dust mask when in the colony room or near a monkey.\textsuperscript{7} Cages were cleaned regularly 2 to 3 times a week. An ambient noise level of 65 db (A), RE 0.0002 d/cm\textsuperscript{2} existed within the room, produced mainly by the exhaust fan and the central furnace-air-conditioning blower system located outside the colony room.

Test Environment

Figures 1 and 2 show the test environment located within a sound-deadened chamber constructed for this study. The interior of the chamber

\textsuperscript{6}Feeder Type LC-249, by Wahmann Manufacturing Company, PO Box 6883, Baltimore, Md.

\textsuperscript{7}Non-Toxic particle mask no. 8500, by Minnesota Mining and Manufacturing Co., 3M Center, St. Paul, Minn. 55101.
Figure 1. Test environment within sound deadened chamber. A, test cage; B, side view of intelligence panel; C, speaker; D, TV camera; E, carrying case; F, water supply for water fount; G, woolen blankets

Figure 2. Front view of intelligence panel. H, presentation cup; I, retractable lever; J, two-chambered fluid reservoir
had a volume of $2.10 \times 1.50 \times 1.98 \text{ m}^3$. The chamber consisted of celotex, gypsum board, and acoustical tile double-wall, two-door construction. The doors, one for each of the inner and outer walls, were 7.6 cm thick, soundproofed with celotex, gypsum board, and acoustical tile, then carefully weather-stripped. The floor was carpeted. Ventilation into the chamber was provided through a baffled air intake and a fan-driven, baffled air outlet. A 100-watt ceiling houselight furnished illumination. The ambient sound level within the chamber was between 30 and 32 db (A) RE $0.0002 \text{ d/cm}^2$. A few low-frequency (1-40 Hz) noise components were present due to structure-borne noise. During winter, relative humidity inside the chamber ranged between 42 and 38 percent. The temperature varied between $24^\circ$ and $27^\circ$C. During the summer months, the relative humidity increased to 60 percent; the temperature was maintained between $24^\circ$ and $27^\circ$C.

The test environment inside the chamber consisted of a test cage that could be exposed to a relatively uniform sound field. A closed circuit television system made constant observation of the test animal possible. The test area was completely surrounded by heavy woolen blankets during test sessions.

A transfer cage is shown temporarily attached to the test cage for animal transport. The test cage was constructed similar to the individual home cage of the monkeys, but larger, and made of aluminum and stainless steel 0.476 cm diameter rods to minimize acoustical interference with the sound field. Two main side frames of the cage were constructed of 1.9 cm angular aluminum stock. The cage width was 43 cm, 10 cm wider than the monkey's individual cage. A removable floor was attached at perch level to
confine the monkey to the upper volume of the cage, 43 x 35 x 23 cm\(^3\) during the test session. Stainless steel rods, 0.476 cm in diameter, were used for the ceiling, perch, and removable floor, while all other sections were of 0.476 cm diameter aluminum rod.

The test cage was supported on a chair frame\(^8\) wrapped with tissue paper to minimize sound reflections and standing waves. An automatic water fount was situated on the right side of the test cage.

An aluminum intelligence panel, 15 cm high, 23 cm wide, and 13 cm deep, was attached to the upper left side of the cage by means of 2 strap hooks. It could be easily removed for cleaning and maintenance. The intelligence panel contained an AC motor-operated retractable lever.\(^9\) A stainless steel liquid presentation cup\(^10\) protruded approximately 2.5 cm beyond the panel. A 2-chambered fluid reservoir\(^11\) was attached to the top of the intelligence panel. The liquid flow to the presentation cup was controlled by a solenoid valve.\(^12\) The presentation cup was situated to the left of the lever. The distance between the presentation cup and the

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\(^8\)Frame model number 194-01, by Lehigh Valley Electronics, Inc., Box 125, Fogelsville, Pa. 18051.

\(^9\)Lever model number 123-05, by Lehigh Valley Electronics, Inc., Box 125, Fogelsville, Pa. 18051.

\(^10\)Presentation cup identical to that supplied with model 114-06 Liquid solenoid valve, by Lehigh Valley Electronics, Inc., Box 125, Fogelsville, Pa. 18051.

\(^11\)Reservoir identical to that supplied with model 114-06 liquid solenoid valve, by Lehigh Valley Electronics, Inc., Box 125, Fogelsville, Pa. 18051.

\(^12\)Valve Model 8262, stainless steel, normally closed solenoid valve, by Asco Valves, Automatic Switch Company, Florham Park, N. J. 07932.
lever measured 7.6 cm center to center. Both were elevated 9.5 cm from the removable floor.

Tone stimuli were presented from a speaker located 58 cm above the test volume. Two speakers were available. A 30.5 cm diameter woofer\textsuperscript{13} with a cone angle of about 150° was used for the frequencies 500, 1000, 2000, and 4000 Hz. A 7.6 cm diameter tweeter\textsuperscript{14} having an approximate cone angle of 160° was used for the frequencies 8000 and 16000 Hz, giving directional characteristics similar to those observed for the lower frequencies.

The range and standard deviation for sound-pressure levels within the test volume measured at 13 positions for the 6 frequencies are shown in Table 1.

Table 1. Mean, standard deviation, and range for sound-pressure levels at 6 frequencies, relative to 50 db at center of test volume

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 Hz</td>
<td>50.0</td>
<td>2.01 db</td>
<td>± 3.5 db</td>
</tr>
<tr>
<td>1000 Hz</td>
<td>50.0</td>
<td>1.21 db</td>
<td>± 2.0 db</td>
</tr>
<tr>
<td>2000 Hz</td>
<td>46.0</td>
<td>2.19 db</td>
<td>± 3.5 db</td>
</tr>
<tr>
<td>4000 Hz</td>
<td>47.0</td>
<td>1.69 db</td>
<td>± 3.0 db</td>
</tr>
<tr>
<td>8000 Hz</td>
<td>48.5</td>
<td>1.71 db</td>
<td>± 2.5 db</td>
</tr>
<tr>
<td>16000 Hz</td>
<td>45.6</td>
<td>3.18 db</td>
<td>± 5.5 db</td>
</tr>
</tbody>
</table>

\textsuperscript{13} Speaker model C12R8, by Jensen, 5655 West 73rd St., Chicago, Ill. 60638.

\textsuperscript{14} Speaker model 3C8T, by Oaktron, 1000 30th St., Monroe, Wis. 53566.
The 2 speakers were mounted on separate sides of a back-enclosed cabinet 40 cm wide, 40 cm high, and 61 cm long. Within this cabinet the tweeter had a separate enclosed compartment 13 cm x 13 cm x 13 cm. All construction was of 1.9 cm thick plywood. The cabinet was rotated to bring the required speaker into position.

The interior of the enclosure was filled with 15 cm$^3$ foam rubber cubes. Such a loudspeaker system with the back of the cone completely enclosed is a simple source. The output is independent of frequency above the resonant frequency of the system (Olson, 1957). The mounting of the loudspeaker in the front wall of the cabinet influences the response due to resonance of the cavity in front. In addition, reflections and diffractions are produced from the front circular cavity. For this reason, the speakers were mounted completely flush with the cabinet walls, to minimize any such cavity.

Behavioral Task

The psychophysical method of constant stimuli was used to measure the monkey's response to a tone stimulus. The start of a test session was signaled by the extension of the response lever. A tone was of 0.1 second duration with every tone indicating a trial. A correct response was for the monkey to report a perceived trial by pressing the extended lever. A correct response was rewarded by opening a solenoid valve to the presentation cup for 0.25 second. This filled the presentation cup with approxi-
mately 0.1 cc of sweetened condensed milk\textsuperscript{15} which was diluted 50 percent with tap water.

If the monkey did not respond within 2.5 seconds after the presence of the stimulus, this was designated as a default, and no reward was earned. A new tone was presented 5.5 seconds after defaulted trials.

Any lever press that was not a correct response was called a spontaneous response and was not rewarded but punished with a subsequent time-out session. Every time-out session was determined randomly and ranged from 30-60 seconds. No further tone was presented to the monkey until the time-out period was successfully served.

Each animal received 70 trials per day, 7 days a week. One frequency was tested each day, selected in sequence from the following table of frequencies: 500, 8000, 2000, 4000, 1000, 16000 Hz. The first 10 trials of each test session were of maximum intensity within an attenuated progression. These trials, called pre-trials, were presented at random time intervals ranging from 8 to 29 seconds. They were scored separately to enable comparison of initial early daily performance with later performance.

The next 60 trials consisted of 10 presentations each of 6 different auditory intensities, presented in pseudorandom order and at random time intervals ranging from 8 to 20 seconds. The auditory intensities differed from each other by 6 db RE 0.0002 d/cm\textsuperscript{2} and were near the monkey's hearing

\textsuperscript{15}Borden Eagle Brand, sweetened condensed milk, by Borden, Inc., New York, N.Y. 10017.
threshold. The condition imposed on the tone intensity order was that no more than 2 like tone amplitudes could appear in succession.

After 70 tone trials, the response lever was retracted into the intelligence panel under computer control, signaling the end of the test session.

Control and Data Acquisition

Figure 3 is a block diagram of the equipment used for stimulus presentation and data acquisition. A sine wave oscillator\(^{16}\) having a total harmonic distortion less than 0.5 percent was used to generate the test tone. Frequency selection was within 1 percent. The electronic audio switch\(^{17}\) gated the sine wave with a rise and decay time of 10 milliseconds. The duration of each tone was 0.1 second. The tone on-off amplitude ratio was specified by the manufacturer to be at least 50 db.

An attenuation network followed the electronic switch. This network had an input impedance of 600 ohms, compatible with the audio switch. Its output impedance was 50 ohms. Six different tone intensities could be selected from this network via computer-controlled relay closures. The attenuation varied in 6 db steps.

An electronic audio switch may have residual switching transients. For this reason, a band pass filter\(^{18}\) was added to the circuit.

\(^{16}\) Oscillator Model III, by Wavetek, 8159 Engineer Rd., San Diego, Calif.

\(^{17}\) Switch model 829E, by Grason-Stadler Co., Inc., 56 Winthrop St., Concord, Mass. 01742.

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After 70 tone trials, the response lever was retracted into the intelligence panel under computer control, signaling the end of the test session.

Control and Data Acquisition

Figure 3 is a block diagram of the equipment used for stimulus presentation and data acquisition. A sine wave oscillator having a total harmonic distortion less than 0.5 percent was used to generate the test tone. Frequency selection was within 1 hertz. An electronic audio switch gated the sine wave with a rise time of 1 microsecond. The duration of each tone was specified by the manufacturer. The amplitude ratio was specified by the manufacturer.

An attenuation network was used. This network had an input impedance of 60 ohms and an output impedance of 50 ohms. Several intensities could be selected from this network via computer-controlled relay closures. The attenuation varied in 6 db steps.

An electronic audio switch may have residual switching transients. For this reason, a band pass filter was added to the circuit.

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16 Oscillator Model III, by Wavetek, 8159 Engineer Rd., San Diego, Calif.

17 Switch model 829E, by Grason-Stadler Co., Inc., 56 Winthrop St., Concord, Mass. 01742.

Figure 3. Block diagram of the stimulus presentation and data acquisition instrumentation
Audio Oscillator → Electronic Switch → Attenuator 0-30 db → Band-Pass Filter

Digital Computer LINC-8 → Power Amplifier

→ Attenuator 10 db → Speaker

→ Intelligence Panel

TEST CHAMBER
The filtered signal was fed through a power amplifier.\textsuperscript{19} Total harmonic distortion of this amplifier was specified by the manufacturer to be no more than 0.6 percent. Because of a minute but constant white noise component in the output of this amplifier, the output was attenuated by approximately 10 dB, utilizing a T network matched for 8 ohms on both its input and output. The signal to noise ratio was consequently improved by about 10 dB. No perceivable white noise remained.

Second harmonic content of the final signal available to energize the speaker was down by at least 65 dB from the fundamental. A harmonic wave analyzer\textsuperscript{20} was utilized to make this measurement at the frequencies of 500, 1000, 2000, 4000, 8000, and 16000 Hz. At these frequencies, second harmonic content within the sound field itself was down by at least 55 dB from the fundamental tone with a sound pressure level of 55 dB RE 0.0002 d/cm\textsuperscript{2}. Sound pressure levels were measured using a sound-level meter\textsuperscript{21} with its microphone attached to a cable. Measurements were made at 13 positions in the cage.

Before each daily session, all electronic equipment was allowed to warm up for at least 1 hour. The tone output of the speaker was calibrated before each session by monitoring the sound field from the center of


\textsuperscript{21} Sound-level meter type 1551-C, by General Radio, West Concord, Mass.
the cage. The sound level meter was calibrated via a sound-level calibrator.

A LINC-8\textsuperscript{23} laboratory computer was interfaced with the intelligence panel, electronic audio switch, and attenuator network for on-line control and data acquisition. Demographic data on each animal were recorded via the computer's teletype before each test session. The data collected in real time were printed out via teletype after the completion of each animal's session.

Response Measures

Two response measures were recorded, namely, the correct responses and the spontaneous responses per 70 trial session. In addition, the total time required to complete a session was logged.

The number of tones reported for each of 6 tone intensities was recorded. The interpolated tone intensity for which 50 percent of the trials were reported was designated as the hearing threshold. Should 2 such points exist in the response data, the louder of the 2 tones was defined as the hearing threshold. Should no such point exist, then the loudest tone of that session was called the hearing threshold.

The loudest tone of an attenuated tone sequence was assigned such that the hearing threshold of an animal fell within the lowest 2 or 3 tone intensities. If this criterion was not met for 2 consecutive testing ses-

\textsuperscript{22} Sound-level calibrator type 1562-A, by General Radio, West Concord, Mass.

\textsuperscript{23} LINC-8 computer, by Digital Equipment Corporation, Maynard, Mass. 01754.
sions, the loudest tone and, consequently, the tone sequence was shifted by 6 db.

Habituation and Initial Training

After the monkeys had become accustomed to their individual cages, their body weight was gradually reduced to approximately 90 percent of their free feeding weight. Sweetened condensed milk diluted with an equal part of tap water was offered to the monkeys during this period. The monkeys learned to eagerly accept this fluid from a syringe.

An appropriate amount of monkey chow, supplemented with 2 peanuts was fed daily to each monkey. This diet was further supplemented every second day with an unpeeled slice of orange. Feeding followed at the end of each day. Each monkey received 2 miniature marshmallows in the morning. All transportation of monkeys between their individual cages and the test chamber was via a small transfer cage. Monkeys were given a miniature marshmallow for leaving the home cage and entering the transfer cage. The monkeys were then taken to a scale where they were weighed.

Following habituation to this procedure, the monkeys were taken to the test chamber. They were offered several marshmallows while in the test cage and then transported back to their home cages, receiving another marshmallow.

Upon entering the test cage on subsequent days, the monkeys were rewarded with sweetened condensed milk within the presentation cup. A view

24 Aluminum transfer cage 31.8 cm long by 23.5 cm wide and 30.5 cm high, compatible with cage type AC-3889-MK(A), both by Acme Research Products, 5500 Muddy Creek Rd., Cincinnati, Ohio 45238.
of the monkey's behavior was available over closed circuit television. The method of successive approximations was used to train the monkey to press the extended lever to earn more milk. Initial training sessions lasted from 15 to 40 minutes, depending on the animal's progress. In some later sessions, a peanut was attached to the response lever to draw the attention of some monkeys.

Animals were trained until they would reliably bar-press. This was indicated by the animals' ability to respond 60 or more times in a 5-minute session.

Subject Attrition

The 13 monkeys initially assigned to this study were unselected from the supplier. Monkeys #101 and #109 had eye injuries, would not work inside the test cage, and were, therefore, dropped from the experiment. Monkeys #111 and #114 could not master the initial phase of training after 12 hours of practice and were also dropped from the experiment. The remaining monkeys mastered the task in less time. From these remaining monkeys, #107 was randomly eliminated to leave 8 animals to be continued on auditory training.

Auditory Detection Training

After the bar pressing response had been acquired, tone training of 8 monkeys was begun. A 500 Hz, 80 db RE 0.0002 d/cm² tone was turned on and remained on until the subject responded by bar-pressing. The bar press resulted in the presentation of the milk reward and the termination of the tone. After the termination of the tone, a 2.5 second time-out period during which no bar presses were made had to occur before the next tone pre-
sentation. The duration of the tone was gradually reduced and the time out period increased to 20 seconds until the monkeys were reliably responding within 2.5 seconds to a 0.1 second tone of either 500, 1000, 2000, 4000, 8000, or 16000 Hz. This phase of the training took sixty 30-minute daily sessions for each animal. Control of the training sessions up to this point was via Massey-Dickinson behavioral programming equipment. Subsequent control was by the LINC-8 laboratory computer.

LINC-8 control introduced random intertrial intervals between 8 and 29 seconds and random time-out punishment sessions between 30 and 60 seconds for false reports.

Later, attenuated but still audible tone sequences were introduced. Thereafter, the final behavioral task was implemented, and baseline hearing thresholds were collected until consistent audiograms, ± 3 db at each frequency, were regularly collected for each monkey. Baseline hearing thresholds were collected for 36 days.

The daily behavioral sessions were begun at 7:00 to 7:30 PM and were continued until around midnight. This schedule was followed on a 7 day/week basis until the study was terminated. The monkeys were run in the same order each day. The light-dark cycle in the colony room was adjusted so the monkeys were working during their normal day period.

Assignment of Treatment and Dosing Procedures

The 8 monkeys were divided into two, 4-animal statistically optimally matched groups based on stable baseline hearing threshold data. Care was

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taken that animals from each group were distributed throughout the caging area. Two animals from each group occupied the top 4 cages. Two animals from each group occupied the bottom 4 cages. The groups were unsymmetrically spaced.

Baseline hearing threshold data revealed that the hearing of Monkey #110, when compared to the hearing of other animals, was impaired by about 10 db at frequencies below 4000 Hz. Because it was felt that hearing of the control animals would not change over the course of the experiment, the group containing Monkey #110 was chosen as control. The other 4 animals constituted the parathion exposed group which was subsequently fed 0.1 mg parathion/kg daily.

Technical parathion was dissolved in 95 percent ethanol in a concentration of 0.1 mg/10 μl. The ethanol only was used for the 0 dose control. Using a microliter syringe\(^\text{26}\), the appropriate volume was injected into a miniature marshmallow. Marshmallows were injected on a weekly basis. They were then stored in small glass vials and refrigerated.

Subsequent to the collection of stable baseline data, injected marshmallows were fed daily to the monkeys. Two monkey dosing schedules were used. For the first 57 exposure days, the monkeys were dosed 3-5 hours before their daily test sessions. During the next 37 days, dosing was 1-1.5 hours before testing. This was again followed by the 3-5 hours schedule for 36 days. For the last 18 days, the 1-1.5 hour schedule was used.

\(^{26}\) Microliter syringe, by Hamilton Company, PO Box 307, Whittier, Calif.
After a monkey had eaten his injected marshmallow, he was rewarded with a plain marshmallow.

The monkeys were tested in the same order each day. In terms of exposed and control animals, the order had the following form where C and D represent a control and exposed animal, respectively: CDCDCDDC.

Analysis of Results

Four corrections of the raw hearing threshold data were performed, as suggested by Peterson and Gross (1967). The corrections are summarized in Table 2. The data were corrected for a 2 db loss in the microphone cable. Since all sound pressure measurements were made relative to db (A) RE 0.0002 d/cm^2, scale A readings were converted to absolute db RE 0.0002 d/cm^2 by utilizing frequency response curves of the sound level meter. A third correction converted pressure response of the calibration microphone to its free field response. Microphone response curves were used. The final correction adjusted the sound pressure measured at the center of the test volume to the average sound pressure within the test volume.

Once parathion exposure was initiated, 3 consecutive hearing thresholds at the same frequency were averaged and their standard deviations calculated. This procedure was carried out over all 6 frequencies for both the control and exposed groups.

A block of 18 days was required to determine 3 consecutive hearing thresholds for each of 6 auditory frequencies tested. Table 3 summarizes the blocked days over the course of the experiment with their dosing schedule. Several blocks contain more than 18 days. The extra days were required to have the end of these blocks coincide with a change in dosing.
Table 2. Corrections for raw data hearing thresholds

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Cable</th>
<th>Absolute db</th>
<th>Free field</th>
<th>Test volume</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 Hz</td>
<td>+2 db</td>
<td>+ 3.0 db</td>
<td>+0.0 db</td>
<td>+0.0 db</td>
<td>+5.0 db</td>
</tr>
<tr>
<td>1000 Hz</td>
<td>+2 db</td>
<td>+ 0.0 db</td>
<td>+0.0 db</td>
<td>+0.0 db</td>
<td>+2.0 db</td>
</tr>
<tr>
<td>2000 Hz</td>
<td>+2 db</td>
<td>- 1.5 db</td>
<td>-1.0 db</td>
<td>-4.0 db</td>
<td>-4.5 db</td>
</tr>
<tr>
<td>4000 Hz</td>
<td>+2 db</td>
<td>- 1.5 db</td>
<td>-3.0 db</td>
<td>-3.0 db</td>
<td>-5.5 db</td>
</tr>
<tr>
<td>8000 Hz</td>
<td>+2 db</td>
<td>+ 1.0 db</td>
<td>-5.0 db</td>
<td>-1.5 db</td>
<td>-3.5 db</td>
</tr>
<tr>
<td>16000 Hz</td>
<td>+2 db</td>
<td>+ 4.0 db</td>
<td>+0.0 db</td>
<td>-4.4 db</td>
<td>+1.6 db</td>
</tr>
</tbody>
</table>

Table 3. Blocks of data and their dosing procedures

<table>
<thead>
<tr>
<th>Block</th>
<th>Dosing procedure</th>
<th>Time interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None (Baseline)</td>
<td>36 days</td>
</tr>
<tr>
<td>1</td>
<td>3-5 hours before testing</td>
<td>18 days</td>
</tr>
<tr>
<td>2</td>
<td>3-5 hours before testing</td>
<td>18 days</td>
</tr>
<tr>
<td>3</td>
<td>3-5 hours before testing</td>
<td>21 days</td>
</tr>
<tr>
<td>4</td>
<td>1-1.5 hours before testing</td>
<td>18 days</td>
</tr>
<tr>
<td>5</td>
<td>1-1.5 hours before testing</td>
<td>19 days</td>
</tr>
<tr>
<td>6</td>
<td>3-5 hours before testing</td>
<td>18 days</td>
</tr>
<tr>
<td>7</td>
<td>3-5 hours before testing</td>
<td>18 days</td>
</tr>
<tr>
<td>8</td>
<td>1-1.5 hours before testing</td>
<td>18 days</td>
</tr>
</tbody>
</table>

Schedule. In these cases, 4 hearing thresholds were averaged instead of the 3. The data of the entire experiment was organized into 8 blocks with an additional block of data for baseline scores.

A response of the animal to no tone was called a spontaneous response. The number of spontaneous responses per session time were averaged in simi-
lar fashion as the hearing thresholds. The standard deviations of each averaged group was calculated.

A transformation was used on the spontaneous response data as recommended by Winer (1971). Count data, such as responses per unit time, were converted by the formula $X' = \sqrt{X^2} + \sqrt{X + 1}$. No advantage was found in using this transformation, and it was consequently dropped from the analysis.

An analysis of variance was performed on hearing threshold, correct pre-trial response, and spontaneous response data, with the blocked days being the repeated measure.
RESULTS

Baseline Hearing Thresholds

Baseline hearing thresholds for the 2 groups of squirrel monkeys are shown in Table 4.

Table 4. Baseline hearing thresholds for the 2 groups of squirrel monkeys in db RE 0.0002 d/cm², 50 percent tone perception

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Mean Group A</th>
<th>Standard deviation Group A</th>
<th>Mean Group B</th>
<th>Standard deviation Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 Hz</td>
<td>27.90 db</td>
<td>5.72 db</td>
<td>26.44 db</td>
<td>2.58 db</td>
</tr>
<tr>
<td>1000 Hz</td>
<td>22.29 db</td>
<td>6.47 db</td>
<td>20.30 db</td>
<td>3.62 db</td>
</tr>
<tr>
<td>2000 Hz</td>
<td>19.42 db</td>
<td>6.14 db</td>
<td>17.40 db</td>
<td>0.82 db</td>
</tr>
<tr>
<td>4000 Hz</td>
<td>21.67 db</td>
<td>3.70 db</td>
<td>21.21 db</td>
<td>3.71 db</td>
</tr>
<tr>
<td>8000 Hz</td>
<td>17.97 db</td>
<td>1.70 db</td>
<td>14.54 db</td>
<td>1.64 db</td>
</tr>
<tr>
<td>16000 Hz</td>
<td>8.29 db</td>
<td>0.86 db</td>
<td>8.71 db</td>
<td>1.25 db</td>
</tr>
</tbody>
</table>

Group A displayed larger standard deviations in hearing thresholds at the lower frequencies than Group B. Group A included Monkey #110 which showed a slight hearing impairment at frequencies below 4000 Hz. Figure 4 is a graphical representation of the hearing thresholds of the 2 groups. An increase in hearing threshold is apparent at 4000 Hz. With the exception of Monkey #110, all monkeys demonstrated this increased hearing threshold. The increase is statistically significant (t = 3.80, 6 d.f., p<0.02) when compared to the hearing threshold at 2000 Hz and excluding
Figure 4. Baseline hearing thresholds for 2 groups of squirrel monkeys. Threshold was defined to be the sound pressure where the monkeys reported 50 percent of the presented tones correctly. Group B was later exposed to 0.1 mg parathion/kg.
Group A
Group B

dB re 0.0002 d/cm²

FREQUENCY (Hz)

500 1000 2000 4000 8000 16000
Monkey #110 from the comparison. Individual animal hearing threshold data is shown in Appendix A.

Post-exposure Hearing Thresholds

Following the collection of baseline hearing threshold data, Group B was exposed for 148 days to 0.1 mg parathion/kg. Collection of hearing threshold data continued for both groups.

The analysis of variance performed on hearing thresholds with blocks of days being the repeated measure (Table 5) demonstrated no significant changes in absolute hearing thresholds between the control and exposed groups at any auditory frequency.

Table 5. Analysis of variance on hearing threshold

<table>
<thead>
<tr>
<th>Source</th>
<th>M.S.</th>
<th>d.f.</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>148.04</td>
<td>1</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Subjects within Groups</td>
<td>1064.12</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aud. Freq.</td>
<td>1443.17</td>
<td>5</td>
<td>37.19</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Aud. Freq. x Treatment</td>
<td>49.24</td>
<td>5</td>
<td>1.27</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Subj. within Groups</td>
<td>38.80</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>6.03</td>
<td>7</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Time x Treatment</td>
<td>18.84</td>
<td>7</td>
<td>1.58</td>
<td></td>
</tr>
<tr>
<td>Time x Subj. within Groups</td>
<td>11.90</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Time</td>
<td>5.65</td>
<td>35</td>
<td>1.77</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Aud. Freq. x Time x Treatment</td>
<td>2.97</td>
<td>35</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Time x Subj. within Groups</td>
<td>3.19</td>
<td>210</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
From Table 5, it can be seen that hearing thresholds varied with the auditory frequencies tested (p<0.005) and that parathion treatment had no effect. Some hearing thresholds showed significant (p<0.025) interaction between time and auditory frequency. These changes were likewise unaffected by parathion treatment. The hearing threshold-frequency by time interaction is shown in Figure 5 and is less than 4 db. Parameters of the tone stimulus over time are given in Appendix B.

Variability in the repeatedly tested post-exposure hearing thresholds showed changes (Figure 6). The analysis of variance (Table 6) performed on the standard deviations of the repeatedly tested hearing thresholds showed a significant difference between the parathion exposed group and the control group (p<0.025).

Table 6. Analysis of variance of the standard deviations of the tested hearing thresholds

<table>
<thead>
<tr>
<th>Source</th>
<th>M.S.</th>
<th>d.f.</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>130.06</td>
<td>1</td>
<td>10.17</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Subjects within Groups</td>
<td>12.79</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aud. Freq.</td>
<td>7.56</td>
<td>5</td>
<td>1.91</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Treatment</td>
<td>4.61</td>
<td>5</td>
<td>1.17</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Subj. within Groups</td>
<td>3.96</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>13.66</td>
<td>7</td>
<td>1.80</td>
<td></td>
</tr>
<tr>
<td>Time x Treatment</td>
<td>13.35</td>
<td>7</td>
<td>1.76</td>
<td></td>
</tr>
<tr>
<td>Time x Subj. within Groups</td>
<td>7.58</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Time</td>
<td>3.23</td>
<td>35</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Time x Treatment</td>
<td>2.65</td>
<td>35</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Time x Subj.</td>
<td>3.77</td>
<td>210</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 5. Plot of hearing thresholds at each frequency versus time for the combined 2 groups.
Figure 6. Plot of the average standard deviation of repeatedly tested hearing thresholds per block of time. The 8 time blocks encompass 148 days of hearing threshold testing of two groups of squirrel monkeys. Block 0 represents baseline data. Following the collection of baseline data, Group B received parathion, 0.1 mg/kg daily. In blocks 1, 2, 3, 6, 7, parathion was fed 3-5 hours before daily testing. For blocks 4, 5, and 8, parathion was fed 1-1.5 hours before daily testing. Vertical lines with brackets indicate ± SE of the means. Each point is the average of 24 measures, 1 measure on each of 6 auditory frequencies for 4 animals.
Group A
Control

Group B
0.10 mg/kg

STANDARD DEVIATION (dB)

GROUPS

BLOCKS OF DAYS

DAYS

0 20 40 60 80 100 120 140
Table 6 shows no significant interaction between standard deviations of hearing thresholds and auditory frequency. Consequently, a pooled averaged measure for all standard deviations of hearing thresholds for all the tested frequencies per time block is presented in Figure 6.

Correct Response Scores

The first 10 tones of each test session were called pre-trials and were of maximum sound intensity within the attenuated series of tones. All animals continued to report a near perfect score throughout the experiment. Analysis of variance on the means and standard deviations of the pre-trial scores are summarized in Table 7 and Table 8. There were no statistically significant differences between the 2 groups.

Table 7. Analysis of variance on correctly reported pre-trials

<table>
<thead>
<tr>
<th>Source</th>
<th>M.S.</th>
<th>d.f.</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1.28</td>
<td>1</td>
<td>1.45</td>
<td></td>
</tr>
<tr>
<td>Subjects within Groups</td>
<td>0.88</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aud. Freq.</td>
<td>0.15</td>
<td>5</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Treatment</td>
<td>0.02</td>
<td>5</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Subj. within Groups</td>
<td>0.17</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>0.12</td>
<td>7</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Time x Treatment</td>
<td>0.30</td>
<td>7</td>
<td>1.35</td>
<td></td>
</tr>
<tr>
<td>Time x Subj. within Groups</td>
<td>0.25</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Time</td>
<td>0.15</td>
<td>35</td>
<td>1.09</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Time x Treatment</td>
<td>0.10</td>
<td>35</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Time x Subj. within Groups</td>
<td>0.14</td>
<td>210</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 8. Analysis of variance on the standard deviations of correctly reported pre-trials

<table>
<thead>
<tr>
<th>Source</th>
<th>M.S.</th>
<th>d.f.</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2.38</td>
<td>1</td>
<td>3.38</td>
<td></td>
</tr>
<tr>
<td>Subjects within Groups</td>
<td>0.70</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aud. Freq.</td>
<td>0.25</td>
<td>5</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Treatment</td>
<td>0.06</td>
<td>5</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Subj. within Groups</td>
<td>0.28</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>0.26</td>
<td>7</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>Time x Treatment</td>
<td>0.28</td>
<td>7</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Time x Subj. within Groups</td>
<td>0.35</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Time</td>
<td>0.26</td>
<td>35</td>
<td>1.15</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Time x Treatment</td>
<td>0.22</td>
<td>35</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Time x Subj. within Groups</td>
<td>0.23</td>
<td>210</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fewer tones were reported within the attenuated tone sequence by the exposed group in time blocks 4 and 5 \(p<0.1\) as compared to the controls. The analysis of variance on the number of 3 loudest presented tones reported in a test session is summarized in Table 9. Table 10 summarizes the analysis of variance of the total number of tones reported within the attenuated tone sequence. The averages of the reported tones in each time block are presented in Figure 7. The number of tones reported was a function of the loudest tone of the attenuated tone sequence. Minimal change occurred with the loudest tone in the blocks 4 and 5 (Appendix B).
Table 9. Analysis of variance on the number of the 3 loudest level presented tones reported in a test session

<table>
<thead>
<tr>
<th>Source</th>
<th>M.S.</th>
<th>d.f.</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>53.95</td>
<td>1</td>
<td>4.40</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>Subjects within Groups</td>
<td>12.26</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aud. Freq.</td>
<td>1.87</td>
<td>5</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Treatment</td>
<td>1.44</td>
<td>5</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Subj. within Groups</td>
<td>2.04</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>10.71</td>
<td>7</td>
<td>2.75</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Time x Treatment</td>
<td>8.07</td>
<td>7</td>
<td>2.07</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>Time x Subj. within Groups</td>
<td>3.89</td>
<td>42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 10. Analysis of variance on the total number of tones reported from the attenuated sequence of tones per test session

<table>
<thead>
<tr>
<th>Source</th>
<th>M.S.</th>
<th>d.f.</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>1.82</td>
<td>1</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Subjects within Groups</td>
<td>212.22</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aud. Freq.</td>
<td>36.04</td>
<td>5</td>
<td>1.13</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Treatment</td>
<td>11.94</td>
<td>5</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Subj. within Groups</td>
<td>31.80</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>69.65</td>
<td>7</td>
<td>3.31</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Time x Treatment</td>
<td>44.02</td>
<td>7</td>
<td>2.09</td>
<td>&lt;0.10</td>
</tr>
<tr>
<td>Time x Subj. within Groups</td>
<td>21.05</td>
<td>42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 7. Plot of correct responses reported during hearing threshold testing. Block 0 represents pre-exposure data. In blocks 1, 2, 3, 6, and 7, 0.10 mg parathion/kg was fed 3-5 hours before daily testing. For blocks 4, 5, and 8, exposure was 1-1.5 hours before daily testing. Data points are the within dosage group averages summarized across a block of time.
Total Number of Reported Tones from the Attenuated Tone Sequence

<table>
<thead>
<tr>
<th>Days</th>
<th>Group A</th>
<th>Group B 0.10 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>1</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>38</td>
</tr>
<tr>
<td>4</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>8</td>
<td>28</td>
<td>28</td>
</tr>
</tbody>
</table>

Responses to the 3 Loudest Level Tones Presented

<table>
<thead>
<tr>
<th>Days</th>
<th>Group A</th>
<th>Group B 0.10 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>1</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>
Spontaneous Response Scores

A lever press by the monkey that was not a correct response was called a spontaneous response. Parathion treatment resulted in no statistically significant change in spontaneous responses/session time (Table 11). Session time typically ranged from 20 to 37 minutes. A significant reduction of spontaneous responses/session time occurred over the course of the experiment (p<0.005) in both the control and exposed groups. The extinction of spontaneous responses was highly dependent on auditory frequency (p<0.005).

Table 11. Analysis of variance on spontaneous responses/session time

<table>
<thead>
<tr>
<th>Source</th>
<th>M.S.</th>
<th>d.f.</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>7.22</td>
<td>1</td>
<td>1.24</td>
<td></td>
</tr>
<tr>
<td>Subjects within Groups</td>
<td>5.81</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aud. Freq.</td>
<td>0.11</td>
<td>5</td>
<td>4.50</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Aud. Freq. x Treatment</td>
<td>0.33</td>
<td>5</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Subj. within Groups</td>
<td>0.02</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>1.60</td>
<td>7</td>
<td>4.73</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Time x Treatment</td>
<td>0.33</td>
<td>7</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>Time x Subj. within Groups</td>
<td>0.34</td>
<td>42</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 8 shows the average number of spontaneous responses/session time over the course of the experiment.

Table 12 demonstrates the spontaneous response by auditory frequency interaction for averages taken over the entire duration of the experiment. Although no statistical group differences could be demonstrated, the para-
Figure 8. Plot of averaged spontaneous responses/min. of session time over the course of the experiment. In blocks 1, 2, 3, 6, and 7, 0.10 mg parathion/kg was fed 3-5 hours before daily testing. For blocks 4, 5, and 8, exposure was 1-1.5 hours before daily testing.
SPONTANEOUS RESPONSES

<table>
<thead>
<tr>
<th>BLOCKS OF DAYS</th>
<th>DAYS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
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<tr>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>120</td>
</tr>
<tr>
<td>7</td>
<td>140</td>
</tr>
</tbody>
</table>

Group A
Control

Group B
0.10 mg/kg
Table 12. Spontaneous responses/session time by auditory frequency interactions for grand means

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Group A and Group B resp/min</th>
<th>Group A only Control resp/min</th>
<th>Group B only 0.1 mg parathion/kg resp/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 Hz</td>
<td>0.876</td>
<td>0.741</td>
<td>1.010</td>
</tr>
<tr>
<td>1000 Hz</td>
<td>0.755</td>
<td>0.638</td>
<td>0.872</td>
</tr>
<tr>
<td>2000 Hz</td>
<td>0.831</td>
<td>0.691</td>
<td>0.972</td>
</tr>
<tr>
<td>4000 Hz</td>
<td>0.837</td>
<td>0.689</td>
<td>0.985</td>
</tr>
<tr>
<td>8000 Hz</td>
<td>0.849</td>
<td>0.700</td>
<td>0.999</td>
</tr>
<tr>
<td>16000 Hz</td>
<td>0.815</td>
<td>0.682</td>
<td>0.948</td>
</tr>
</tbody>
</table>

The thion-exposed group seems to have more slowly extinguished spontaneous responding.

During baseline hearing threshold testing, Group A had a higher overall spontaneous response rate (1.288/min) than Group B (1.098/min).

Body Weight Data

No significant body weight gains or losses occurred during the course of the experiment. Control Group A maintained an average body weight near 829.1 g with a standard deviation of 99.3 g. The animals in the control group ranged from 700-931 g. Exposed Group B maintained its average body weight near 789.6 g with a standard deviation of 44.4 g. The animals in the exposed group ranged from 742-850 g. No statistical differences existed between the weights of control and exposed groups. Figure 9 presents the mean body weights for the 2 groups over the 148 days.
Figure 9. Body weight curves during the 148 days of the experiment. Data points are the within dosage group averages summarized across a block of time. Vertical line with brackets indicates ±SD.
DISCUSSION

Daily hearing tests on the 2 groups of squirrel monkeys indicated that no significant changes occurred between the mean hearing thresholds of the control and exposed animals. Furthermore, the combined hearing thresholds of the 2 groups varied by no more than 4 db during the experiment. However, the exposed group showed a significantly increased standard deviation in the averaged hearing thresholds after 40 days of parathion exposure. The size of the standard deviation continued to grow for 54 additional days and thereafter declined.

The hearing threshold data were obtained with the psychophysical method of constant stimuli. Tones were reported by the monkey via a bar-pressing response.

Fewer tones were reported by the exposed animals during the period of increased standard deviations in hearing thresholds. A large number of unreported tones were from the 3 loudest tone levels. However, all monkeys reported throughout the experiment a near perfect score for the 10 pre-trials. The pre-trial tones were 20-25 db above the monkeys' hearing threshold. No group difference existed for either means or standard deviations of these pre-trial scores.

Since the exposed group reported fewer tones from the 3 loudest tone levels, one would also expect a shift in the mean hearing thresholds. This, however, was not the case. Rather, the mean hearing thresholds did not deviate significantly from the pre-exposure levels but acquired a larger standard deviation, indicating an increased variability in the data. Essentially, the effect of reporting fewer of the 3 loudest tones was not
a change in hearing thresholds but a flattening of the psychophysical function.

The results of this study indicate that daily doses of parathion at 0.10 mg/kg causes a decrement in the squirrel monkey's tone reporting behavior during hearing threshold testing. This decrement becomes apparent through an increased variability in repeatedly tested hearing thresholds and a reduced correct response rate. The decrement becomes significant at tones presented near the animal's hearing threshold, while no significant disruption exists for tones presented 20-25 db above threshold.

A complete explanation of the mechanism involved which would account for the appearance of the decrement in behavior is not possible. Changes in conditioned behavior have been reported to become evident with a 40-60 percent inhibition of serum and erythrocyte cholinesterases as reviewed by Medved et al. (1964) or 60 percent reduction of brain cholinesterase (Russell, 1969).

Significant reductions in cholinesterase have been reported with parathion exposure of 0.10 mg/kg and have been reviewed in the Introduction. Frawley and Fuyat (1957) have observed a 60-70 percent inhibition of dog plasma cholinesterase with as little as 0.047 mg parathion/kg in 6 weeks. It is conceivable, therefore, that the squirrel monkey's cholinesterase level may have fallen to a critical disruptive level in 40 days. No cholinesterase data had been obtained in this study for fear that taking blood samples from the animals would disrupt behavior.

Adaptation to cholinesterase inhibition has been previously shown to occur after long exposure to organophosphate insecticides (Sumerford et al., 1953). Likewise, a 6-day subacute parathion treatment study
demonstrated compensation for prolonged depression of cholinesterase activity (Reiter et al., 1973b).

The mechanism of adaptation is not completely understood but apparently involves the adjustment of central synapses to low levels of acetylcholinesterase (Stavinoha et al., 1969). It is possible that an elevated level of acetylcholine is important in initiating the adaptive process. Enzyme induction caused by phenobarbital increases significantly the dose of parathion required to produce acute toxicity in rats (DuBois and Kinoshita, 1968). The behavior of the squirrel monkeys could return to an undisrupted level assuming a similar adaptation.

This study has demonstrated a decrement in behavior caused by parathion at a level of exposure well below that of any other study to date, with the exception of Bignami and Gatti (1967), who found that as little as 0.05 and 0.25 mg parathion/kg/day caused reduced acquisition of avoidance conditioning. Their experience with pre-trained animals exposed after daily testing, however, gave negative results. It may be instructive to suggest reasons which may be related to the results observed in the present experiment, which involved pre-trained monkeys.

Exposure to parathion in this experiment took place either 3-5 hours or 1-1.5 hours before the testing session. It is known that at least in male rhesus monkeys a dose of parathion, 0.3 mg/kg, causes an abrupt decrease in whole blood cholinesterase in the first 30 minutes with a maximum depression after 6 hours (Copeland et al., 1971). Sumerford et al. (1953) thought that the rate of fall in acetylcholinesterase rather than the level of acetylcholinesterase determined whether or not poisoning symptoms could be expected. No different trends between the two dosing schedules employed
in this experiment could be detected. Nevertheless, the dosing time sched­
ules fall in the vicinity of low and most varying levels of acetylcholines­
terase. The possibility exists that low and varying acetylcholinesterase
levels are more effective in producing decrements in behavior than a low
and stable level. Such a possibility has not been mentioned in the behav­
ioral literature on experimental organophosphate poisoning (Clark, 1971).

Another reason for the positive findings in this experiment could be
that the animals were not completely trained when parathion exposure was
initiated. Although all animals reliably reported consistent baseline
hearing thresholds, their spontaneous response rate had not reached a mini­
mum level at the beginning of the experiment. The animals gradually extin­
guished this response to half of the original level. The extinction indi­
cates that the monkeys became more conditioned to the experimental task
and refrained from bar-pressing when there was no tone. In this sense,
some learning of the task had taken place over the 148 days. The principle
expressed by Medved et al. (1964) that learning acquisition is a more sen­
sitive indicator of toxic effects than the maintenance of a learned task
appears to be illustrated in this experiment.

During the entire period of the study, all the monkeys were in excel­
 lent health. Parathion exposure at 0.10 mg/kg did not cause any signs of
clinical toxicosis throughout the study. This lack of clinical toxicosis
is consistent with a study by Santolucito and Morrison (1971). They fed
0.10 mg parathion/kg to rhesus monkeys and observed no clinical toxicosis
over an 18-month period. It is interesting to note, however, that after
the first week of parathion exposure in this experiment, the treated ani­
mals became reluctant to consume their daily parathion dose. Along with
this reluctance, 2 exposed animals developed what is best described as a conditioned salivary response. This response occurred whenever the experimenter entered the colony room to feed the parathion containing marshmallow on a regular schedule. The monkey would temporarily salivate and refuse the marshmallow. After cleaning away the saliva with his hand, the monkey would reluctantly accept and ingest the marshmallow.

This study has demonstrated that parathion exposure at a rate of 0.10 mg/kg per day had an adverse effect on the tone-reporting behavior of squirrel monkeys. Such decrement in tone-reporting behavior could easily have gone unnoticed with less automated testing techniques which do not fully control stimulus presentation and data acquisition. An increased variability in auditory data may easily be overlooked as possible inconsistencies in experimental procedures. Such variability is minimized with automation.

It is difficult to speculate whether the decrement in behavior is actually caused by a dysfunction in the monkey's auditory system or whether the dysfunction is of more general origin. In this regard, this type of study has not been exhausted.

Maximum safe levels of daily human pesticide intake are determined in part by the World Health Organization Expert Committee on Pesticide Residues. The acceptable daily intake is that dosage which, during an entire lifetime, appears to be without appreciable risk. The acceptable daily intake is usually calculated from the "no effect" level that does not cause any biologic change in the most susceptible animal. When no human data is available, the acceptable daily intake is 1/100 of the "no effect level" measured in mg/kg body weight. When available, human data, although cover-
ing a relatively short period, takes precedence over animal data (Fitzhugh, 1965).

The acceptable daily intake of parathion is given as 0.005 mg/kg and has been calculated from the "no effect" level of 0.05 mg/kg x 1/10 based on a limited 2 months human cholinesterase study. The "no effect" level of parathion for the dog is the lower value of 0.025 mg/kg (Fitzhugh, 1965).

This experiment demonstrated that parathion in the dose of only 20 times the acceptable daily intake for humans has significantly disrupted behavior in the subhuman primate *Saimiri sciureus*.
SUMMARY

Eight male squirrel monkeys were assigned to 2 dosage groups and systematically exposed to daily oral doses of parathion, an organophosphate insecticide. There were 4 monkeys in the 0 dose control group and 4 in the 0.10 mg/kg group. The exposure period was for 148 days. The 0 dose group received placebos for the entire 148 days.

Prior to dosage assignment, the monkeys were adapted to the laboratory and trained for hearing threshold testing. Daily threshold testing employed the method of constant stimuli. Monkeys were required to reliably report hearing thresholds at 500, 1000, 2000, 4000, 8000, and 16000 Hz. Parathion exposure began after the 8 monkeys could be divided into 2, four-animal statistically matched groups based on stable baseline hearing threshold data.

An analysis of variance was performed on hearing thresholds and standard deviations of hearing thresholds. The parathion exposed group showed a significant (p<0.025) increase in the standard deviation of hearing thresholds after 40 days of parathion exposure. The magnitude of the standard deviation continued to grow for 54 additional days and thereafter declined. Mean hearing thresholds between control and the exposed group did not vary significantly.

It was concluded that daily oral doses of parathion at 0.10 mg/kg caused a decrement in the squirrel monkey's tone-reporting behavior during hearing threshold testing.
REFERENCES


ACKNOWLEDGMENTS

With the hope that the ideas and concepts presented in this dissertation will inspire others to continue and to improve upon the findings presented herein, I wish to express my sincere appreciation to my graduate committee, Dr. Gary A. Van Gelder, Dr. Neal R. Cholvin, Dr. George G. Karas, Dr. William H. Brockman, and Dr. Charles C. Bowen for their continuous assistance, support, and encouragement throughout all phases of this research.

My major professor, Dr. Gary A. Van Gelder, introduced me to the discipline of toxicology. His direction of the Behavioral Toxicology Laboratory made the entire facilities and technical staff readily available for the pursuit of this investigation. I am deeply grateful for the countless hours he has devoted to this research and for the guidance he has given me.

Dr. Karas has made numerous visits to the Behavioral Toxicology Laboratory to attend research meetings associated with this research. His contributions were greatly appreciated.

Dr. Brockman's assistance with the instrumentation for this research will not be forgotten.

Dr. Cholvin has encouraged my educational progress, and his interest will always be remembered.

Dr. Bowen's interest in this research and non-technical contributions were sincerely appreciated.

I am indebted to Dr. Leroy Wolins for the many hours he devoted to the statistical analysis of the experimental data. A thank you to Mrs.
Charlotte Bentley of the statistical section for handling the data at the Computation Center. Mr. Marvin Freeman's programming help was appreciated.

I am grateful for the technical contributions of individual staff members of this laboratory. Mr. Ronald Munsinger's maintenance of the electronic equipment was indispensable, as was the help of Mr. Arnold Severson in constructing the sound chamber. A thank you to Mrs. Liz Wiedenman and Miss Ginger Horak for their help in the laboratory and in the office.

A special thank you goes to my friends, who visited the laboratory, who helped with the initial experiments, and who have presented helpful suggestions and ideas.

The financial support for this investigation was provided by E.P.A. Contract 63-03-0036.
APPENDIX A: BASELINE HEARING THRESHOLD DATA,
IN db RE 0.0002 d/cm²
Table 13. Table of mean baseline hearing thresholds ± SD by animal. Number in parentheses denotes number of observations

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Frequency</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>500 Hz</td>
<td>1000 Hz</td>
<td>2000 Hz</td>
</tr>
<tr>
<td>#103</td>
<td>25.02±2.35 (5)</td>
<td>20.50±2.50 (5)</td>
<td>15.70±1.64 (6)</td>
</tr>
<tr>
<td>#105</td>
<td>24.00±1.00 (3)</td>
<td>21.02±3.46 (5)</td>
<td>16.90±3.63 (6)</td>
</tr>
<tr>
<td>#108</td>
<td>26.20±2.79 (4)</td>
<td>16.20±3.03 (5)</td>
<td>16.47±1.29 (6)</td>
</tr>
<tr>
<td>#110</td>
<td>36.37±2.84 (4)</td>
<td>31.44±2.74 (5)</td>
<td>28.60±1.66 (6)</td>
</tr>
</tbody>
</table>

|        | 500 Hz            | 1000 Hz                 | 2000 Hz                 | 4000 Hz | 8000 Hz | 16000 Hz |
| #102   | 23.80±2.14 (4)    | 15.55±3.31 (4)          | 16.34±3.04 (5)          | 16.80±4.15 (5) | 12.54±3.90 (5) | 8.90±2.13 (4) |
| #104   | 29.97±1.27 (4)    | 22.20±2.77 (5)          | 17.33±2.36 (6)          | 20.62±2.59 (6) | 16.16±2.50 (5) | 7.32±1.30 (4) |
| #106   | 26.25±1.45 (4)    | 19.60±2.24 (4)          | 17.60±2.74 (6)          | 25.82±2.26 (5) | 15.56±0.63 (5) | 10.30±2.25 (4) |
| #113   | 25.75±1.95 (4)    | 23.86±3.87 (5)          | 18.33±2.34 (6)          | 21.60±3.49 (5) | 13.90±1.14 (5) | 8.30±3.26 (4) |
APPENDIX B: TONE PARAMETERS
The loudest tone of an attenuated tone sequence was assigned such that the hearing threshold of an animal fell within the lowest 2 or 3 tone intensities. If this criterion was not met for 2 consecutive testing sessions, the loudest tone and, consequently, the tone sequence was shifted by 6 db. The analysis of variance performed on the loudest presented tones is summarized in Table 14.

Table 14. Analysis of variance on the loudest tones presented in an attenuated tone sequence

<table>
<thead>
<tr>
<th>Source</th>
<th>M.S.</th>
<th>d.f.</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>161.02</td>
<td>1</td>
<td>0.283</td>
<td></td>
</tr>
<tr>
<td>Subjects within Groups</td>
<td>569.58</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aud. Freq.</td>
<td>1746.27</td>
<td>5</td>
<td>36.99</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Aud. Freq. x Treatment</td>
<td>26.40</td>
<td>5</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Subj. within Groups</td>
<td>47.21</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>19.13</td>
<td>7</td>
<td>3.10</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Time x Treatment</td>
<td>4.31</td>
<td>7</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>Time x Subj. within Groups</td>
<td>6.17</td>
<td>42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Time</td>
<td>2.00</td>
<td>35</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Time x Treatment</td>
<td>2.07</td>
<td>35</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Aud. Freq. x Time x Subj.</td>
<td>2.11</td>
<td>210</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From Table 14, the loudest tone intensities varied with the auditory frequency tested (p<0.005) as did the hearing thresholds. The loudest tone intensities varied with time (p<0.025). Figure 10 demonstrates the loudest tone intensities at each of the 6 auditory frequencies tested over the time span of the experiment. Although no statistically significant group
differences existed, Figure 11 shows the loudest tone levels of the individual groups, averaged per time block over all 6 frequencies.
Figure 10. Sound pressure curves over 148 days of the loudest tones presented during hearing threshold testing. Data points are the averages of a test day, summarized across a block of time.
Figure 11. Sound pressure curves over 148 days of the loudest tones presented to the 2 groups of squirrel monkeys during hearing threshold testing. Data points are averages across all 6 frequencies tested per time block. Vertical lines with brackets are ± SD and indicate variability of the tone parameter from static conditions.
Tones to Group A
Control

Tones to Group B
0.10 mg/kg

SOUND PRESSURE (dB RE 0.0002 d/cm²)

BLOCKS OF DAYS

DAYS