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Effects of chronic streptomycin treatment on the free-fall response in rats

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EFFECTS OF CHRONIC STREPTOMYCIN TREATMENT ON THE FREE-FALL RESPONSE IN RATS

A Thesis

Presented to

the Faculty of the Department of Psychology

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

by

Patricia Ann Taber

May 1998

APPROVED FOR THE DEPARTMENT OF PSYCHOLOGY

Robert Fox Ph. D.

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Abstract

EFFECTS OF CHRONIC STREPTOMYCIN INJECTIONS

ON THE FREE-FALL RESPONSE IN RATS

by Patricia Ann Taber

The free-fall response (FFR), an electromyographic response elicited by sudden fall, has been shown to be an otolith-spinal reflex in man, baboons, and cats. Streptomycin has been found to preferentially destroy the otolith hair cells of pigmented rats. To test the hypothesis that the FFR is an otolith-spinal response in rats, Long Evans rats were injected with intramuscular doses of either streptomycin (400 mg/kg/day) or sterile normal saline for 34 consecutive days. To assess behavioral changes, the animal's ability to air-right was observed. Electromyogram (EMG) electrodes were implanted in the lateral gastrocnemius and tibialis anterior muscles. The rats were subjected to repeated trials of free-fall while the EMG was monitored. Rats treated with streptomycin had an EMG response of a significantly lesser amplitude than control rats and disruption in their ability to air-right completely. These findings suggest the free-fall response is an otolith-driven reflex in rats.

ACKNOWLEDGMENTS

I would like to thank Dr. Robert Fox for his guidance and never-ending patience in the design and processing of data in this experiment, and writing of this thesis. I would also like to thank the Pfizer Pharmaceutical Company for their generous donation of streptomycin without which this research could not have been done.

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Abstract

The free-fall response (FFR), an electromyographic response elicited by sudden fall, has been shown to be an otolith-spinal reflex in man, baboons, and cats. Streptomycin has been found to preferentially destroy the otolith hair cells of pigmented rats. To test the hypothesis that the FFR is an otolith-spinal response in rats, Long Evans rats were injected with intramuscular doses of either streptomycin (400 mg/kg/day) or sterile normal saline for 34 consecutive days. To assess behavioral changes, the animal's ability to air-right was observed. Electromyogram (EMG) electrodes were implanted in the lateral gastrocnemius and tibialis anterior muscles. The rats were subjected to repeated trials of free-fall while the EMG was monitored. Rats treated with streptomycin had an EMG response of a significantly lesser amplitude than control rats and disruption in their ability to air-right completely. These findings suggest the free-fall response is an otolith-driven reflex in rats.

Effects of Chronic Streptomycin Treatment on the Free-Fall Response in Rats

Unexpected falls evoke an electromyographic (EMG) response in the legs of humans, monkeys, cats, and rats. The response is of large amplitude; it is biphasic and synchronized, in which many muscle fibers are recruited. This response occurs in flexor and extensor muscles of the lower limbs and results in the stiffening of the limbs (Jones & Watt, 1971). Activation of this EMG response, known as the free-fall response (FFR), is thought to be of vestibular origin in monkeys (Lacour, Xerri, & Hugon, 1978). The FFR has been identified as an otolith-spinal reflex in humans (Greenwood and Hopkins, 1976), cats (Watt, 1976), and monkeys (Lacour et al., 1978). The reflex has also been shown to be present in rats (Gruner, 1989). However, the origin of the response has not been determined for this species. The objective of this study was to evaluate whether the FFR is an otolith-spinal reflex in the rat. This was done by selectively destroying the otolith portion of the vestibular system of the rat using chronic injections of streptomycin (STP).

Streptomycin is a well-documented ototoxic agent in many species. After STP was administered chronically to adult pigmented rats, Meza, Bohne, Daunton, Fox, and Knox (1996), examined behavioral changes using swimming analysis. Animals treated with STP showed more abnormalities in swimming: vertical swimming with rolls, barrel rolling, corkscrew swimming, and forward and backward looping. Histological examination via electron microscopy of the utricular macula sensory cells showed fused stereocilia cells and pyknotic nuclei. In contrast, sections of the cristae and organ of Corti appeared normal. In a prior study, Meza, Daunton, Lopez-Griego, and Salas (1993), found STP selectively disrupted the otolith organ function while sparing the function of the semicircular canal (assessed with postrotary nystagmus) and auditory function (assessed with evoked auditory potentials).

Air-righting (Hard & Larson, 1975; Pellis & Pellis, 1994; Pellis, Pellis, & Nelson, 1992) is a reflex in rats that is fully developed by postnatal day 19 (Hard & Larsson,

1975). There is thought to be a vestibular component to the reflex (Pellis, et al., 1992). Therefore, it is used as a behavioral measure to assess vestibular changes in rats which have been exposed to hypergravity. After exposure to hypergravity, a smaller percentage of these rats air-right and when they do, they take longer to right (Fox, Daunton, & Corcoran, in press). Fox, et al., (in press) suggest that this increased latency to right may be due to increased threshold for detecting linear acceleration secondary to a decrease in synapses on Type II hair cells in the utricular maculae.

Given that the FFR has been shown to be present in rats and is thought to be of vestibular origin in other animals, and that it has been shown that STP selectively destroys the utricular macula sensory cells in pigmented rats, if STP is injected into rats the utricular macula sensory cells should be destroyed. Further, if the utricular macula sensory cells are destroyed, the FFR should be disrupted. That is, there should be a change in the EMG response. It was hypothesized that there would be a reduction in the peak-to-peak amplitude and an increase in the latency of the FFR response in animals treated with STP. In addition to the EMG response, air-righting was also assessed. It was hypothesized that the ability to air-right would be decreased in rats treated with STP.

Method

Subjects

A total of 19 male rats of the Long Evans strain (Simonsen, Gilroy, CA) were randomly assigned to treatment (N = 12) and control (N = 7) groups. The animals, which were 21 days old at the onset of the experiment, were housed three per cage (one control and two treatment, except for one cage with only one rat) in "shoe box" plastic cages and maintained on standard laboratory chow. The rats had access to food and water ad libitum throughout the experiment. The animals were treated in accordance with the ethical standards of treatment of non-human animals in the "Ethical Principles of Psychologists and Code of Conduct" (APA, 1992).

Drug Injection

Progressive destruction of the vestibular hair cells was accomplished by administering intramuscular injections of STP twice daily at a total daily dosage of 400 mg/kg/day for 35 consecutive days. The injections were made 8 to12 hr apart. To prevent irritation and necrosis of tissue of the hind limbs, and possible effects on the EMG, the injections were alternated between the triceps muscles of the fore limbs using a 25 gauge needle. Rats in the control condition were given twice-daily injections of an equal volume of sterile normal saline.

Behavioral Assessments

The air-righting reflex (Pellis & Pellis, 1994; Pellis, et al., 1992), was used to test vestibular function and whole-body coordination on experimental days 21 and 35. To test these two behaviors, a swim tank was used. The swim tank was a 60.5-cm plexiglas cube with a metal rod placed 54 cm above the water line. The water was 37°C and 45 cm deep. The animals were held in a supine position at the level of the rod until they were relaxed and then were dropped into the water. A minimum of three drops was used with each rat. On the initial drop, the animal was allowed to swim for 45 s. The free-fall and the swimming were video-taped at a speed of 30 frames/s from frontal and side views and recorded as a split screen image. On the second and third drops, the animals were removed from the water immediately. At the completion of the three drops, the animals were towel dried and returned to their home cages. The release, segment righting, and landing were analyzed frame-by-frame at a later time.

EMG Electrode Implantation

Electrodes for monitoring electrical activity were implanted in the lateral gastrocnemius and tibialis anterior. Electrodes were formed by baring the tips of insulated 40 AWG wires (Part No. AS765-40, Cooner Wire Chatsworth, CA) for 1 mm on the recording tip and 5 mm on the transmission end of a 25-cm wire. Wires were positioned in

pairs and staggered so that when the recording tips were bent back on themselves forming a hook, there was a 2-mm distance between the recording tips of the wires. The electrodes were threaded through a 23 gauge x 1 in. hypodermic needle and positioned such that the apex of the bend was at the tip of the needle.

Anesthesia was accomplished with inhalation of 2% isoflurane. A 1-cm long incision was made in the rostral to caudal direction on the lateral aspect of the left hind limb proximal to the course of the lateral marginal vein. After the biceps femoris muscle was exposed by blunt dissection, electrodes were placed in the gastrocnemius and tibialis anterior by insertion in the appropriate muscle through a 1-cm incision in the biceps femoris. The electrodes were anchored with single stitches at the point of insertion through the biceps femoris using 5-0 silk suture. The skin incision was closed with two or three interrupted stitches as required using 5-0 silk suture and lidocaine ointment (2.5%) was applied to the skin incision.

EMG Recording and Testing

Immediately after removal from anesthesia the rat was placed in a full-body jacket and was suspended from an apparatus for inducing sudden drops. A modification of the apparatus described by Gruner (1989) was used. The jacket was attached to a suspension device that was held by an electromagnet to the drop frame. Attached to the suspension device were cables for monitoring the EMG and accelerometer signals and a spring which gently broke the animal's fall prior to reaching the floor. When the electromagnet was de-energized, the animal was exposed to a sudden, 40-cm free-fall.

Electromyogram signals were bandpass filtered (10 to 3KHz), amplified (x1000 or x500 dependent on signal amplitude) using GRASS P511 amplifiers, and then converted to digital signals using the PEAK Technologies Analog to Digital Sampling Module. Digital conversion was with a sampling rate of 10Hz. Signals were recorded for 50 ms prior to initiation of the sudden drop and for 300 ms following the drop. The signal from the

accelerometer was recorded simultaneously to determine the precise time of the initiation of the drop.

Three types of tests were conducted on each rat: dummy, auditory startle, and free-fall. Recordings of muscle activity with no stimulation, referred to as "dummy" trials, were conducted to assess baseline muscle activity. In startle trials, muscle activity resulting from a loud auditory stimulus was recorded. The stimulus was a single clang produced by hitting a metal container against a metal handle behind the animal. This test was performed to verify the auditory system was not significantly affected by treatment with STP. In free-fall trials, EMG response was elicited by a sudden fall using the suspension apparatus previously described. Additionally, a calibration pulse was recorded prior to and following each test series. The pre- and post- calibration was taken to ensure there was no drift in the signal during the testing. The mean of the pre- and post-calibration pulses was used to convert digital values of the EMG response to actual voltage in millivolts.

The sequence of testing for each rat with the number of tests in parentheses was as follows: calibration (1), dummy (1), startle (3), FFR trials (minimum of 20), startle (3), dummy (1), and calibration (1). The initial startle response was recorded during the time the animal was recovering from anesthesia. The trials were not performed until the animal was recovered from anesthesia at about 9 to 10 min after removal from anesthesia. This precaution was taken to decrease the possibility of confounding the effects of STP and the anesthesia. However, in one particularly active animal, the trials were begun at 5 min post anesthesia. All tests were performed in a well-lighted room. Throughout testing, ongoing EMG activity of either the gastrocnemius or tibialis anterior was monitored on an oscilloscope and the rat was also monitored to ensure sudden falls occurred only during periods of inactivity. At the end of testing, the rat was anesthetized with inhalation therapy and sacrificed with an intracardiac infusion of sodium pentobarbital. The hind limb was

then dissected and EMG electrode placement in the gastrocnemius and tibialis anterior muscles was verified.

Results

During the course of the injections, two of the treated animals died, possibly secondary to the toxic effects of STP. In a third animal, the lateral marginal vein was inadvertently nicked during the electrode placement. Therefore, the data from these animals were not used in all of the subsequent FFR analyses. All animals with the exception of two control animals were assessed for the air-righting ability and EMG response during free-fall on days 35 or 36. These two control animals were assessed for the same functions on day 41.

The EMG response to free-fall is a large synchronized response in the leg muscles that is a very consistent in control rats. Figure 1 shows the two measurements that were taken to measure the response, latency and peak-to-peak amplitude. Latency was defined as the first deflection, either positive or negative, that was equal to or larger than four standard deviations from the baseline recording. Peak-to-peak amplitude was defined as the difference between the highest peak and the lowest peak in a response. This was later converted to millivolts using the mean calibration value for a particular series of trials. Five consecutive trials in a control rat are shown in Figure 2.

Auditory Startle Response

The effects of treatment (Control/STP) and whether the auditory startle response (ASR) was tested before (pretest) or after (posttest) the FFR trials were evaluated. The data from one of the STP animals were discarded due to the loss of information in the posttest trials, therefore 16 animals were used for the analyses. A 2 (treatment) x 2 (test) mixed analyses of variance (ANOVA) with test as the repeated measure was conducted on EMG peak-to-peak voltage as the dependent variable. There was a significant main effect of test in which the posttest responses had a much larger amplitude ($\underline{M}$ = 7.34, $\underline{SD}$ = 3.59), than

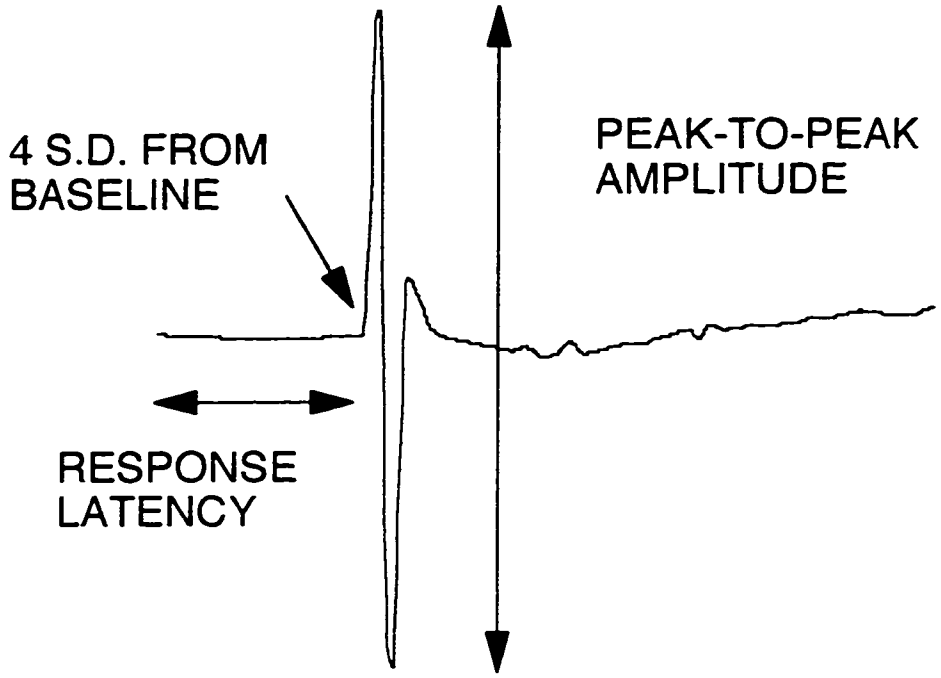

Figure 1. Schematic representation of an EMG illustrating the two measures used to characterize each response. Response latency is the time (ms) from the start of linear acceleration (the beginning of the trace) to the point where the EMG exceeds 4 SDs from the baseline level of EMG. Peak-to-peak amplitude is the voltage (mv) between the maximum and the minimum values of the EMG.

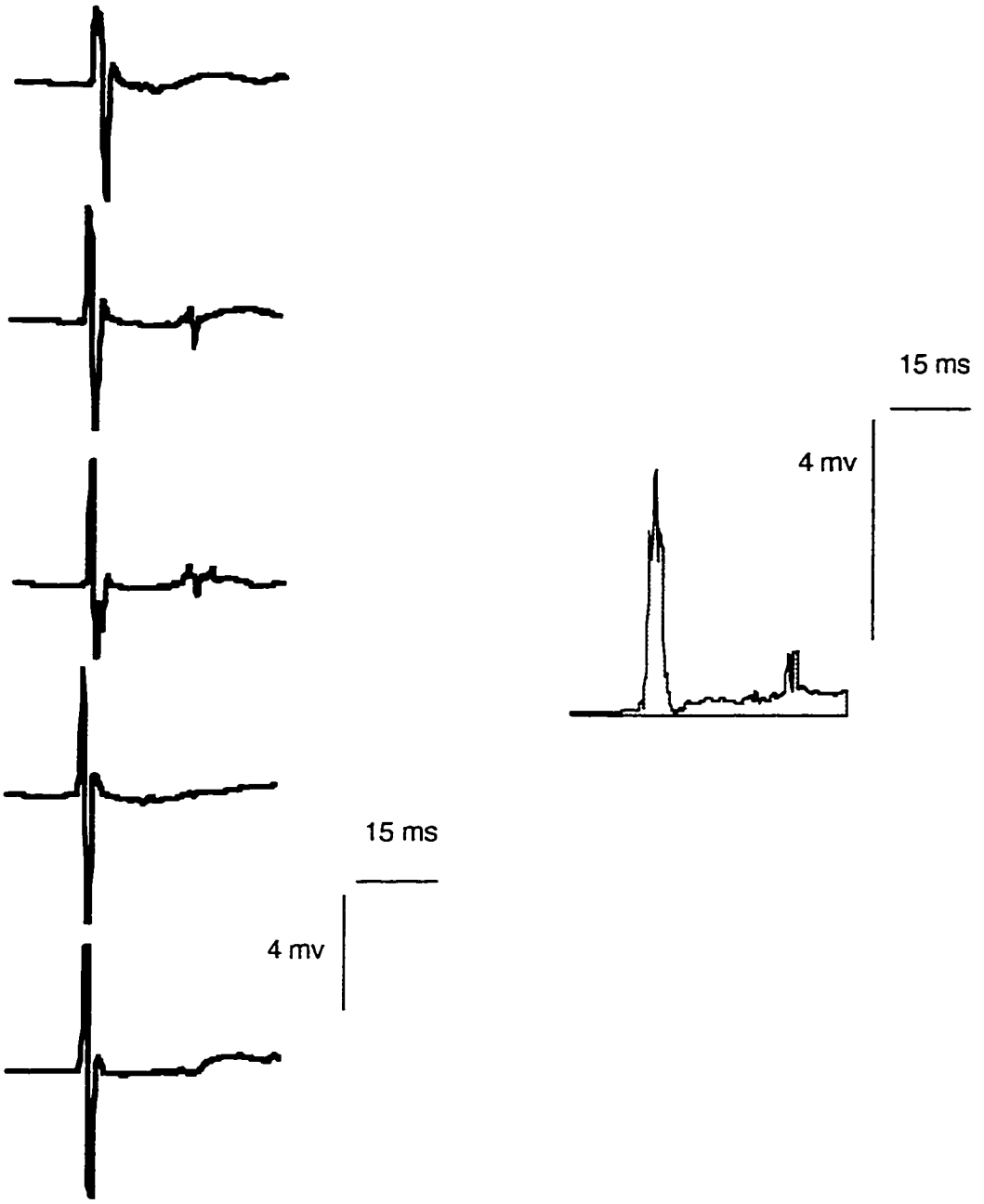

Figure 2. The raw EMG recorded on five consecutive tests for Control Rat 5B is shown in the left panel. Each trace shows 50 ms of data with linear acceleration beginning at the start of the trace. The average rectified EMG for Rat 5B based on 15 tests is shown in the right panel.

the pretest responses, (M= 2.74, SD = 3.37, F(1,13) = 51.04, p <.001). There was neither a significant effect of the treatment (STP-treated M = 4.89, SD = 3.89)/ control M = 5.24, SD = 4.69, F < 1), nor a significant Treatment x Test interaction (F < 1). Thus, the auditory startle response did not differ between the control and STP treated groups. To illustrate the response more clearly, the data were rectified. For this process, all negative deflections are changed to positive. The individual EMGs are then summated and averaged over three trials. Figure 3 shows the average rectified data from both control and treated groups for pretest and posttest trials. The appearance of this figure is consistent with the significant time effect, and the failure to find a significant treatment or interactive effect.

Free-fall Response

All animals were tested for a minimum of 20 free-fall trials. Due to complications or poor EMG recordings, several recordings were discarded. Trials were considered unacceptable if voluntary movement occurred during the recording of the baseline or if there was electrical artifact. The fewest number of acceptable trials was 13 for a single animal. To avoid bias, the first 13 acceptable trials of each animal were used in the analyses for the free-fall response. The data from 16 animals were analyzed to determine the effects on the peak-to-peak amplitude and latency of the FFR.

One-way ANOVAs with type of treatment as the independent variable were performed on the EMG measures of latency and peak-to-peak amplitude. There was no significant difference in latency of the EMG response between the control (M = 14.52, SD = .40) and STP groups, (M = 14.96, SD = 1.53, F < 1). However, the STP-treated animals had a considerably smaller peak-to-peak amplitude (M = 2.08, SD =1.71) than the control animals (M = 6.79, SD = 2.67, F (1,14) = 18.91, p < .001). Figure 4 shows the rectified EMG responses for control and STP treated animals To illustrate the change in the peak-to-peak amplitude following treatment with STP, the amplitudes of the

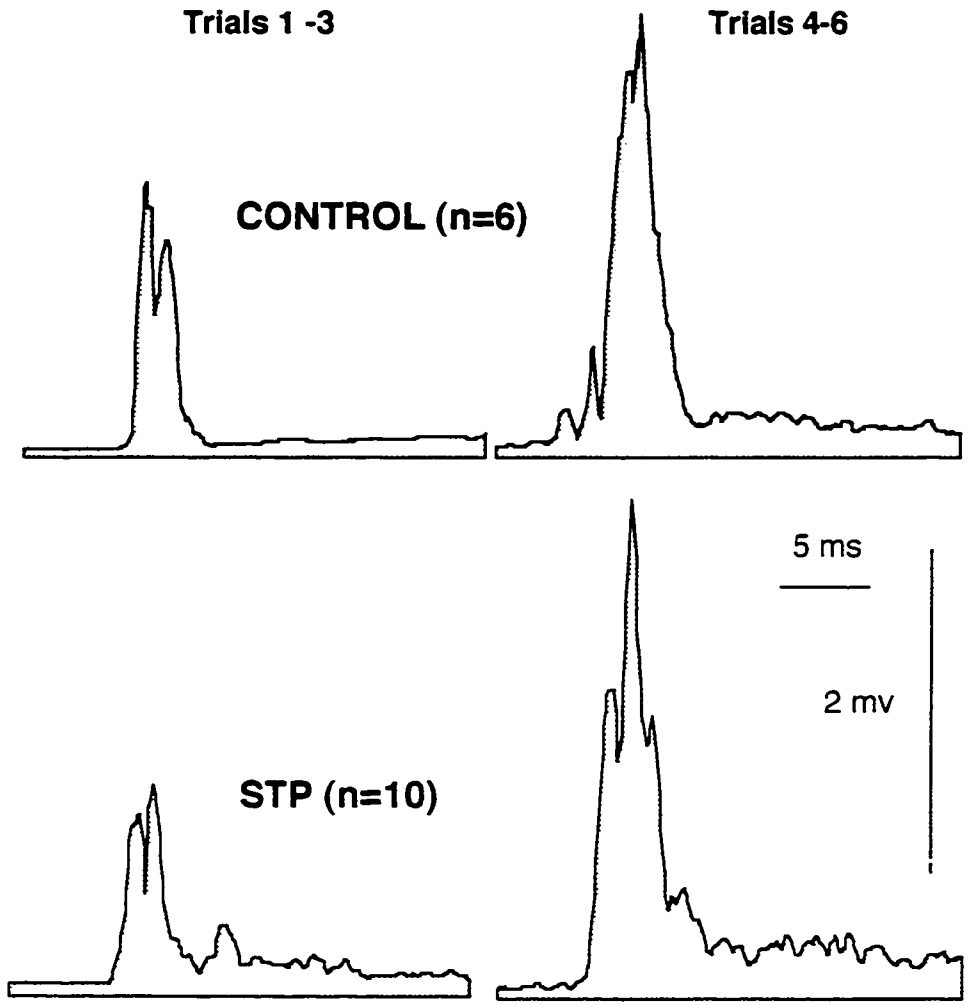

Figure 3. Average rectified EMG in the gastrocnemius muscle of Control and Treated animals on auditory startle trials. Pre-FFR trials are in the two left panels and Post-FFR trials are in the right panels.

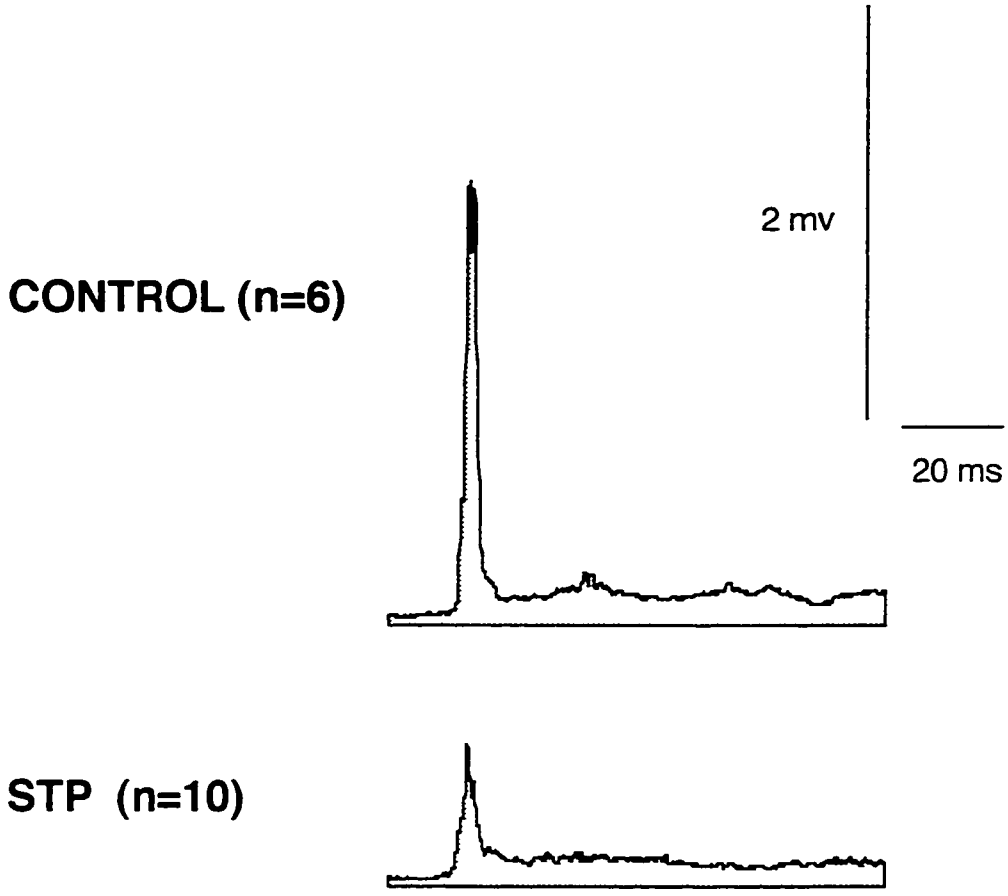

Figure 4. Average rectified EMG of the gastrocnemius muscle in Control and Treated animals on FFR trials.

FFR for each animal were analyzed to determine the frequency of FFRs occurring in 2-mv intervals. As can be seen from the averages of these frequencies (Figure 5), the control animals had the greatest percentage of responses with amplitudes of greater than 6 mv, and STP-treated animals had the greatest percentage of responses with amplitudes of less than 2 mv.

Air Righting

There were three air-righting trials for each animal. Videotapes of these trials were analyzed frame-by-frame to assess the time at which air-righting was complete. For the control rats, complete air-righting occurred on 94% of the tests. (One rat failed to right on one test). For the rats treated with STP, complete air-righting occurred in only 67% of the tests. Two of the treated rats failed to right on all three tests, one rat righted on only one test, two rats righted on two tests, and five rats righted on all three tests. To investigate the relationship between ability to air-right (a behavioral test of vestibular function) and the FFR, the mean latency of the FFR for each animal was correlated with the number of trials in which complete righting occurred. As the latency of the FFR increased, the number of trials in which the animal successfully air-righted decreased, $r = -.70$.

Discussion

Lacour et al. (1978), Watt (1976), and Greenwood and Hopkins (1976), have shown that the EMG response to sudden fall is dependent on labyrinthine function and, more specifically, is an otolith-originating reflex in baboons, humans, and cats. In previous experiments which have tested this, vestibular inputs have been eliminated in animals via vestibular neurectomies, both bilateral and unilateral, and canal plugging of the semicircular canals followed by total labyrinthectomy. In the case of humans, the role of vestibular input has been studied using vestibular deficient patients. In all cases, the EMG response to free-fall has been smaller in amplitude in the participant or the contralateral limb in the case of unilateral neurectomies. In this experiment sensory input from the vestibular

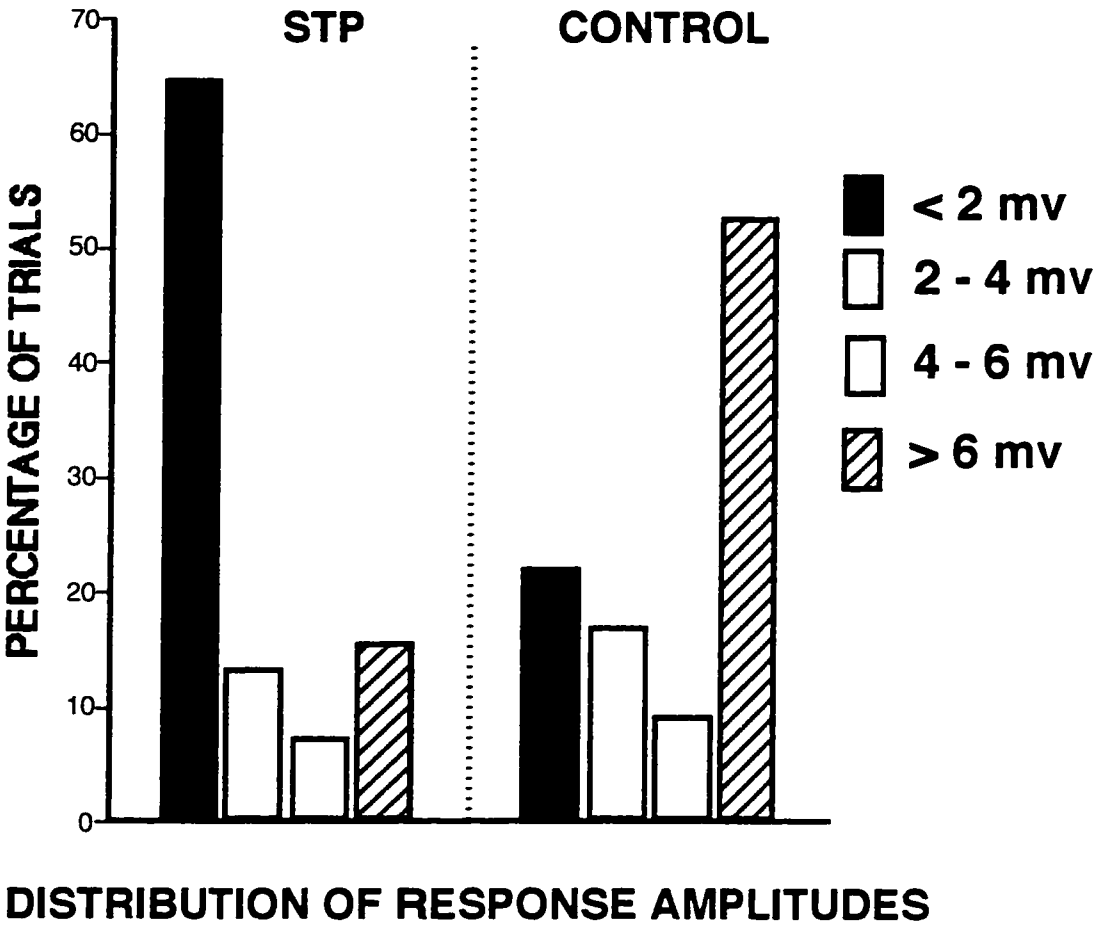

Figure 5. Bars respresent the percentage of trials with response amplitudes of varying amplitudes. Streptomycin treated animals had the greatest percentage of responses in the less than 2-mv range: Control animals had the greatest percentage of responses in the less than 6 mv range.

system was disrupted by chronic injections of STP. It was hypothesized that the injections of STP would result in a decrease in amplitude of the FFR. The findings were consistent with this hypothesis. It also was hypothesized that the latency of the FFR would be increased. This hypothesis was not supported. One possible explanation for this may be the manner in which latency was determined; only trials with an identified FFR were used to calculate the mean latency. Another possible explanation is that the variability of the latencies was so small that our sampling rates could not detect changes that were less than 0.1 ms. The last hypothesis was that air-righting would be disrupted. Although statistically not significant, the percentage of animals which air-righted completely was much smaller in animals treated with STP.

Neurophysiological mechanisms

In the rat it has been shown that there are primary vestibular afferents going chiefly to the rostral ventral portion of the lateral vestibular nucleus (Mehler & Rubertone, 1985). Neurons from the LVN descend in the ipsilateral vestibulospinal tract and project in a somatotopic fashion with the hindlimb spinal levels in the dorsalcaudal region and the forelimbs in the ventral rostral area. The vestibulospinal connections have not been studied extensively in the rat, but are in lamina 7 and 8 in the cat. It has been shown in the cat that stimulation of Dieter's nucleus increases activity in the extensor muscles of the hind limb due, in part to monosynaptic excitatory post synaptic potentials (EPSP) in some motor neurons (Wilson & Melvill Jones, 1979). This has been found mainly in the quadriceps and gastrocnemius muscles, activation of which results in extension of the knee and ankle. Although care must be taken in generalizing findings between species, one might speculate the pathways in the rat and the cat are similar. This inference, in combination with the findings that STP preferentially destroys otolith hair cells, supports the hypothesis that the FFR in the rat is a otolith-spinal reflex.

While stimulation of the LVN leads to excitation of extensor muscles, it also leads to inhibition of the flexor muscles. Inhibitory post synaptic potentials (IPSP) are evoked via Ia interneurons of the segmental reciprocal inhibitory pathways in the cat (Wilson & Melvill Jones, 1979). This leads to inhibition of the knee flexors and hip extensor flexor motor neurons. As of yet, these connections have not been verified in the rat.

Actions of Streptomycin

Chronic intramuscular injection of STP reduces the EMG response to sudden free-fall without changes to the latency of the response. These changes occur concomitantly with otolith dependent behavioral changes such as disruption in swimming and air righting. These same types of changes, both EMG and behavioral, were seen in rats exposed to hyper-gravity (Fox et al., in press). Fox et al. speculated that these disruptions in behavioral responses may be due to changes in the otolith hair cells caused by changes in gravity. They further suggest that the reduced EMG response may be due to a reduction in gain in the vestibular component of the sensorimotor system. The demonstration that chronic treatment with STP which preferentially destroys the otolithic hair cells of the pigmented rat (Meza et al. (1996), leads to suppression of the FFR lends support to this position. The absence of a significant difference in the auditory startle response in the control and STP treated rats in this experiment also is consistent with normal auditory evoked response seen in rats treated with STP (Meza et al., 1996).

The findings in this experiment are in disagreement with results by Schaeppi, Krinke, FitzGerald, and Classen (1991), who reported that STP destroyed both the auditory and vestibular hair cells of the rat. There could be two explanations for this. First, Schaeppi et al. (1991) used the Sprague Dawley strain of rats rather than the Long Evans strain used in the present experiment. Second, Schaeppi et al. (1991), injected rat pups on postnatal days 11 to 22 when both the auditory and the vestibular hair cells are developing. In this experiment, injections began on postnatal day 21 after auditory and

semicircular canal hair cells are mature, but before utricular hair cells reach maturity (Dechesne, Mbiene, & Sans, 1986).

In addition to the ototoxic effects of STP, it is also known that STP has an effect at the neuromuscular junction via its effect on calcium metabolism or its acetylcholine-blocking properties (see Pittinger & Adamson, 1972 for a review). These properties may lead one to question whether the changes in the FFR were due to STP's ototoxic effects or its effects on the neuromuscular junction. Although the question cannot be answered unequivocally by the methods used in this experiment, two facts suggest that it was due to the ototoxic effect of STP. First, the animals were tested for FFR 24 hours after their last injection of STP. This is well over the half-life of STP (Fujita & Fujita, 1985). Therefore, the systemic level of STP would be low at the time of testing, while the destruction to the hair cells would remain essentially unchanged. Second, if the changes in the EMG response were primarily at the neuromuscular junction, one would expect there would be changes in the EMG in the auditory startle response between the control and treated animals. This did not occur. Thus, these findings suggest the changes in the FFR were due primarily to a change in the input at the otolith level.

While the decrease in amplitude in the EMG response may not be solely due to destruction of the otolith hair cells, it is clear this destruction plays a major role. It is also clear that the effect of STP can be detected in the EMG response at an earlier time than behavioral changes. Whether lack of behavioral changes are due to incomplete destruction of hair cells or the ability of the animal to adapt its behavior to the gradual destruction of hair cells via oculomotor and proprioceptive inputs is not known and needs to be addressed in future research. Additionally, the actions of STP at the neuromuscular junction in conjunction with hair cell destruction need to be quantified in future research. In spite of these questions, there is clear indication that chronic injections of STP do affect the FFR response in rats.

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San Jose State University
Institutional Animal Care and Use Committee

LETTER OF OFFICIAL PROTOCOL REVIEW

Date: April 13, 1997

Dear ROBERT A. FOX:

The animal care and use portion of your research proposal indicated below was reviewed by the Institutional Animal Care and Use Committee (IACUC). The IACUC Protocol Number indicated below should be used when ordering animals for this study, and on grant and contract proposals to fund this study. This protocol number may be used ONLY by the principal investigator and other participants included in the protocol. **The IACUC must be notified in writing of any proposed changes to this approved protocol, and approval must be granted in writing before any change is instituted.**

If you have any questions, please contact Dr. Miriam Saltmarch at **924-3118.**

APPROVAL FOR THE IACUC COMMITTEE

(JDM)

Miriam Saltmarch, Ph.D., Chair, IACUC

PROTOCOL #: 670

INVESTIGATOR NAME ROBERT A. FOX

TITLE OF PROTOCOL: BEHAVIORAL AND PHYSIOLOGICAL EFFECTS OF HAIR CELL DAMAGE IN RATS

Approval Date: APRIL 12, 1996 **Expiration Date:** MARCH 31, 1997

Species To Be Used: RATS **Total No. of Animals:** 48

Principal Investigator: ROBERT A. FOX

Department: Psychology **Phone:** 4-5652

Co-Investigator: N/A

XXXXX This application was approved without modification.

_____ This application was approved with the following mandatory changes:

_____

_____

_____ This application was **not** approved for the following reasons:

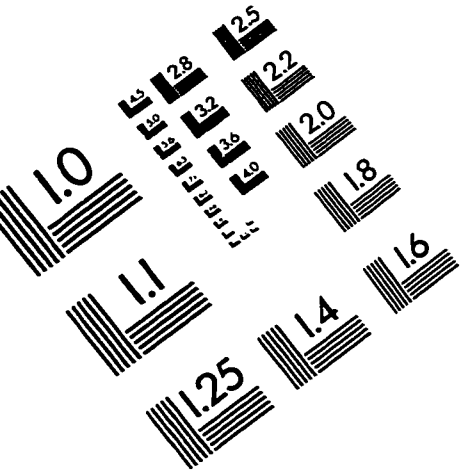
_____

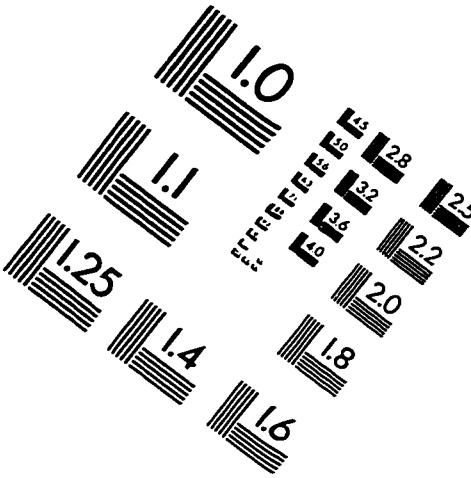
_____

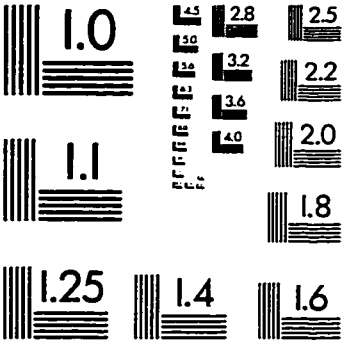
cc: University Animal Care Office
Chair: Investigator's Department, or Departmental Animal Committee

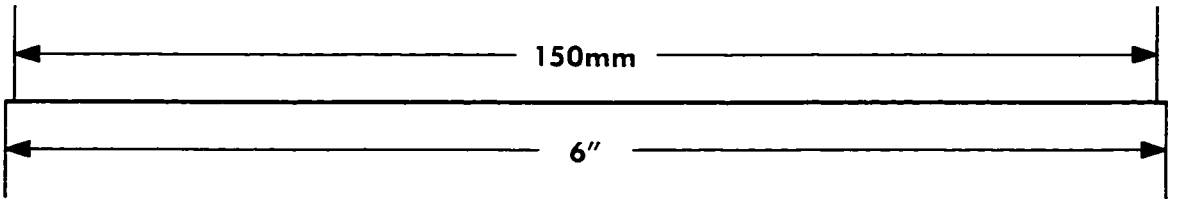
SJSU/UAC/1/96 rev.
jdm

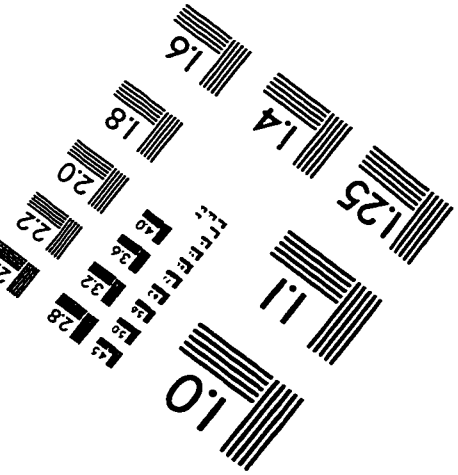
IMAGE EVALUATION
TEST TARGET (QA-3)

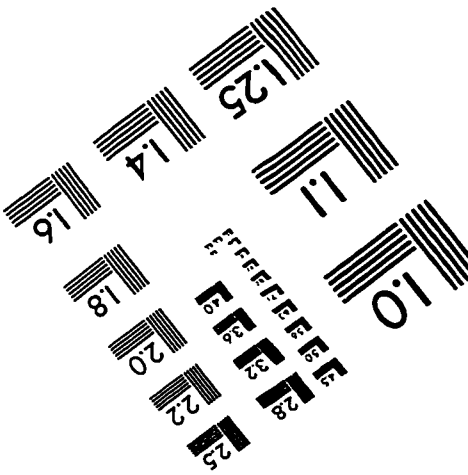












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