Recovery of Semicircular Canal Primary Afferent Activity in the Pigeon After Streptomycin Ototoxicity

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Li, Weidong and Manning J. Correia. Recovery of semicircular canal primary afferent activity in the pigeon after streptomycin ototoxicity. J. Neurophysiol. 80: 3297–3311, 1998. The electrophysiological activity of horizontal semicircular canal primary afferents (HSCPA) was investigated in vivo in the barbiturate-anesthetized pigeon by means of extracellular single-fiber vestibular nerve action potential recordings. The spontaneous and driven discharges to pulse (step/trapezoid waveform, peak velocity = 120°/s) and sum-of-sines (0.03, 0.09, 0.21, 0.39, 0.93, 1.83 Hz, peak velocity = 30°/s for each frequency) rotations were measured both in normal control animals and a group of animals at 30, 40, 50, 60, 71, and 150 days postinjection sequence (PIS) of streptomycin sulfate. Prior to 30 days PIS, the activity in the nerve was not appropriately modulated during and after rotation. At 30 days PIS and thereafter, the responses resembled those observed in control animals but with systematic changes in parameters of fitted pulse responses and fitted Bode plots as days PIS increased. The return of parameters characterizing the neural dynamics of the semicircular canals were monotonic and could be best described by either linear or exponential functions. After 30 days PIS, the mechanical cupula-endolymph system, the function of which can be inferred from the cupula long time constant (τL) following step velocity, did not change systematically (τL = 6.92 ± 3.96, 8.64 ± 5.52, 8.35 ± 4.21, 10.00 ± 2.79, 9.05 ± 3.67, 7.05 ± 2.72; means ± SD). However, the mean gain (G) of the HSCPA response to pulse rotation nearly doubled between 30 and 150 days PIS (from 1.31 ± 0.39 to 2.40 ± 1.04) and returned linearly to control values (G = 2.39 ± 0.77) over this period [G = 1.33 ± 0.009 (PIS = 30), R² = 0.92, P < 0.05]. Meanwhile, neural adaptation as quantified using a fractional operator, k, decayed exponentially (single exponential) to an asymptote. The time constant of this exponential was ~55 days [k = 0.034 ± 0.033 e^(-(PIS–30)/55.4), R² = 0.99, P < 0.01]. Features of the spontaneous discharge previously shown to be correlated with k changed appropriately. That is, the coefficient of variation (CV) and frequency of firing (FF) decayed and grew asymptotically, respectively. These parameters also exhibited an exponential time course of return to control values from 30 to 150 days PIS [CV = 0.44 ± 0.06 e^(-(PIS–30)/21.5), R² = 0.96, P < 0.01, and FF = 39.97 ± 101.42 (1 – e^-(PIS–30)/32.5), R² = 0.97, P < 0.01]. The trends of recovery for G, k, and τL derived from analysis of the pulse response were confirmed by strong positive correlations with best fitted parameters obtained from analysis of the sum-of-sines frequency domain response of HSCPAs. There were statistically significant correlations (r = 0.90, P < 0.05 and r = 0.93, P < 0.05) between parameters (G, k) derived from pulse responses and those (G’, k’) from sum-of-sines responses, respectively. The cupula time constant based on sum-of-sines data (τL) showed no statistically significant change between 30 and 150 days PIS (P > 0.05, analysis of variance). Thus the results in present study indicate that both the spontaneous discharge and the driven response to rotation of pigeon HSCPAs recovered their normal physiological status between 30 and 150 days PIS after hair cell death due to aminoglycoside ototoxicity. The recovery was systematic for the parameters chosen to be tested with the exception of the cupula long time constant, τL. The mechanisms (changes in ciliary dynamics, changes in hair cell ionic currents, changes in bouton terminals, etc.) underlying these changes await further morphophysiological studies.

INTRODUCTION

In recent years, it has been clearly documented that the sensory cells of the cochlea and vestibular apparatus in the avian inner ear regenerate spontaneously (Jorgensen and Mathiesen 1988; Kil et al. 1997), after loud sounds (Corwin and Cotanche 1988; Ryals and Rubel 1988,) and after aminoglycoside-induced death (Cruz et al. 1987; Hashino et al. 1992; Janas et al. 1995; Lippe et al. 1991; Weisleder and Rubel 1993). The regenerated hair cells develop typical anatomical features and eventually are reinnervated by neural terminals (Blumberg et al. 1997; Masetto and Correia 1997a; Ryals and Westbrook 1994). In the avian semicircular canals, after streptomycin ototoxicity, the type II hair cells reacquire basolateral ion channels and show the different ensemble of ionic currents for cells in different regions of the neuroepithelium as normal animals (Masetto and Correia 1997a,b). A number of studies (Girod et al. 1991; Hashino and Sokabe 1989; Marean et al. 1993, 1995; Norton and Rubel 1990; Tucci and Rubel 1990) have shown recovery of auditory function as indicated by responses to pure tones, otoacoustic emissions, brain-stem-evoked potentials, and behavior. Recovery of vestibular function also has been studied. Jones and Nelson (1992) reported that a sixfold decrease in the sensitivity of vestibular evoked potentials to linear horizontal stimulation immediately after ototoxicity. Thresholds of response returned within 2 wk, and the amplitudes and latencies of the components of the responses returned in 8–10 wk. Recovery of the vestibulococular reflex (VOR) also has been studied. Initially, the VOR gain was essentially zero as a result of streptomycin-induced hair cell loss. As hair cells regenerated, the VOR gain recovered to near normal values (Carey et al. 1996). Preliminary studies (Boyle et al. 1994) were carried out to study the recovery of primary afferent discharge during hair cells regeneration. It was concluded that anterior semicircular canal afferent spontaneous...
activity and responses to duct indentation were reestablished progressively after streptomycin ototoxicity. The purpose of this study was to extend those findings by quantifying the nature of the recovery of spontaneous afferent discharge and the transfer function relating head velocity and primary afferent discharge in the adult pigeon after streptomycin ototoxicity.

The transfer function relating angular head motion and primary afferent discharge has been studied extensively and in a number of species including: elasmobranchs (Ó Leary and Honrubia 1976), teleosts (Boyle and Highstein 1990; Hartmann and Klinke 1980; Hightstein et al. 1996; Rabbitt et al. 1995), amphibians (Honrubia et al. 1989; Precht et al. 1991), reptiles (Brichta and Goldberg 1996), birds (Anastasio et al. 1985; Dickman and Correia 1989a,b, 1993; Landolt and Correia 1980), rodents (Baird et al. 1988; Schneider and Anderson 1976; Yagi and Ueno 1988), felines (Anderson et al. 1978; Tomko et al. 1981), and primates (Correia et al. 1992; Fernández and Goldberg 1971; Keller 1976; Lysakowski et al. 1995). The transfer functions vary both qualitatively and quantitatively from species to species and in some cases from afferent to afferent, but they share several common features. First, all include a term that represents the motion of the cupula and endolymph (Van Egmond et al. 1949), and second, all include a term that describes neural adaptation (Thorsen and Bierdeman-Thorsen 1974). Differences in the regularity of action potential trains in spontaneously active vestibular afferents were initially quantified by Goldberg and Fernández (1971). The usual measure of regularity is the coefficient of variation (CV) of the firing rate corrected or uncorrected for the mean discharge rate (Goldberg and Fernández 1971). Parameters corresponding to the regularity of spontaneous discharge and the transfer function relating head velocity and rotational velocity have been correlated with the morphology of terminals synapsing with hair cells. Goldberg et al. (1992) concluded that in chinchilla, a bouton unit (bouton terminal innervation of type II hair cells) had a regular spontaneous discharge, supplied the peripheral portion of the crista, and had tonic (no gain enhancement and phase advance at high frequencies) transfer characteristics. Central dimorphs (terminals innervating type I and type II hair cells) were irregularly discharging and were phasic (gain enhancement and phase advancement at high frequencies), whereas peripheral dimorphs were regularly discharging and tonic. Calyx units (calyx terminals innervating type I hair cells) that supplied the center of the crista were the most irregularly firing and the most phasic of the afferent types. Calyx units had the lowest rotational gains at 2.0 Hz ($\pm 20^\circ$/s), combined with the other characteristics of irregularly firing units.

In the present study, we killed the hair cells in the pigeon semicircular canal neuroepithelia using a 10-day injection sequence of streptomycin sulfate. We then recorded spontaneous activity and responses to rotation from afferents innervating the horizontal semicircular canals (HSCPs) at different days postinjection sequence (PIS). We fitted a time domain and frequency domain representation of a transfer function that we have used in the past (Landolt and Correia 1980) to describe responses of pigeon primary afferents to rotation, and we have concluded that after 30 days PIS all but one of the parameters characterizing spontaneous and driven afferent responses systematically return toward values obtained from untreated animals.

METHODS

All procedures used in the studies reported herein were within the guidelines of humane animal experimentation as specified by the American Physiological Society and the National Institutes of Health. All procedures were approved in advance by the Animal Care and Use Committee of the University of Texas Medical Branch at Galveston.

Experimental subjects

The animals used in these experiments were adult white king pigeons (Columba livia) that ranged in weight between 400 and 550 g. Twenty-nine pigeons that were injected with streptomycin sulfate (Pfizer, 400 mg/ml) provided useful data, which are presented herein. A group of animals of comparable ages ($n=22$) served as normal controls. A previous study (Masetto and Correia 1997c) determined that the maximum dose over the minimum time period that killed almost all hair cells was 250 mg·kg$^{-1}$·day$^{-1}$ injected intramuscularly into the breast muscle for 10 consecutive days.

Surgical preparation

In addition to ear bars and a beak clamp, supplementary head restraint and chronic access to the vestibular nerve in each animal was achieved by a surgical procedure quite similar to that developed for alert pigeon experiments (Anastasio et al. 1985) with several modifications as described in the following text. Under deep barbiturate anesthesia, slots were cut in the skull behind the eyes and two stainless steel screws (No. 2-56, 6 mm), the heads of which had been machined flat, were inverted and inserted in the slots between the dura and the cranium and secured with nylon nuts. Near the front of the skull and at the base of the beak, a third stainless steel screw (No. 2-56, 8.5 mm) was tapped into a naturally occurring triangular pocket of bone. This three-point triangular support was bridged by dental acrylic into which was placed a stud that fit into a head holder mounted on the stereotaxic frame. This arrangement facilitated accurate reinstallation of the animal into the stereotaxic apparatus. The stereotaxic coordinates of a mark on the skull cap could be repeatedly achieved over days with an accuracy to within 100 $\mu$m. A hole was trephined in the left side of the skull at stereotaxic coordinates previously determined to intersect the vestibular nerve (see next paragraph). A small piece (11 mm in length) of premachined nylon tube (6 mm OD) was placed over the hole in the cranium and secured to the anchoring screws using dental acrylic. The nylon tube was sealed between recording sessions.

Before beginning the chronic experiments on streptomycin-treated animals, we conducted a series of acute experiments on a large group (~50 animals) of pigeons. We used the electrode trajectory that we (Dickman and Correia 1993) previously had confirmed histologically as entering the vestibular nerve. This trajectory produced positive-going action potentials, spontaneous discharge with regularly and irregularly firing units and units that increased discharge on ipsilateral horizontal rotation (toward the nerve from which recordings were made), and decreased discharge on contralateral horizontal rotation (Anastasio et al. 1985; Dickman and Correia 1989a,b, 1993). These features of the response are characteristic of vestibular primary afferent recordings. The stereotaxic coordinates of the point of entry of the electrode into the skull were recorded. Based on these coordinates, a series of animals were tested, and their data comprise the control data. These animals did not receive streptomycin. One animal provided data for both
shaped action potential pulses and an analog representation of a and barbiturate-anesthetized pigeons (Correia and Landolt 1977; Correia et al. 1992) generated the stimulus waveforms, CV. We have used these measures in the past to characterize the spontaneous firing from alert pigeons (Anastasio et al. 1985), controlled the DC torque motor (30 ft-lb) of the rotator, acquired shaped action potential pulses and an analog representation of a rate gyroscope (Watson Industries).

### Table 1. Animals, neurons, and observations that comprise the means presented in Table 2

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Animals Repeatedly Tested*</th>
<th>Number of Neurons</th>
<th>Spontaneous discharge</th>
<th>Driven discharge</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 days PIS</td>
<td>9</td>
<td>6</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>40 days PIS</td>
<td>11</td>
<td>5</td>
<td>19</td>
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<td>35</td>
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</tr>
<tr>
<td>60 days PIS</td>
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<td>5</td>
<td>35</td>
<td>17</td>
</tr>
<tr>
<td>71 days PIS</td>
<td>8</td>
<td>8</td>
<td>35</td>
<td>17</td>
</tr>
<tr>
<td>150 days PIS</td>
<td>13</td>
<td>8</td>
<td>55</td>
<td>20</td>
</tr>
<tr>
<td>Control</td>
<td>22</td>
<td>1</td>
<td>48</td>
<td>75</td>
</tr>
</tbody>
</table>

**SS**, sum of sines; PIS, postinjection sequence. *Animals repeatedly tested in this and other time windows and giving usable data.

the streptomycin and control groups (served as his own control). There were no differences between the responses of this animal as a control and the other members of the control group.

### Time windows

Recordings were obtained from treated animals in the windows 30, 40, 50, 60, 71, or 150 days PIS. A summary of recordings made in each of these windows is presented in Table 1. Eight animals were tested in each of two windows, two animals were tested in each of three windows, two animals were tested in each of four windows, and one animal was tested in five windows. Additional animals were tested in only one window to bring the groups up to 9 animals in the 30-day group, 11 animals in the 40-day group, 10 animals in the 50-day group, 6 animals in the 60-day group, 8 animals in the 71-day group, and 13 animals in the 150-day group (PIS intervals ranging from 110 to 177 days, mean = 150 days PIS).

### Experimental procedure

Before each test session, the pigeon was anesthetized initially with an injection of pentobarbital sodium (10–15 mg/kg) into the medial wing vein. Supplementary injections of ketamine hydrochloride (10–20 mg/kg) were given intramuscularly as needed throughout the experiment. The unconscious animal then was placed in a body-support system attached to a stereotaxic device, and its head was secured in the stereotaxic device via the head-restraint system and the ear and beak bars. The major plane of the horizontal semicircular canals was coplanar to an earth horizontal plane. The stereotaxic device was securely mounted onto a Contra-Ves Goertz rotator (model 823), with the animal’s head centered on the axis of rotation. The stereotaxic frame was enclosed in a gimbal system so that the animal could be statically tilted to any angle between ±90° in either pitch or roll. Before recording, the cap over the recording well was removed. A stainless steel guide tube (26 gauge) containing a tungsten microelectrode (see following text) then was advanced through the cortex and optic tectum to a position ~2 mm above the vestibular nerve. The microelectrode was advanced further into the nerve from inside the guide tube with a hydraulic microdrive. Receptor of origin of individual SCPAs was determined by a series of manual yaw, pitch, and roll rotations and tilt (Blanks and Precht 1976; Estes et al. 1975; Perachio and Correia 1983). A suite of C ++ language computer programs (Correia et al. 1992) generated the stimulus waveforms, controlled the DC torque motor (30 ft-lb) of the rotator, acquired shaped action potential pulses and an analog representation of a rate gyroscope (Watson Industries).

### Test protocols

The test protocols included 1) spontaneous activity—recordings were made for 30 s with the rotator stationary. 2) Step or trapezoid test—a yaw velocity pulse rotation was delivered. Then after a 60-s interpulse interval, an identical pulse rotation but in the opposite direction was delivered. The steps were from zero to a constant peak velocity of ±120°/s in 120 ms, and the trapezoids were to the same velocity in 250 ms, each pulse had a duration of 60 s. No statistical difference was noted between the response of the steps and fast trapezoids and the responses were pooled. 3) sum-of-sines (SSs) test—three cycles of yaw rotation, consisting of the sum of six frequencies, containing a base frequency and five odd prime multiples of the base frequency (0.03, 0.09, 0.21, 0.39, 0.93, and 1.83 Hz), were delivered. The peak amplitude of each frequency was 30°/s. The three cycles were combined into one cycle histogram. For all protocols, extracellular action potentials were first amplified and filtered (band-pass 300–10,000 Hz) using a Grass preamplifier (model P-15) before being passed across the rotator slip rings. Once amplified, the neural signal was displayed simultaneously on a Tektronix oscilloscope, passed through an audio monitor, and sent to a Bak Instruments (model DIS-1) time-amplitude window discriminator, the output of which was displayed on the oscilloscope. Action potentials from isolated single afferent fibers then were identified by using consistent properties of spike amplitude, spike duration, and spike waveform. The action potential train and stimulus reference signal (rate gyroscope signal) from the rotator were taped using an Instrutech model VR-100A 8-channel digital recorder connected to a Sharp VCR. The rate gyroscope was directly coupled to the stereotaxic device, which in turn was directly coupled to the rotator, so its signal reflected head velocity. The suite of above-mentioned programs used a CED (Cambridge Electronic Design) 1401 interface, and a PC/80386. The programs acquired the action potential shaped pulses and stimulus reference signals; stored them in files; and produced cycle (C), peristimulus time (PST), and interspike interval (ISI) histograms on-line. The programs contained special weighted curve-fitting algorithms that during calibration permitted the fitting of sinusoidal cycle histograms from electronically generated pulse trains that were at worst 50% rectified with a phase error of <1° and an amplitude error of <1%.

### Data analysis

The measures we used to characterize the spontaneous firing rates of HSCPA fibers were mean frequency of firing (FF) and CV. We have used these measures in the past to characterize the spontaneous discharge from alert pigeons (Anastasio et al. 1985), and barbiturate-anesthetized pigeons (Correia and Landolt 1977; Dickman and Correia 1989a,b). Two responses (usually replicated...
once) to pulse rotations were obtained for most of afferents tested. These responses were an increase in firing during ipsilateral rotation and a decrease in firing during contralateral rotation. We quantified parameters of the PST response to pulse rotations by fitting each of the four responses to pulse rotation with a model previously used (Landolt and Correia 1980) to describe the transfer characteristics of the HSCPA fibers to pulse rotations. The model contains a gain term (G), a term to express response baseline (DC), and terms that describe the cupula long time constant (τ_C) and neural adaptation (k). The model is described by the following equation

\[ r(t) = (G/t^t) \left[ \gamma(-k, -t/\tau_C)e^{kt} \right] + DC \] (1)

where \( \gamma(a, t) = \left[ \Gamma(a)/\Gamma(a) \right] \int\, x^a e^{-x} \, dx = \) incomplete gamma function (which is single-valued and finite in terms of \( a \) and \( t \)); \( k \) is a parameter that characterizes neural adaptation; \( G \) is the gain and \( DC \) is the resting firing level.

The measures used to characterize the single-unit response to passive sum-of-sines rotation were gain and phase. They were obtained by fitting each of the component frequencies of a folded binned cycle histogram (based on 3 cycles) and then comparing this response to a best-fitted curve of the rate gyrocope voltage trace. Gain was calculated by dividing the peak amplitude of the best-fitted sine curve to the cycle histogram by the peak amplitude of the best-fitted curve of rate gyro voltage (head velocity). Phase was calculated as the difference angle between the peak amplitudes of the fitted functions. Positive phase angles represent a phase lead of the best-fit amplitude of the binned response relative the velocity trace (rate gyro voltage). Gain and phase values were used to calculate the frequency domain equivalent of Eq. 1

\[ H(s) = G's^{a+1}(\tau_C^2s + 1)^{-1} \] (2)

Where \( G' \) represents the frequency independent gain; \( s = \tau_C^2s + 1, \) an operator describing neuronal adaptation and \( \tau_C, \) the viscoelastic (long) time constant of the cupula-endolymph system (Anastasio et al. 1985; Landolt and Correia 1980). The terms in Eq. 2 that have corresponding terms in Eq. 1 are primed for clarity.

All parameters describing the spontaneous activity and those derived from Eqs. 1 and 2 were grouped according to PIS day and animals are presented in the legends. An example of the equation given in the figure legend. An appropriate was successfully used subsequently at 30 days PIS. But we were unable to record action potentials with modulated responses. At 30 days PIS, in some cases (4/12 neurons), we observed spontaneous activity with firing patterns that were in bursts of doublets of action potentials. The ISI histogram for this activity was multimodal. In the other cases, the histograms of the spontaneous activity were generally unimodal as illustrated in Fig. 2A (top). In all cases, the response to a pulse of rotation was a momentary increase or decrease in firing rate (Fig. 2A, bottom). The firing rate increased during ipsilateral horizontal rotation (toward the nerve from which recordings were made) and decreased during contralateral horizontal rotation, respectively. Additionally, at 30 days PIS, 4/12 neurons exhibited a bursting response to pulse rotation (see Fig. 4). As time progressed from 30 to 150 days PIS, the responses more closely resembled those observed in control animals but with systematic changes in parameters of the fitted time and frequency domain representations of the transfer function.

**Spontaneous discharge**

The FF and CV were calculated for each neuron. Histograms illustrating the distribution of CVs for control and 150 days PIS groups are presented in Fig. 1, A and B, respectively. The FF for HSCPA in the control group varied from 18.6 to 179.2 spikes/s, with an average for all 48 recordings of 108.4 ± 6.4 (mean ± SE). The CV for the control group ranged between 0.06 and 0.98, with a mean CV for all the best-fitted sine curve to the cycle histogram by the peak amplitude of the best-fitted curve of rate gyro voltage (head velocity). Phase was calculated as the difference angle between the peak amplitudes of the fitted functions. Positive phase angles represent a phase lead of the best-fit amplitude of the binned response relative to the velocity trace (rate gyro voltage). Gain and phase values were used to calculate the frequency domain equivalent of Eq. 1

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Where \( G' \) represents the frequency independent gain; \( s = \tau_C^2s + 1, \) an operator describing neuronal adaptation and \( \tau_C, \) the viscoelastic (long) time constant of the cupula-endolymph system (Anastasio et al. 1985; Landolt and Correia 1977; Dickman and Correia 1989a). These CVs binned to the left of the first arrow would be considered regularly firing and those to the right of the second arrow would be considered irregularly firing. Those CVs binned between the first and second arrows would be considered as firing with intermediate regularity. The percentage of afferents in each of the three categories is presented in parentheses in the legends. An example of ISI histograms for 30, 71, and 150 days PIS and for control animals are presented in the top panel of each of the components of Fig. 2 (A–D). The histogram examples were chosen to illustrate the fact that as PIS days increase, the firing rate rises exponentially to an asymptote and the CV falls exponentially to an asymptote. These trends are demonstrated in Fig. 3, A and B, respectively. In these and subsequent graphs, the error bars represent ±1 SE, and the numbers in parentheses represent the number of observations that contributed to the mean. The line drawn through the points represents the best-fit curve resulting from evaluation of the equation given in the figure legend. An appropriateness of the model statistic, the coefficient of determination (\( R^2 \)), and an associated probability value also are presented in the legend. In all cases a linear and an exponential model were evaluated, and the model with the \( R^2 \) closest to 1.0 is presented. The FF grows asymptotically with an exponential time constant of \( \sim 33 \) days PIS (Fig. 3A), whereas the CV decays asymptotically with an exponential time constant of \( \sim 22 \) days PIS. It appears that both the FF and CV curves are beginning to asymptote at 150 days PIS. But the control values for FF and CV are statistically significantly different from those obtained at 150 days PIS. This difference is likely due to the fact that the sample of spontaneous discharge from HSCPA in the control group contained a greater number ( \( \sim 20\% \) more) of irregularly firing afferents (cf. Fig. 1,
A and B. Irregularly firing afferents in pigeon (e.g., Dickman and Correia 1989a), as in most other species, have a lower mean firing rate and a larger CV. Thus parameters reflecting the spontaneous discharge characteristics systematically change from 30 to 150 days PIS. The FF rises and the CV falls exponentially to an asymptote.

Driven discharge

PULSE STIMULATION (STEPS/TRAPEZOIDS). Figure 4 is a photograph of modulation of action potentials from a single HSCPA during a pulse rotation. This response was obtained from an animal 30 days PIS. Even though the response contains bursts of action potentials during excitation, it demonstrates that beginning at 30 days PIS appropriately directed responses could be obtained. That is, ipsilateral rotation (Fig. 4A) produced an increase in discharge rate, whereas contralateral rotation produced an inhibition in response (Fig. 4B). Figure 2A is a PST histogram of this afferent’s response. It can be noted that when compared with HSCPA pulse responses from animals at 71 days PIS (Fig. 2B), 150 days PIS (Fig. 2C), and from the control group (Fig. 2D), the response is less uniform in appearance, has a lower peak amplitude, and has more asymmetry between the excitatory and the inhibitory phases.

A plot of the time course of recovery of the pulse gain (amplitude $\cdot 120^\circ \cdot s^{-1}$) as quantitated by the gain term ($G$) in Eq. 1 is presented in Fig. 5A. It can be noted that the rate of recovery of the gain of the pulse response is nearly linear between 30 and 150 days PIS. At 150 days PIS, the value of the gain is not statistically significantly different from that of control values. Neural adaptation, as quantitated by the $k$ term in Eq. 1, decayed exponentially (single exponential) to an asymptote. The time constant of the exponential was $\sim 55$ days. After a delay of about seven time constants ($\sim 1$ yr), it is presumed that the value of $k$ would approach the control values.

Interestingly, there was not a long-term systematic trend of change of the parameter ($\tau_L$), which describes the cupula time constant in Eq. 1, as a function of PIS. Rather, the mean values for the time constants varied between 6 and 11 s over the time course from 30 to 150 days PIS. At 150 days PIS, the time constant value was not statistically significantly different from that of the control group. The best-fit straight line over the range from 30 to 150 days PIS also is presented in Fig. 5C. It can be noticed that the slope of this line is close to 0. This lack of change in the parameter representing the cupula time constant between 30 and 150 days PIS was confirmed by analysis of the sum-of-sines frequency domain response of HSCPAs (see below). Figure 5D is the plot of $\tau'_L$ derived from evaluation of Eq. 2 based on sum-of-sines responses versus days PIS. It can be noticed in this figure that the range of mean values of $\tau'_L$, similar to those of $\tau_L$ (Fig. 5D), is quite narrow and

![Distribution of coefficient of variation (CV) for horizontal semicircular canal primary afferents (HSCPA) fibers](image-url)
essential constant over the range from 30 to 150 days PIS. There was no statistically significant difference in these values (ANOVA).

**SUM-OF-SINES STIMULATION.** Other best-fit parameters of the transfer function described by Eq. 2 were calculated, and they, along with the best-fit parameters for the pulse response and spontaneous discharge, are summarized in Table 2.

The sum-of-sines parameters were calculated by comparing the binned cycle histogram response of individual HSCPs to the rate gyro signal, deriving gain and phase values, and then fitting Eq. 2 to the corresponding frequency response functions. Figure 6 illustrates, for a HSCPA from a 30-day-PIS animal, an ISI histogram (top), a binned cycle histogram of a rate gyro voltage response (middle), and a binned cycle histogram of the action potential response (bottom). Figure 7 presents Bode plots for individual afferent responses for the 30-day-PIS group (A), the 71-day-PIS group (B), the 150-day-PIS group (C), and the control group (D). The mean (±SD) gain and phase are indicated in red. Equation 2, with best-fit parameters and the goodness of fit estimate ($R^2$) also are presented in each panel. The curves representing evaluation of the equation through mean data are indicated by bold green lines in each panel. First, as has been clearly documented in the past (Dickman and Correia 1989b), it can be observed that afferents in all groups show individual variability in their gain and phase response. Second, it can be noted that in all PIS groups,
there appears to be a slight mean gain enhancement and slight mean phase advance for the two highest frequencies. However, when an alternative transfer function containing an additional zero \( \tau s + 1 \) (Fernández and Goldberg 1971) was fitted to these data, no significant improvement in the \( R^2 \) was noted. Third, it can be noted that the mean phase advance at the lowest frequency decreased (except for values at 71 and at 150 days PIS, which are roughly equal), the asymptote of the phase advance at higher frequencies become less positive (less lead with regard to velocity) and the linear slope of the gain decreases as days PIS increase. These three features suggest that the neural adaptation parameter \( k' \) is greater for the 30-day-PIS group (Thorson and Biederman-Thorson 1974) and decreases to an asymptote as

FIG. 3. Time course of recovery of parameters of spontaneous discharge of HSCPAs as a function of PIS days. A: best-fit curve (---) for frequency of firing (FF). B: best-fit curve (-----) for CV. In these and subsequent figures, symbols and error bars represent means ± SE, and the number in each parenthesis above the error bar is the number of observations comprising the mean. Also the nonlinear equation (with best-fit coefficients) is given as is a goodness-of-fit estimate, the coefficient of determination, \( R^2 = 1 - \frac{SSE}{SSM} \); where SSE = error sum of squares and SSM = mean sum of squares. Corresponding probability level derived from an \( F \) statistic also is given. \( F = \frac{\text{mean square of the regression}}{\text{the mean square error}} \). Degrees of freedom = number of fitted coefficients, number of mean data points.
days PIS increase. The best-fit parameters for $k'$ decrease as days PIS increase as demonstrated by values of $k'$ in the figure legends and by the statistically significant correlation of the best-fit parameters of $k$ derived from pulse response ($k$) and sum-of-sines responses ($k'$; Fig. 8A). Also it can be noted as for the pulse gain, $G$, the best-fit parameter for the frequency independent gain ($G'$) in equations in Fig. 7, increase as days PIS increase. A statistically significant correlation between $G$ and $G'$ is illustrated in Fig. 8B.

Thus both frequency and time domain measures of the response to rotation support the same conclusion that while the cupula–endolymph mechanical time constant ($\tau_m$) does not change between 30 and 150 days PIS, the gain of the response increases and an operator representing neural adaptation decays to control levels by 150 days PIS.

**Discussion**

The results of the present study provide convincing evidence that signals carried by avian horizontal semicircular canal primary afferents recover their usual electrophysiological function in a systemic fashion during and after the reinnervation of regenerating hair cells damaged by aminoglycoside ototoxicity. More precisely, the parameters that characterize the mean firing rate (FF) and irregularity of spontaneous discharge ($CV$) grow and decay to an asymptote, respectively, in an exponential fashion (Fig. 3, A and B) with time constants of $\sim 33$ and 22 days, respectively, between 30 and 150 days PIS. The gain of pulse and sum of sinusoidal rotational responses returns almost linearly between 30 and 150 days PIS, and an indicator of neural adaptation, the $k$ operator, in the pulse and sum of sinusoidal responses decreases during this time period. Interestingly, the time constant that characterizes the mechanical properties cupula-endolymph system does not appear to change systematically after 30 days PIS.

Although it is difficult to say exactly when normal neural activity reappeared, we could only record spontaneous discharge and appropriately directed afferent responses at 30 days PIS. Repeated attempts at 20 and 25 days PIS failed. It has been reported (Baird et al. 1996) that in frog, hair cells identified by short well-formed bundles, appear as early as 1–2 days after gentamicin treatment, and extensive restoration of hair bundle density in the saccule and utricle occurs by 7–9 days after treatment. Weisleder et al. (1995) reported the presence of type II hair cells with identifiable cuticular plates and stereociliary bundles in 5- and 10-day survival chicks. Lopez et al. (1997) noticed the beginning of anatomic recovery of type II hair cells at 28 days postinjection of gentamicin in the crista of mammals. Masetto and Correia (1997a) reported the return of stereocilia and a normal complement of basolateral ion channels in type II hair cells in the pigeon by 21 days after streptomycin treatment. Afferent and efferent reinnervation of chick basilar papilla hair cells has been reported to be as early as days 1–7 after treatment (Duckert and Rubel 1993). However, Blumberg et al. (1997), studying the pigeon semicircular canals and using the same ototoxic methods as used in this paper, noticed that at 5 days PIS, few nerve fibers were present in the neuromast of the cupula–endolymph system does not appear to change systematically after 30 days PIS.

The gross change in the response dynamics to rotation as
a function of days PIS is best illustrated by comparing the Bode plots and best-fit equations in Fig. 7. First, it can be noticed from the best-fit equations, that the frequency-independent gain increases from 4 (Fig. 7A) to 7 (Fig. 7C) as days PIS increase from 30 to 150. Second, it can be noticed that the slope of the mean gain decreases as PIS day increases (cf. Fig. 7, A and C). Also the phase lead at 0.03 Hz and the phase lead across all frequencies generally become less as days PIS increase. These characteristics of gain and phase are indicative of a decrease in neural adaptation (Thorson and Biederman-Thorson 1974) as confirmed by the decrease in the best-fit value of \( k' \) from 0.38 at 30
days to 0.19 at 150 days PIS. Similar changes in comparable mean parameters of the pulse rotation response can be noted by comparing correlations of $k$ and $k'$ in Fig. 8A and $G$ and $G'$ in Fig. 8B and plots of $\tau_L$ and $\tau'_L$ in Fig. 5, C and D, respectively.

The mean gain of the HSCPA response to rotation nearly doubles between 30 and 150 days. The gain returns to control values (Fig. 5A) almost linearly over this time period. The gain derived from pulse rotations and sum-of-sines rotations is correlated (Fig. 8A), implying that the same linear relationship of gain increase as a function of days PIS is probably amplitude independent because the peak rotational velocity
TABLE 2. Summary for each parameter for different days PIS and the control group

<table>
<thead>
<tr>
<th>Conditions</th>
<th>n</th>
<th>ISI (ms)</th>
<th>CV (%)</th>
<th>FF (pps)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 days PIS</td>
<td>13</td>
<td>31.59 ± 23.28</td>
<td>1.12 ± 1.08</td>
<td>45.27 ± 23.85</td>
</tr>
<tr>
<td>40 days PIS</td>
<td>19</td>
<td>28.67 ± 48.98</td>
<td>0.78 ± 0.45</td>
<td>57.41 ± 37.64</td>
</tr>
<tr>
<td>50 days PIS</td>
<td>35</td>
<td>28.85 ± 51.28</td>
<td>0.70 ± 0.52</td>
<td>82.86 ± 54.59</td>
</tr>
<tr>
<td>60 days PIS</td>
<td>23</td>
<td>13.23 ± 11.21</td>
<td>0.68 ± 0.43</td>
<td>106.69 ± 54.33</td>
</tr>
<tr>
<td>71 days PIS</td>
<td>19</td>
<td>10.70 ± 5.43</td>
<td>0.51 ± 0.28</td>
<td>116.54 ± 55.44</td>
</tr>
<tr>
<td>150 days PIS</td>
<td>55</td>
<td>8.57 ± 3.69</td>
<td>0.43 ± 0.25</td>
<td>136.63 ± 52.30</td>
</tr>
<tr>
<td>Control</td>
<td>48</td>
<td>12.60 ± 8.15</td>
<td>0.56 ± 0.24</td>
<td>108.93 ± 44.09</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Conditions</th>
<th>n</th>
<th>( r_c ) (ms)</th>
<th>Gain ((G))</th>
<th>( k )</th>
<th>DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 days PIS</td>
<td>23</td>
<td>6.92 ± 3.96</td>
<td>1.31 ± 0.39</td>
<td>0.37 ± 0.21</td>
<td>59.99 ± 27.06</td>
</tr>
<tr>
<td>40 days PIS</td>
<td>32</td>
<td>8.64 ± 5.52</td>
<td>1.36 ± 0.71</td>
<td>0.29 ± 0.24</td>
<td>66.55 ± 37.94</td>
</tr>
<tr>
<td>50 days PIS</td>
<td>56</td>
<td>8.35 ± 4.21</td>
<td>1.39 ± 0.74</td>
<td>0.25 ± 0.17</td>
<td>100.00 ± 58.53</td>
</tr>
<tr>
<td>60 days PIS</td>
<td>39</td>
<td>10.00 ± 2.79</td>
<td>1.82 ± 0.82</td>
<td>0.24 ± 0.17</td>
<td>121.27 ± 44.75</td>
</tr>
<tr>
<td>71 days PIS</td>
<td>33</td>
<td>9.05 ± 3.67</td>
<td>1.73 ± 0.79</td>
<td>0.19 ± 0.09</td>
<td>129.87 ± 53.35</td>
</tr>
<tr>
<td>150 days PIS</td>
<td>93</td>
<td>7.05 ± 2.72</td>
<td>2.40 ± 1.04</td>
<td>0.07 ± 0.05</td>
<td>125.90 ± 46.81</td>
</tr>
<tr>
<td>Control</td>
<td>77</td>
<td>6.59 ± 2.85</td>
<td>2.39 ± 0.77</td>
<td>0.05 ± 0.05</td>
<td>118.76 ± 48.37</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Conditions</th>
<th>n</th>
<th>( r'_c ) (ms)</th>
<th>Gain ((G'))</th>
<th>( k' )</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 days PIS</td>
<td>8</td>
<td>5.53 ± 0.69</td>
<td>3.93 ± 4.46</td>
<td>0.38 ± 0.02</td>
</tr>
<tr>
<td>40 days PIS</td>
<td>9</td>
<td>5.82 ± 0.52</td>
<td>4.82 ± 3.6</td>
<td>0.35 ± 0.01</td>
</tr>
<tr>
<td>50 days PIS</td>
<td>14</td>
<td>5.15 ± 0.54</td>
<td>3.77 ± 3.66</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td>60 days PIS</td>
<td>6</td>
<td>5.87 ± 0.75</td>
<td>5.43 ± 5.21</td>
<td>0.33 ± 0.02</td>
</tr>
<tr>
<td>71 days PIS</td>
<td>5</td>
<td>6.60 ± 0.50</td>
<td>6.40 ± 3.90</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>150 days PIS</td>
<td>26</td>
<td>5.74 ± 0.50</td>
<td>7.28 ± 4.31</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td>Control</td>
<td>35</td>
<td>8.50 ± 1.03</td>
<td>10.03 ± 7.91</td>
<td>0.19 ± 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SD; \( n \), number of observations; ISI, interspike interval; CV, coefficient of variation; FF, frequency of firing; \( r_c \), cupula long time constant; DC, response baseline.

at any frequency of the sum-of-sines was 30°/s, whereas the peak velocity of the pulse rotations was 120°/s. The underlying cause of the gain increase is unknown, but it may be that as regeneration and reinnervation proceeded and as the number of single and multiple type I hair cells increased, the proportion of dimorph terminals increased relative to the bouton only terminals and therefore the probability of recording from a more sensitive afferent increased.

The fact that the cupula time constant remained essentially the same and close to control values during testing from 30 to 150 days PIS (Fig. 5, C and D) suggests that after 30 days there was little change in the mechanical properties of the cupula–endolymph system and the stereocilia–cupula interface after the stereocilia of new regenerated hair cells were reinserted in the cupula.

However, because the neural adaptation operator, \( k \), changed (decreased) as days PIS increased, it is probable that the electrical properties of either the transduction process (hair cells/
ciliary bundle unit), the ionic currents in the hair cells, the hair cell-primary afferent synapse and/or the primary afferent terminals continually changed during 30–150 days PIS. These changes could occur at the neural links that have been inferred or shown to contribute to adaptation. These include the hair cell mechanoelectrical transduction process (Eatock et al. 1987; Ricci and Fettiplace 1997), the hair cell basolateral ionic currents (Masetto et al. 1994, 1995), the hair cell-primary afferent synapse (Ross 1994), and/or as a result of changes in postsynaptic potentials on the unmyelinated terminals of the primary afferents in the neuroepithelium (Taglietti et al. 1977). Each of these links in the transduction chain have been shown to be plastic or contribute a relaxation process (or a high-pass filter) (Landolt and Correia 1980), which when taken together could form a spatially distributed series of relaxation processes thought to underlie the neural adaptation operator (Thorson and Biederman-Thorson 1974). This operator has been used frequently to describe the adaptive properties of the semicircular canal primary afferent response in rhesus monkeys (Correia et al. 1981, 1992), rodents (Baird et al. 1988; Schneider and Anderson 1976), and birds (Dickman and Correia 1989a; Landolt and Correia 1980).
In conclusion, the data presented herein show that both the spontaneous discharge and the driven response to rotation of pigeon horizontal semicircular canal primary (HSCPA) afferents recover after hair cell death due to aminoglycoside ototoxicity. Normal responses are delayed for ~30 days, and the return to control values is nearly complete in 150 days. The return of parameters characterizing the responses is monotonic and can be described by a linear or an exponential function. By curve fitting functions previously shown to characterize the HSCPA response and using the resulting mean best-fit parameters, statements can be made about the recovery of elements that contribute potentials to the primary afferent response. It appears that after 30 days PIS, the cupula-endolymph system, the function of which is inferred from the cupula long-time constant, $\tau_L$, does not change, but the gain of the response and the neural adaptation of the response do change. The gain increases and the neural adaptation operator, $k$, decreases. Parameters of the spontaneous discharge previously shown to correlate with $k$ change appropriately. That is, the coefficient of variation and frequency of firing decrease and increase correspondingly. Whether these changes reflect reconstruction of the afferent terminals or molecular changes in the transduction and synaptic connections await further intra-axonal dye injection morphophysiological studies.

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