"A Cochlear Nucleus Auditory prosthesis based on microstimulation"

Contract No. NO1-DC-8-2102
QUARTERLY PROGRESS REPORT #2
Oct 1, 1998 - Dec 31, 1998

HUNTINGTON MEDICAL RESEARCH INSTITUTES
NEUROLOGICAL RESEARCH LABORATORY
734 Fairmount Avenue
Pasadena, California 91105

D.B. McCreery, Ph.D.
W.F. Agnew, Ph.D.
T.G.H. Yuen, Ph.D.
L.A. Bullara, B.S.

HOUSE EAR INSTITUTE
2100 WEST THIRD STREET
Los Angeles, California 90057

Robert Shannon, Ph.D
Jean Moore, Ph.D
Steve Otto. M.S.

ABSTRACT AND SUMMARY

These studies are a continuation of our program to determine safe and effective stimulus parameters for an auditory prosthesis based on multisite microstimulation in the ventral cochlear nucleus. We examined the physiologic and histologic effects of prolonged microstimulation with chronically-implanted iridium microelectrodes in the posteroventral cochlear nucleus (PVCN) of cats, using stimulus pulses of relatively high amplitude (up to 63 μA) and short duration (40 μs/ph). The arrays of 3 or 4 iridium microelectrodes were pulsed in the interleaved mode, with controlled-current pulses, at a rate of 250 Hz per electrode, for 7 hours per day on 3-16 successive days. In most experiments, the amplitude of the stimulus pulses was modulated according to the logarithmically compressed artificial voice signal. However, in two animals, the microelectrodes were pulsed continuously at 250 Hz and at 63 μA.

In the protocols in which the stimulus was modulated by the artificial voice signal, the study was executed in two phases separated by at least 26 days. In Phase 1, the stimulus pulse duration was 150 μs/ph (as in our previous studies) and the artificial voice was allowed to modulate the stimulus pulse amplitudes across the range of 6-20 μA, a protocol which, in previous studies, has been shown not to induce excessive depression of neuronal excitability, and not to produce histologically-detectable changes in the PVCN. In Phase 2, the 7-hour stimulations were conducted using a stimulus pulse duration of 40 μs/ph and the artificial voice was allowed to modulate the pulse amplitudes over a range of amplitudes that was selected for each individual animal, so as to duplicate the amount of neural activity induced by the 150 μs pulses. This "neural excitability scaling factor" (NESF) ranged from 2.2 to 3, in different animals. This is less than the value of 3.75 which would be expected on the basis of a 1:1 correspondence between charge per phase and neural activity, and it demonstrates that the short-duration pulses are relatively more efficient from the standpoint of charge per phase. At the end of Phase 2, the animals were sacrificed for histologic evaluation of the electrode sites in the PVCN.

In two animals (CN119 and CN131), in which the NESF was 2.2 to 2.4, the amount of long-lasting stimulation-induced depression of neuronal excitability (SIDNE) was very similar in both phases of the study (the amount of SIDNE was essentially independent of the stimulus pulse duration). In these animals, the amplitude of the 40μs pulse never exceeded 50 μA. The subsequent histologic analysis did not reveal any changes that could be attributed to the prolonged electrical stimulation. In one of

these cats, the dynamic response of the neurons in the PVCN was monitored during the high-rate stimulation using the embedded response recording technique. These data showed that short duration temporal interactions between stimulus pulses were not excessive, whether the stimulus pulse duration was 150 or 40 μs/ph.

In two other cats (CN125 and CN126), the NESF was 3.0, and the amplitude of the 40 μs stimulus pulses therefore reached 60 μA. In one of these animals (CN125), the SIDNE was considerably greater in Phase 2 of the protocol, when the stimulus pulse duration was 40 μs/ph, and the stimulus amplitude reached 60 μA. In Cat CN126, the SIDNE was only slightly greater in Phase 2. In cat CN125 the histology revealed a proliferation of glial cells (probably astrocytes) extending up to 50 μm from the site of the tip of only the pulsed microelectrodes, a phenomenon that we have not previously observed.

In two cats (CN127 and CN129), the electrodes were pulsed continuously at 63 μA for 7 hours per day on 9 successive days at 250 Hz. This regimen induced severe SIDNE in the neurons of the PVCN and the SIDNE was fully manifested by the end of the first day of stimulation. In Cat CN129, there was also some histologic evidence that iridium oxide had been spalled from the tips of at least one of the pulsed microelectrodes. In Cat CN127, there was a marked proliferation of astrocytes extending up to 50 μm from the pulsed electrode site. Curiously, this gliotic reaction was not seen in Cat CN127, which received the same regimen of stimulation. Taken together, these findings suggest that if short duration stimulus pulses are used in a clinical auditory prosthesis based on intranuclear microstimulation, then the stimulus pulse amplitude should not be allowed to exceed approximately 50 μA.

Prior to its being used in the study in which the microelectrodes were pulsed continuously at 63 μA, Cat CN129 had been used in a study in which we attempted to reduce the amount of SIDNE produced by prolonged stimulation at 250 Hz, by "conditioning" the neurons during several sessions of stimulation at a lower rate. The stimulus pulsing rate was increased from 100 to 250 Hz, in steps, over 15 days. This protocol did not appear to reduce the severity of the SIDNE produced by prolonged stimulation at 250 Hz.

INTRODUCTION

Most of our studies of the effects of prolonged microstimulation in the feline cochlear nucleus have used stimulus pulses of rather long duration (150 μs/ph), and low pulse amplitudes (McCreery et al, 1992,1994, 1997). The slow kinetics of charge injection from iridium oxide microelectrodes favors a long pulse duration, to allow the most efficient injection of charge across the electrode-electrolyte interface. However, the first clinical trials of the intranuclear microstimulation system will use a speech-processor and stimulating system that was designed to drive intracochlear electrodes over a larger range of stimulus currents, and which is not well adapted to modulating the pulse amplitude over the range of 0-20 μA. We therefore have begun to investigate the physiologic and histologic effects of prolonged intranuclear stimulation using relatively short (40 μs/ph) pulses (and a correspondingly greater range of pulse amplitudes).

METHODS

Fabrication of stimulating microelectrodes.

Activated iridium stimulating microelectrodes are fabricated from lengths of pure iridium wire, 50 μm in diameter. A Teflon-insulated lead wire is welded to one end of the iridium shaft, and the other end is shaped to a conical taper, by electrolytic etching. The microelectrodes have relatively blunt tips (a radius of curvature of approximately 6 μm) to reduce tissue injury during insertion into the brain. The entire shaft and wire junction then is coated with 3 thin layers of Epoxylite 6001-50 heat-cured electrode varnish. The insulation is removed from the tip by dielectric destruction, leaving an exposed geometric surface area of approximately 1000 μm^2. In the most recent animals, the insulation was removed by ablation with an Erbium laser. The individual electrodes are assembled into an integrated array of 4 microelectrodes spaced approximately 400 μm apart. The integrated array, with its closely-spaced microelectrode shafts, is designed to approximate the dimensions of an array that can

be implanted into the human posteroventral cochlear nucleus using a tool inserted through the translabyrinthine surgical approach to the CP angle.

The iridium electrodes are "activated" to increase their charge capacities, then soaked in deionized water for 120 hours, and sterilized with ethylene oxide.

Implantation of stimulating and recording electrodes

Young adult cats are anesthetized with Pentothal sodium, with transition to a mixture of nitrous oxide and Halothane. Implantation of the electrodes is conducted using aseptic surgical technique. The cat's head is placed in a stereotaxic frame, and the skull is exposed as far back as the posterior fossa by reflecting the scalp and muscles. A pair of stainless steel recording electrodes is implanted by stereotaxis into the right inferior colliculus through a small craniectomy. The compound action potential induced by a train of clicks delivered to the left ear is used to position the recording electrodes.

A small craniectomy is made in the posterior fossa over the cerebellum, through which the array of iridium stimulating electrodes is inserted by stereotaxis into the left posteroventral cochlear nucleus (PVCN). Since the feline cochlear nucleus lies on the lateral surface of the brainstem and the human cochlear nucleus is buried behind the middle cerebellar peduncle, the feline array of 4 mm microelectrodes was inserted through a portion of the overlying cerebellar flocculus, so that we could evaluate electrodes whose length was appropriate for use in humans. The microelectrodes were positioned first by stereotaxic coordinates and the final positioning was achieved by observing the compound potential evoked in the inferior colliculus while stimulating with the microelectrode.

Stimulation protocols and data acquisition

For the prolonged stimulation regimen, we have simulated an acoustic environment with a computer-generated artificial voice, which reproduces many of the characteristics of real speech, including the long-term average spectrum, the short-term

spectrum, the instantaneous amplitude distribution, the voiced and unvoiced structure of speech, and the syllabic envelope. The artificial voice signal is passed through a full-wave rectifier and then undergoes logarithmic amplitude compression, before being sent through an appropriate anti-aliasing filter. The amplitude of the filtered signal modulates the amplitude of the charge-balanced stimulus pulses which are delivered to each electrode at 250 Hz, in an interleaved manner. The range of spike amplitudes is shifted so that acoustic silence is represented by a stimulus amplitude which is close to the response threshold of the neurons near the tip of the properly functioning microelectrodes (6 μA when the stimulus pulse duration was 150 μs/phase, and at a higher amplitude when the pulse duration was 40 μsec/phase, as described below in RESULTS). The maximum amplitude of the stimulus pulses was set to 20 μA (3 nC) when the stimulus amplitude was 150 μsec/phase, and at a higher amplitude when the pulse duration was 40 μsec/phase.

The artificial voice signal was presented for 15 seconds followed by 15 seconds in which the stimulus amplitude was held near the threshold of the evoked response. This 50% duty cycle is intended to simulate a moderately noisy acoustic environment. Stimulation and data acquisition was conducted using the two-way radiotelemetry stimulation and data acquisition system described previously. This telemetry system and its companion software allows continuous monitoring of the voltage waveform across the stimulating microelectrodes, and of the compound evoked potential induced in the inferior colliculus by the stimulating microelectrodes.

Before and after each daily session of stimulation, the recruitment curves of the evoked responses were recorded in the inferior colliculus. The responses evoked by 1024 to 4096 consecutive charge-balanced, controlled-current stimulus pulses applied to the stimulating microelectrodes were averaged to obtain an averaged evoked response (AER). For each AER, the amplitude of the first and second component was measured after the averaged response is filtered through a low-pass filter with a bandpass of 250 Hz to 2.5 kHz. The amplitude of the early and second components is measured from the peak of the positivity on the leading edge to the trough of the

subsequent negativity (RESULTS, Figure 1). The response growth function (recruitment curve), which represents the recruitment of the excitable neural elements surrounding the microelectrode, is generated by plotting the amplitude of the first or second component of each of several AERs against the amplitude of the stimulus pulse that evoked the responses.

The conventional (non-embedded) recruitment curves were generated before and immediately after the sessions of prolonged stimulation. The stimulus frequency was 50 Hz, which is much lower than during the 7-hour test session. In addition, a limited number of "embedded" recruitment curves were acquired during the first or last 45 minutes of some of the 7-hour sessions of stimulation from the pulses comprising the 250 Hz, amplitude-modulated pulse train . This procedure, which requires approximately 45 minutes per microelectrode, was described in detail in QPR #5. Briefly, when the appropriate pulse amplitude (e.g., 6 μA) is generated by the artificial voice, subsequent stimulus pulses are suspended for 10 msec, which is long enough for the first and second components of the evoked response to be recorded in the inferior colliculus. Pulsing at the full stimulus rate then resumes, and the computer waits 150 msec before resuming its surveillance for the next 6 μA pulse, at which time another evoked response is recorded. This is repeated until the required number of responses to 6 μA has been collected and averaged, and the process is repeated for each of 7 stimulus amplitudes.

The conventional (non-embedded) recruitment curves allow detection of depression of neuronal excitability that persists after the termination of the high-rate stimulation with the artificial voice signal. If it is severe, this type of depression may persist for several days. Changes in the non-embedded recruitment curves identify stimulation protocols that place significant stress on the neurons of the lower auditory system. The embedded recruitment curves, in contrast, allows us to determine how the regimen of prolonged stimulation affects the neuronal response to the actual artificial voice signal. The non-embedded and embedded responses, and the histologic evaluation of the implant sites, together provide a more complete picture of the safety

and efficacy of the stimulation regimen.

To allow for greater consistency between animals, the non-embedded responses are generated from the 2nd component of the evoked response, which is of higher amplitude than the 1st component, and therefore allow for a greater signa-to-noise ratio in the AERs. We have shown previously that when the stimulus frequency is low (50 Hz), there is an excellent correspondence between the changes in the first and 2nd components during prolonged stimulation regimens. In some animals , the amplitude of the first component may be too small to allow its recruitment characteristics to be generated reliably and reproducibly. On the other hand, the 2nd (trans-synaptically-evoked) component tends to become dispersed during the high-rate (250 Hz) simulation that is used to generate the embedded responses, and we therefore feel that the early (directly- evoked) component is a better index of the effects on the electrical excitability of cochlear nucleus neurons, as they are revealed by the embedded response.

Within 15 minutes after the end of the last day of stimulation, the cats were deeply anesthetized with pentobarbital and perfused through the aorta with ½ strength Karnovsky's fixative (2.5% glutaraldehyde, 2% paraformaldehyde and 0.1M sodium cacodylate buffer). The cochlear nucleus and adjacent portion of the brainstem were resected, embedded in paraffin, sectioned serially in the frontal plane (approximately parallel to the shafts of the stimulating microelectrodes) at a thickness of 8 μm, and stained with Cresol Violet (Nissl stain) or with hematoxylin and eosin.

RESULTS

Cat CN119.

This cat was used in a two-phase experiment in which the microelectrodes were first pulsed for 7 hours per day on 3 successive days using a stimulus pulse duration of 150 μs/ph. In the second phase of the study, the microelectrodes were pulsed on 10 successive days using a pulse duration of 40 μs/ph. Finally, the cat was sacrificed for histologic evaluation of the electrode sites.

Figure 1 shows the average evoked responses (AERs) recorded in the right inferior colliculus while pulsing microelectrode #2 in the left posteroventral cochlear nucleus. The AERs evoked from microelectrode 2 by pulses either 150 or 40 μs/ph in duration are very similar, and consist of an early (first) component with an onset of less than 1 ms. On the basis of its short duration, we assume that this first component represents neuronal activity induced directly (i.e., not transsynaptically) by the intranuclear microstimulation. The amplitude of the first component is quantified as P1-N1. The second component, with a longer latency, probably represents neuronal activity that is induced transsynaptically in the neurons of the central nucleus of the inferior colliculus. Its amplitude is quantified as P2-N2.

In Cat CN119, the first regimen of prolonged stimulation was initiated 200 days after implantation of the microelectrode arrays. Microelectrodes 2,3, and 4 were pulsed in the interleaved mode at 250 Hz per electrode for 7 hours per day on each of 3 successive days. During the 7 hours of stimulation, the stimulus pulse amplitude was modulated across the range of 6-20 μA according to the amplitude of the logarithmically compressed artificial voice signal. The signal was present for 10 seconds then absent for 10 seconds (50% duty cycle). This protocol is designed to simulate a moderately noisy acoustic environment. During the <u>off</u> phase, the stimulus pulse amplitude was a constant 6 μA. Figure 2 shows the response growth curves (non-embedded recruitment curves) of the second component of the AERs evoked from Microelectrode #2, before the start of the 3-day regimen and at the end of the first and third daily sessions. By the end of the first day, the threshold of the recruitment curve was shifted slightly to the right, indicating a small amount of persisting stimulation-induced depression of neuronal excitability (SIDNE). The shift is due primarily to the offset of the curves near threshold, and we interpret this to mean that the effect of the prolonged stimulation was primarily to increase the electrical threshold of the neurons that are very close to the microelectrodes, and to leave largely unaffected the electrical excitability of neurons that are more distant from the tip. The amount of shift in the recruitment curves did not change between the end of the first and third day. By 10

days after the end of the 3-day regimen, the neuronal excitability had partially recovered from the SIDNE. The slow recovery is typical of SIDNE in the cochlear nucleus. The findings were essentially the same for Microelectrodes #2 and #4 and are typical of what we have observed on many occasion using this stimulation regimen.

The second phase of this study was initiated 20 days after the end of Phase #1 when the SIDNE had completely disappeared. It was first necessary to establish the correspondence between the neuronal activity evoked by the 150 and 40 μs stimulus pulses. Figure 3A shows the recruitment curves of the 2nd component of the AERs invoked by 150 and by 40 μs stimulus pulses. Figure 3B was generated from the data shown in Figure 2A. It is a plot of the amplitudes of the 40 and 150 μs pulses that waere required to evoke AERs of the amplitudes that are indicated at various points along the plot. The best fit for the plot is a line with a slope of 2.2 (NESF = 2.2). Thus, the 40μs pulses are relatively more efficient than are the 150 μs pulses, in terms of the charge per phase. This implies that the microelectrodes are exciting neural elements whose chronaxies are considerably shorter than 150 μs, (probably myelinated axons rather than neuronal cell bodies).

In the 2nd phase of the study, the stimulus pulse duration was 40 μs/ph and using a NESF of 2.2, the stimulus pulses were modulated over the range of 13-44 μA (2.2 × 6-20 μA). Figure 4 shows the non-embedded recruitment curves of the 2nd component of the AERs . By the end of the first day of stimulation, the recruitment curve was shifted slightly to the right of the prestimulus (control) curve, in a manner very similar to what occurred after the first day of pulsing with the 150 μs pulses (Figure 2). Throughout the 9 days of the regimen, the threshold of the AER did not increase further and, in fact, the upper part of the recruitment curves moved back toward the prestimulus (control) curve. Thus, the amount of SIDNE induced by prolonged stimulation with the 40μs pulses was certainly no greater than what occurred during the first phase of the study when the stimulus pulse duration was 150 μs/ph.

In Cat CN119, the early component of the AER was quite large, and therefore, we were able to reliably generate the embedded recruitment curves. Figure 5 shows

the recruitments of the embedded and non-embedded responses of the 1st component of the AER, as recorded during the last hour of the first day of stimulation and also near the end of the 3rd, 6th and 10th day of stimulation. The non-embedded recruitment curve recorded near the end of the first day is shown for comparison. As in previous animals, the threshold of the embedded response is somewhat higher than that of the non-embedded response, but above this threshold, the slopes of the two types of curves are similar. This behavior is similar to what we have seen previously when the stimulus pulse duration is 150 μs/ph, and it indicates that the 40μs stimulus pulses should be able to convey acoustic information into the cochlear nucleus with approximately the same efficiency as would pulses 150 μs in duration.

Immediately after the last day of the stimulation regimen, the cat was deeply anesthetized and perfused through the aorta, for histologic evaluation of the electrode sites. Figure 6A (Nissl stain) shows the sites of the tips of two of the pulsed microelectrodes. The neuropil and neurons (N) surrounding the electrode tips (their active stimulation sites) appears to be normal and healthy. There is some healed glial scarring in the neuropil above the tips. The scarified region extends symmetrically from the track of the electrode shafts and may represent a toxic reaction to the Epoxylite insulation. We have subsequently modified our procedure for curing the Epoxylite insulation, and the results of this modification are presently being evaluated.

Cat CN131.

A similar two-phase study was conducted with cat CN131, beginning 76 days after implantation of an array of 3 microelectrodes into the left posteroventral cochlear nucleus. In the first phase of this study, the stimulation regimen was identical to that used in Cat CN119 described above. All 3 microelectrodes were pulsed in the interleaved mode at 250 Hz per electrode, during a single 7-hour session. The pulse duration in Phase 1 was 150 μs/ph. The pulse amplitude was modulated over the range of 6-20 μA, according to the logarithmically compressed artificial voice signal, and the duty cycle of the artificial voice was 50%. Figures 7A and 7B show the non-

embedded recruitment curves of the second component of the AER that was evoked from microelectrodes #2 and #3 and recorded in the contralateral inferior colliculus.

The second phase of this study was initiated 20 days after Phase 1. In Phase 2, the stimulus pulse duration was 40 μs/ph and by using the procedure depicted in Figure 3B, the NESF was determined to be 2.4. Therefore the artificial voice was modulated over the span of 14-48 μA. The 3 microelectrodes were pulsed for 7 hours per day on each of 11 successive days. Figures 8A and 8B show the non-embedded recruitment curves of the 2nd component of the AERs evoked from Microelectrodes 2 and 3, and recorded in the inferior colliculus. The amount of SIDNE at the end of the 1st, 3rd and 11th days is certainly no greater than that which occurred when the stimulus pulse duration was 150 μs/ph (Figure 7).

Immediately after the last session of stimulation, the cat was sacrificed for histologic evaluation of the electrode sites. Figure 9A, 9B and 9C show the sites of the tip of the 3 microelectrodes (all 3 microelectrodes were pulsed in this animal). The tips were in the ventral part of the PVCN and there were relatively few cell bodies in the neuropil. However, a few neurons (N) are in the general vicinity of the tips, and these appear to be normal, as does the surrounding neuropil. There was no reactive gliosis around the tip sites and no evidence of spalled iridium oxide.

Cat CN125.

In Cats CN119 and CN131 described above, the NESF for scaling between stimulus pulse duration of 150 and 40 μs/ph, was 2.2 to 2.4. In Cat CN125, the NESF was greater, and the correspondingly higher stimulus current may have induced some undesirable histologic and physiologic changes.

The first phase of the 2-phase protocol was initiated 118 days after implantation of the microelectrode array into the PVCN. Microelectrodes 2,3 and 4 were pulsed for 7 hours/day at 250 Hz in the interleaved mode. The stimulus pulse duration was 150 μs/ph, and as in the previous animals, the stimulus pulse amplitude was modulated over the range of 6-20 μA according to the logarithmically compressed

artificial voice signal. The duty cycle of the artificial voice was 50%. Figure 10A and 10B show the recruitment curves of the 2nd component of the AERs evoked from Microelectrodes 2 and 3, and recorded in the contralateral inferior colliculus.

The second phase of the protocol, in which the stimulus pulse duration was 40 μs/ph was initiated 37 days after the end of Phase 1. In this cat, the NESF was quite high (3.0). Therefore, the artificial voice signal was allowed to modulate the stimulus pulses over the range of 18-60 μA. During the interval between Phases 1 and 2, Microelectrode 4 failed at the connection to the percutaneous connector, and the second phase of the study was conducted using only Microelectrodes 2 and 3. Figures 11A and 11B show the recruitment curves of the 2nd component of the AERs for these two microelectrodes. The curves acquired after the first, third and tenth day of stimulation are shifted considerably to the right of the prestimulus (control) curve, indicating somewhat more SIDNE than occurred during Phase 1 of the study, when the stimulus pulse duration was 150 μs/ph.

Immediately after the last day of stimulation, the cat was sacrificed for histologic evaluation of the electrode sites. Figure 12A (Nissl stain) shows the site of the tip of Electrodes 1 and 4, neither of which were pulsed during Phase 2 of the protocol. Electrode 4 had been pulsed on 12 successive days during Phase 1 and electrode 1 had never been subjected to prolonged pulsing. The neurons (N) and neuropil adjacent to both electrode tip sites appeared to be normal. Figure 12B shows the sites of the tips of Electrodes #2 and #3, both of which were pulsed during Phases 1 and also during Phase 2 of the protocol. There is some proliferation of glia (seen as darkly stained entities in this Nissl material) near the electrode tips. Neurons (N) and neuropil immediately outside of this area appear to be normal. We have not previously seen this type of gliotic reaction near the tips of electrodes that have been subjected to prolonged pulsing at up to 20 μA with a pulse duration of 150 μs/ph. If the glial proliferation is related to the high current density (up to 6 amps/cm^2 for microelectrodes with a tip area of 1000 μm^2) then it might be alleviated by increasing the geometric area of the tips. In our most recent implant (CN133), the geometric area of the electrode tips

has been increased to 2000 μm², and we plan to repeat the protocol in which the microelectrodes are pulsed over the range of 18-60 μA using a pulse duration of 40 μs/ph. Figure 12C shows the neuropil adjacent to the tip of the electrode shaft. In this animal, there was no toxic reaction to the Epoxylite insulation. The glial sheath surrounding the shaft is thin and compact, and the surrounding neuropil appears normal.

CN126.

In this cat, phase 1 of this study was initiated 119 days after implantation of the array. Microelectrodes 1, 2 and 3 were pulsed for 7 hours per day at 250 Hz, using a stimulus pulse duration of 150 μs/ph. As in the previous animal, the stimulus pulse amplitude was modulated over the range of 6-20 μA, according to the amplitude of the logarithmically compressed artificial voice signal. This pulsing regimen was administered on 3 successive days. Figure 13 shows the recruitment curves of the 2nd component of the AERs evoked from Microelectrode #2 and recorded in the contralateral inferior colliculus. The recruitment curves acquired after the first, second and third days are shifted only slightly to the right of the prestimulus (control) curve, thus indicating only a small amount of SIDNE.

The second phase of the protocol was initiated 26 days after the end of Phase 1. The stimulus pulse duration was 40 μs/ph and the NESF was 3.0 (as in cat cn125), so the artificial voice was allowed to modulate the stimulus pulses over the range of 18-60 μA. Figure 14 shows the recruitment curves acquired before the first day of Phase 2 and after the first and 11th day of stimulation. The latter are shifted to the right of the prestimulus curve, indicating a small amount of SIDNE which is only slightly greater than that which developed during Phase 1 when the stimulus pulse duration was 150 μs/ph. This is in contrast to the experience with cat cn125 , in which the same protocol induced considerable more SIDNE. The reason for this difference is unclear. Unfortunately, the histologic material for this animal was lost due to problems with tissue processing.

CN129 .

Cat CN129 was used in two different studies. The first was a "conditioning" study which, in the interest of organization, is described at the end of this report.

49 days after the end of the conditioning study, and 120 days after implantation of the electrode array, microelectrodes 1 and 4 were pulsed for 7 hours per day, on 9 successive days, at 250 Hz and at a constant amplitude of 63 μA. This is the maximum current amplitude that can be generated by our telemetry system when it is operating in the microstimulation mode. The stimulus pulse duration was 40 μs/ph and the charge per phase therefore was 2.5 nC. This study was conducted in order to examine the extent of the SIDNE and the histologic changes that are produced by continuous high-amplitude pulsing, which is intended to approximate the level of stimulation that might occur in a patient with a microstimulation-based prosthesis, and who inhabits an extremely noisy environment. We have shown previously that 7 hours of continuous stimulation at 500 Hz and with a charge per phase of 3 nC can induce vacuolation of myelinated axons close to the stimulating microelectrode (McCreery et al, 1994). The frequency and charge per phase used in the present study were less than those which had been previously shown to induce such vacuolations, but the total duration of the stimulation was greater than in these previous studies. Figures 15A and 15B show the non-embedded recruitment curves of the second component of the AER recorded in the inferior colliculus when stimulating in the PVCN. By the end of the first 7-hour session, the recruitment curves of the response were greatly depressed, and the response thresholds were significantly elevated, indicating severe SIDNE. The reduction in the slope of the recruitments curves suggests that the depression of neuronal excitability extends to neurons that lie a considerable distance from the microelectrodes. The AER in cat CN129 had a small but usable early component. The recruitment curves of this early component are plotted in Figure 15C and 15D. By the end of the first day of continuous stimulation at 63 μA, the early components of the AERs evoked from microelectrodes 1 and 4 were markedly suppressed. The signal-to-noise ratios of these attenuated AERs was rather poor, but it appears that the

threshold of the early component is greatly elevated and above the threshold, the slope of its recruitment curve also is depressed. Since the first component of the AER is not generated transsynaptically, its marked suppression indicates that the continuous high-rate stimulation produced marked depression of the direct electrical excitability of the neurons in the PVCN. Based on our previous studies, we would expect that the cochlear nucleus neurons would recover only very slowly from such severe SIDNE, and therefore, that some precautions should be taken to ensure that patients with this type of microstimulation-based prosthesis are not exposed to stimulation that approximates these conditions. In this context, it is notable that the severe SIDNE developed after only 7 hours of continuous stimulation.

Immediately after the last day of stimulation, the cat was sacrificed for histologic evaluation of the electrode sites. In this cat, all 4 microelectrodes were in the most ventral part of the PVCN. Figure 16A shows the site of the tip of 2 of the microelectrodes. Microelectrode #3 had been pulsed during the 16-day conditioning regimen which terminated 12 days before the cat was sacrificed. Microelectrode #1 was pulsed during the conditioning regimen (described below, which ended 50 days before the cat was sacrificed) and also during the 9 days immediately before sacrifice, in which the cat was stimulated for 7 hours per day at 63 μA. The tissue surrounding both sites appears to be quite normal. However, there is some very dense material at the tip of Microelectrode 1, and this may be iridium oxide that had spalled from the tip. Figure 16B shows a section through the track made by the shaft of Microelectrode 2. The shaft was surrounded by a compact glial sheath and the surrounding neuropil appears to be normal and healthy.

Cat CN127.

In cat CN127, we repeated the study in which the microelectrodes in the PVCN were pulsed continuously for 7 hours per day at 63 μA using a pulse duration of 40 μs/ph. In this animal, this regimen was administered on 9 successive days, beginning 140 days after implantation of the array, and only one electrode (#4) was pulsed.

Figure 17 shows the non-embedded recruitment characteristics of the 2nd component of the AER evoked from Microelectrode #4. By the end of the first day, the recruitment curves were shifted markedly to the right. The SIDNE, while quite marked, was not as severe as in cat CN129. This may be due to the fact that in the present animal, only one microelectrode was pulsed. If this is indeed what is responsible for the additional SIDNE in cat CN129, then it represents a situation in which interleaving the stimulation across the array of electrodes did not completely negate the additive effect of pulsing the closely-spaced electrodes. Virtually all of the SIDNE was apparent by the end of the first day of stimulation and the severity of the SIDNE was virtually unchanged by the end of the 9th day.

Immediately after the last day of stimulation, the cat was perfused for histologic evaluation of the electrode sites. Figure 18A shows the site of the tip of a pulsed electrode (Electrode #4). There was an active proliferation of glial cells (gliosis) in the tissue immediately surrounding the tip. In contrast, the tissue surrounding this electrode's shaft appears to be quite normal (Figure 18B). There was, however, one isolated region of neuropil surrounding a short section of the electrode shaft, in which there was a active gliosis, and also some perivascular cuffing of lymphocytes, which suggest an active inflammatory process, 150 days after implantation of the array. This response, far above the tip site, appears to be a reaction to the Epoxylite insulation, or perhaps to a contaminant on the surface of the electrode shaft. In the tissue immediately outside of this focus, the neurons and neuropil (N) appear to be quite normal, except for a minimal proliferation of small blood vessels.

CN129 (conditioning study)

Prior to its use in the high-amplitude stimulation study described above, cat CN129 was used in a "conditioning" study to determine if it is possible to reduce the amount of SIDNE by gradually increasing the stimulus pulsing rate over a span of many days. The experiment was begun 53 days after implantation of the array. The stimulus was the same as has been used in our previous studies; namely, charge- balanced

pulses whose amplitude is modulated according to the logarithmically compressed artificial voice signal with a 50% duty cycle. The stimulus pulse duration was 40 μs/ph. The NESF (for scaling between pulse durations of 150 and 40 μs) was 2.4, so the artificial voice was allowed to modulate the pulse train over a range of 14-40 μA. The full range of pulse amplitudes was used throughout the entire 16-day regimen, but the pulsing frequency was increased in 3 steps. All 4 microelectrodes were pulsed in the interleaved mode for 7 hours per day. The microelectrodes were pulsed at 100 Hz for the first 4 days, at 150 Hz for the next 5 days, at 200 Hz for the next 4 days, and at 250 Hz for the final 3 days of the 16-day protocol. Figures 19A and 19B show the recruitment curves of the 2nd component of the AERs evoked from Microelectrodes 1 and 4 before the start of the study, and at the end of several of the 7-hour sessions. Note that stimulating at 100 Hz produced only a small shift of the recruitment curve (indicating very little SIDNE), but that the SIDNE became progressively more pronounced as the stimulus rate was increased in steps during the 16-day regimen. The amount of SIDNE produced by the 250 Hz stimulation (which was begun after 13 days of "conditioning") is comparable to what we have observed previously in other animals in which the prolonged pulsing regimen was initiated at a rate of 250 Hz. Thus, this conditioning regimen did not significantly reduce the severity of the SIDNE produced by the 250 Hz. We plan to repeat the study in at least one additional animal, and to increase the number of days in which the stimulation rate is less than 250 Hz.

DISCUSSION

The findings presented in this report indicate that prolonged microstimulation in the feline cochlear nucleus using short-duration (40 μs/phase) charge-balanced current pulses of relatively high amplitude (up to approximately 48 μA) can be just as safe and effective as is prolonged stimulation with longer 150 μsec/ph pulses. These conclusions are based on the results from 3 animals (CN-119, CN-131 and CN-129) in which the amplitude range of the 40 μs pulses was scaled up from previous criteria for safe and effective stimulation with 150 μs pulses, according to a neural excitability

scaling factor (NESF). In the 3 cats noted above, the NESF factor ranged from 2.2 to 2.4.

In 2 other cats, the NESF was higher (approximately 3.0). The most likely explanation for this difference is that in these cats the microelectrodes were in a region of the PVCN in which the myelinated axons of the efferent pathways were smaller, and their average chronaxies were correspondingly greater. In these cats, some problems were noted when the stimulus pulse amplitude was scaled up according to the NESF, allowing the pulse amplitude (as modulated by the artificial voice signal) to reach 60 μA (2.5 nC/ph) and an average charge density of 240 μC/cm². In one cat (CN-125), there was excessive stimulation-induced depression of neuronal excitability as well as active gliosis around the tip of the pulsed microelectrodes. In another cat, continuous pulsing at 63 μA (2.5 nC/ph) and an average charge density of 250 μC/cm² may have caused some iridium oxide to be spalled from the microelectrode. We should note that when the NESF = 3, the stimulus charge per phase and the charge density delivered by the 40 μsec pulses is still less than what would be delivered by 150 μs pulses of comparable efficacy (the charge per phase would be identical for the two pulse durations when NESF = 3.75). However, the current density of the 40 μs pulses is much greater. Also, the charge density is not uniform over the surface of our blunted-cone electrode tips, and at the very tips of the expose areas, it may have been considerably greater then 240 μC/cm² . The average current density can be reduced by increasing the geometric surface area of the microelectrode tips. In our most recent implant (CN133), the microelectrodes have geometric surface areas of 2000 μm² (vs. 1000 μm² for the animals described in this report). Microelectrodes with this larger surface area will have only slightly less spatial resolution and they may afford an additional safety factor when the stimulus current is high. We are continuing to use microelectrodes with relatively blunt tips (radius of curvature of 6 μm). These blunter tips should minimize the inequities in the current and charge density over the surface of the electrode.

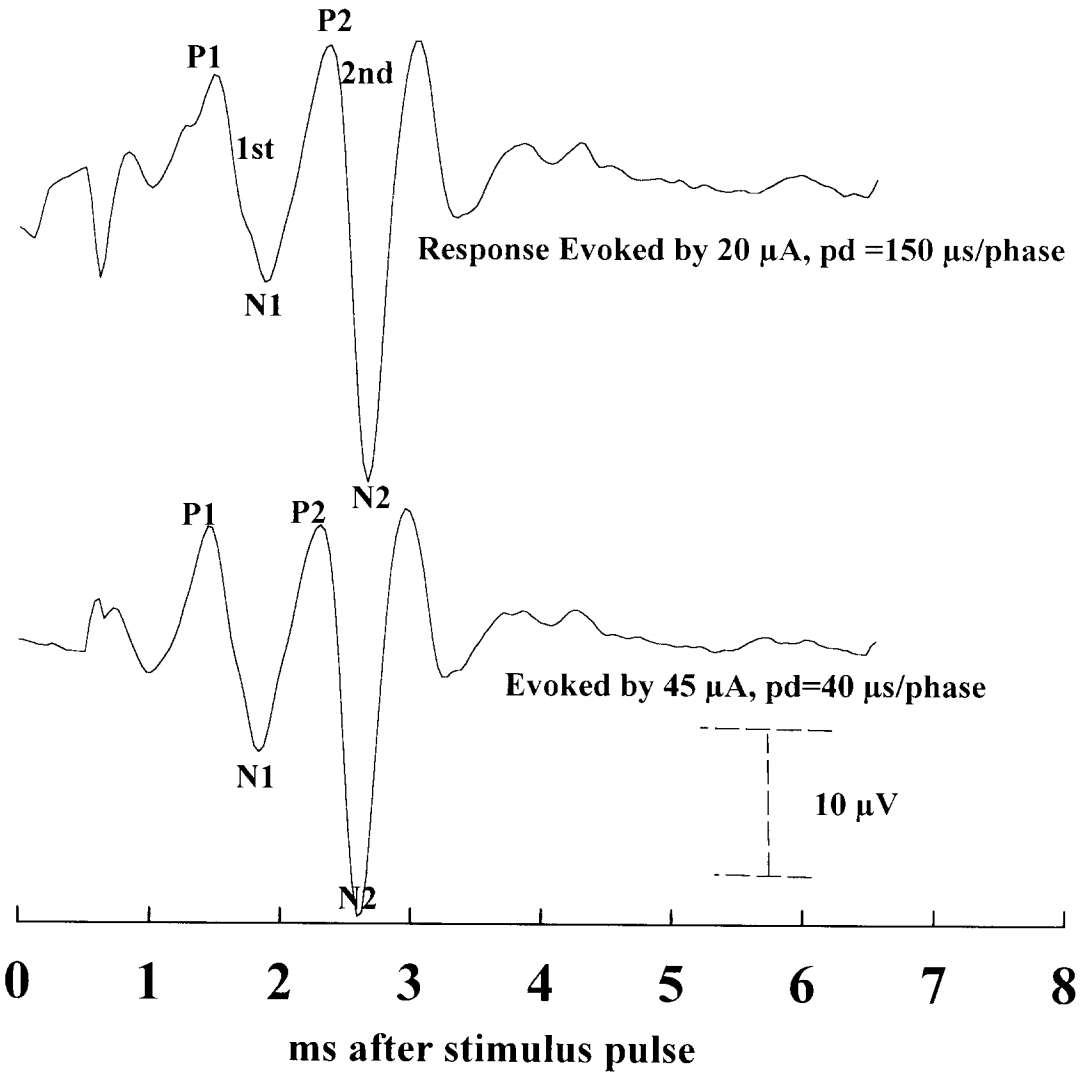
REFERENCES CITED

McCreery, D.B., Yuen, T.G.H., Agnew, W.F., and Bullara, L.A. (1992) Stimulation with chronically implanted microelectrodes in the cochlear nucleus of the cat: Histologic and physiologic effects. Hearing Res. 62:42-56.

McCreery, D.B., Yuen, T.G.H., Agnew, W.F. and Bullara, L.A. (1994) Stimulation parameters affecting tissue injury during microstimulation in the cochlear nucleus of the cat Hearing Res. 77:105-115 .

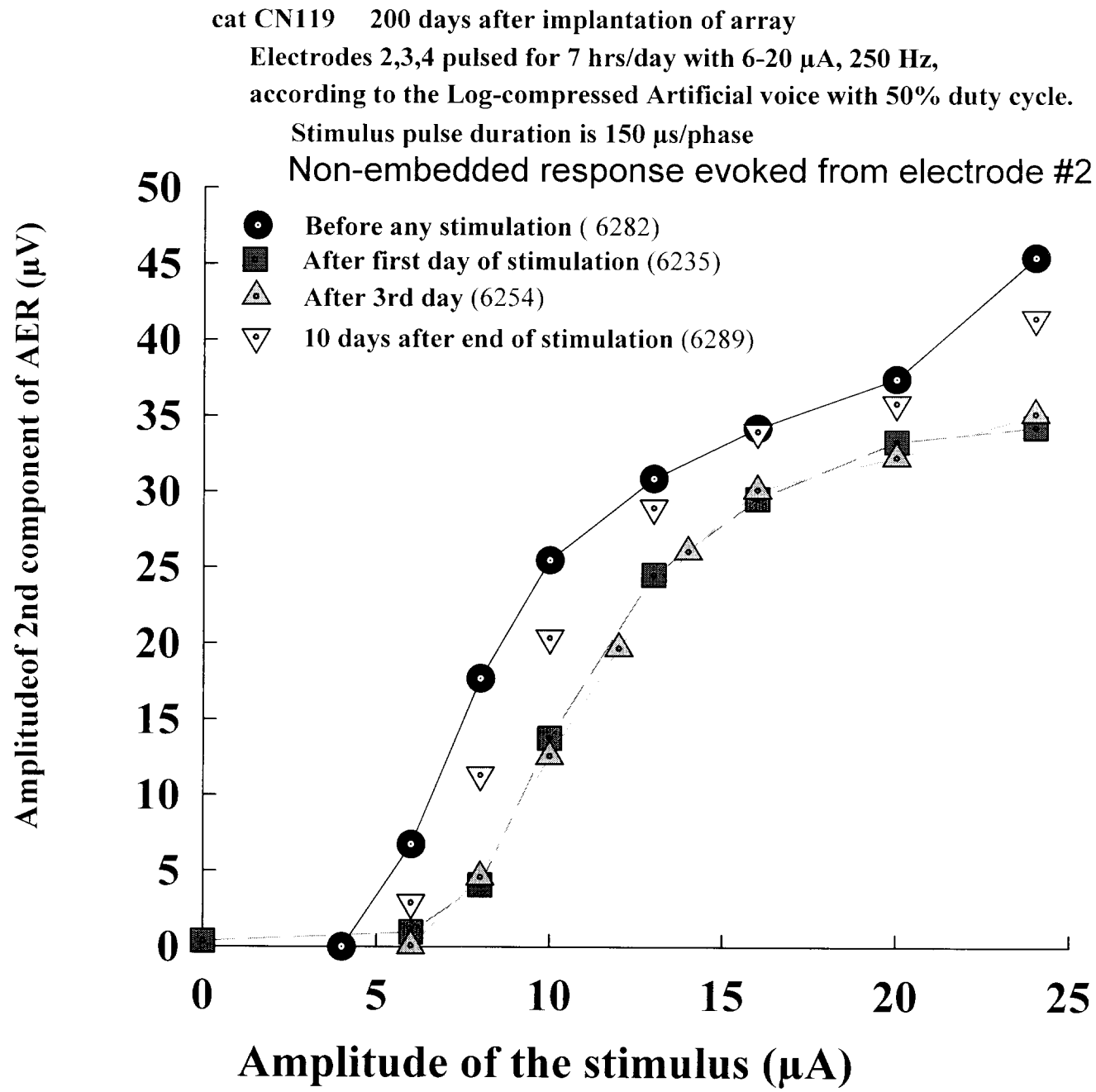
McCreery, D.B., Yuen, T.G.H., Agnew, W.F. and Bullara, L.A. (1997) A characterization of the effects on neuronal excitability resulting from prolonged microstimulation with chronically implanted microelectrodes. IEEE Trans. Biomed. Eng. 44:931-939.

Cat cn119 Averaged evoked responses (AERs) recorded in the IC while pulsing microelectrode #2 in the PVCN



cn119q2e.spw

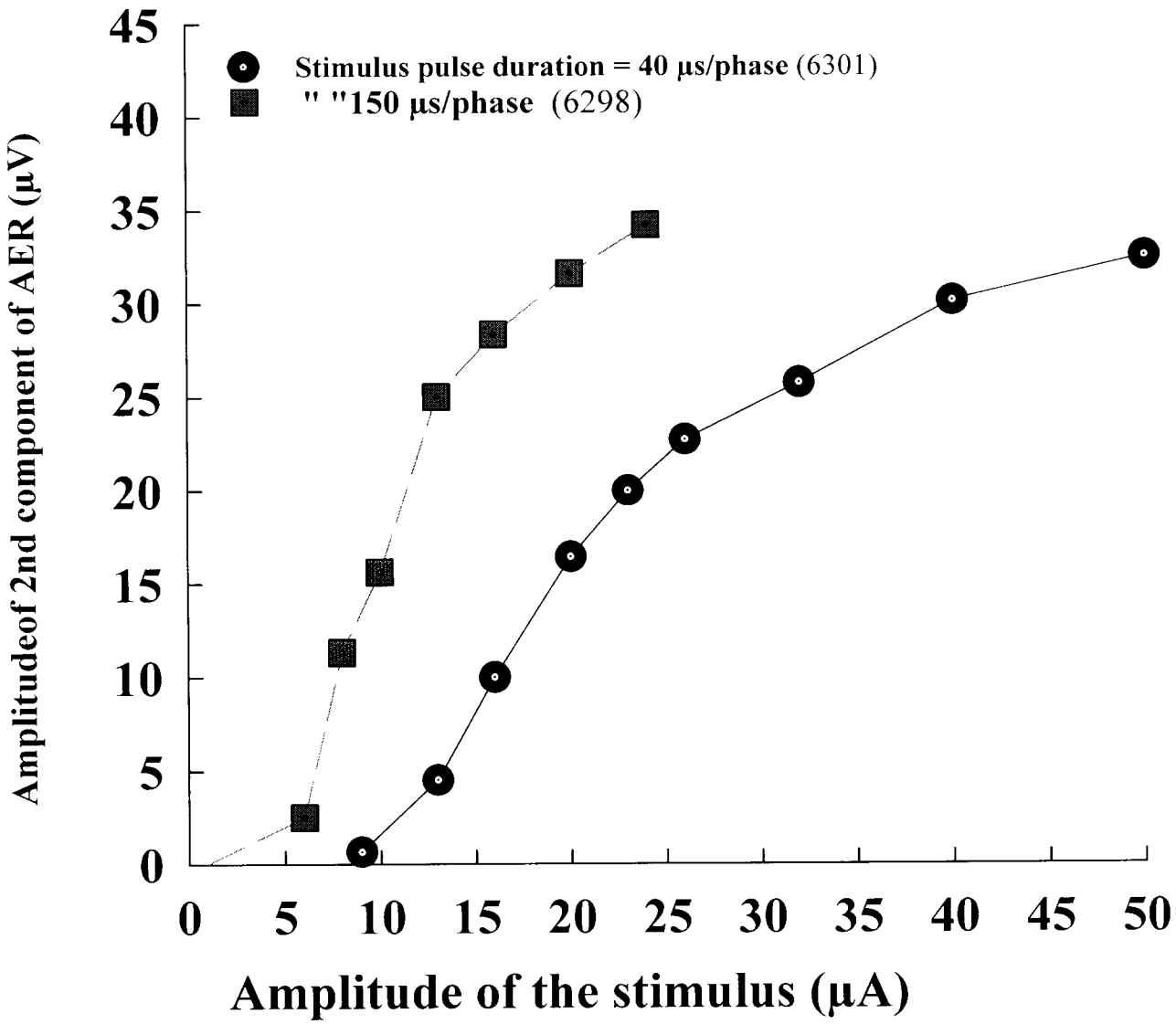
Figure 1



cn119l2a.spw

Figure 2

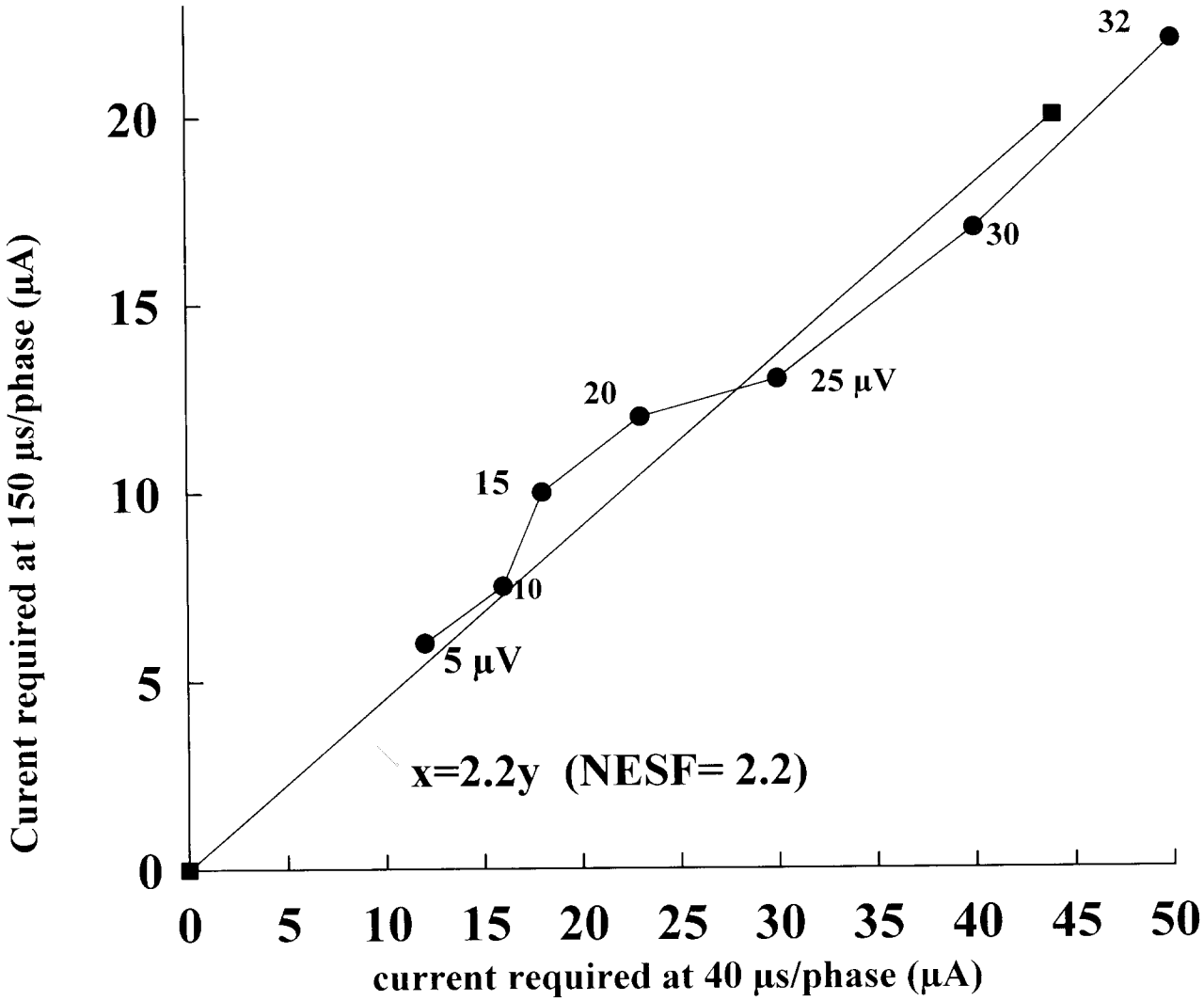
Cat CN 119. Non-embedded recruitment of 2nd phase of AERs evoked by microelectrode #2 in the PVCN and recorded in the IC



cn119qb.spw

Figure 3A

Cat cn119.
Derivation of Neural Excitability Scaling Factor (NESF)
for microelectrode #2



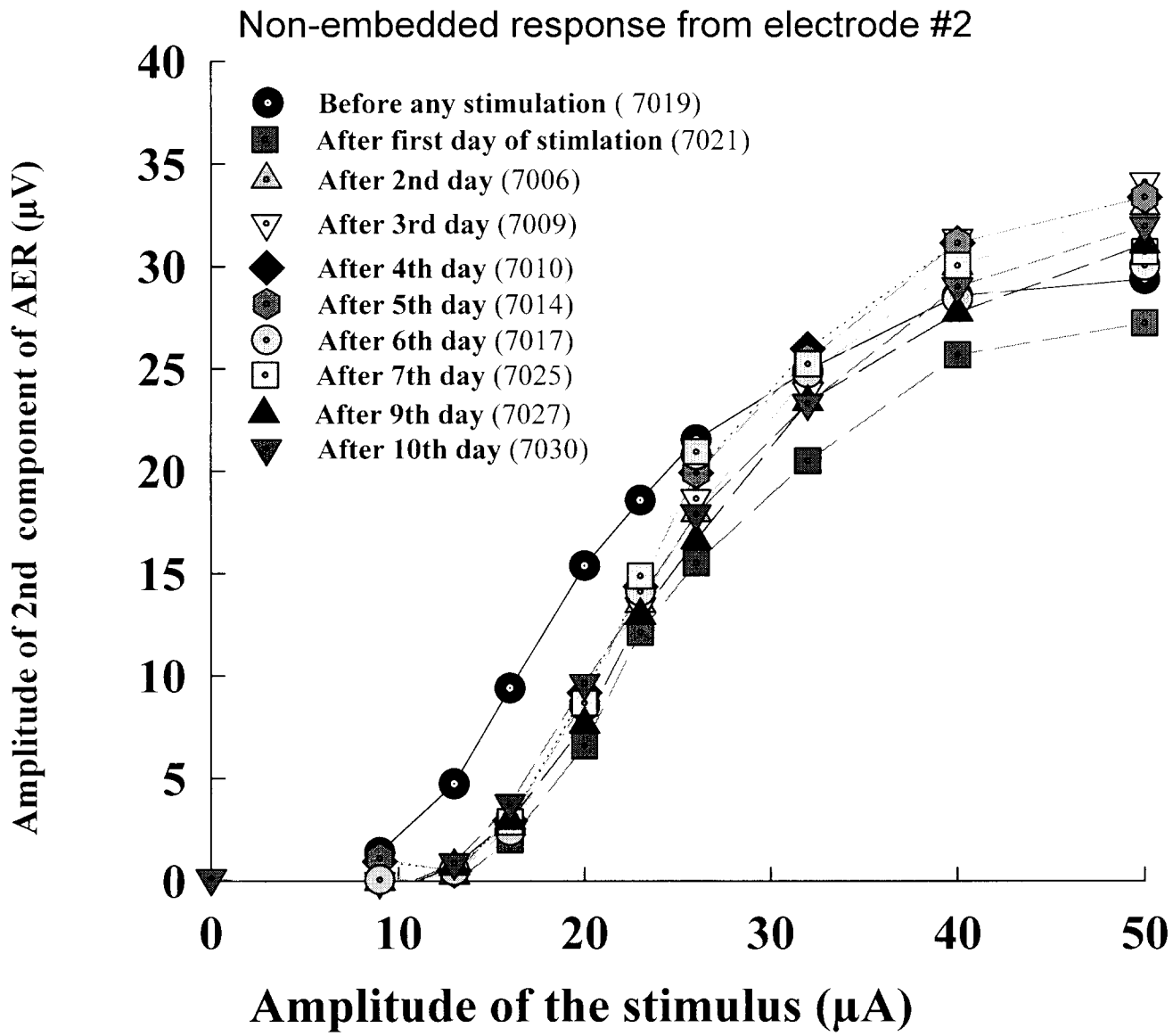
cn119q2d.spw

Figure 3B

cat CN119

Electrodes 2,3,4 pulsed 7 hrs/day with 13-44 μA, 250 Hz, Log-compressed artificial voice with 50% duty cycle

Stimulus pulse duration is 40 μs/phase



cn119u2b.spw

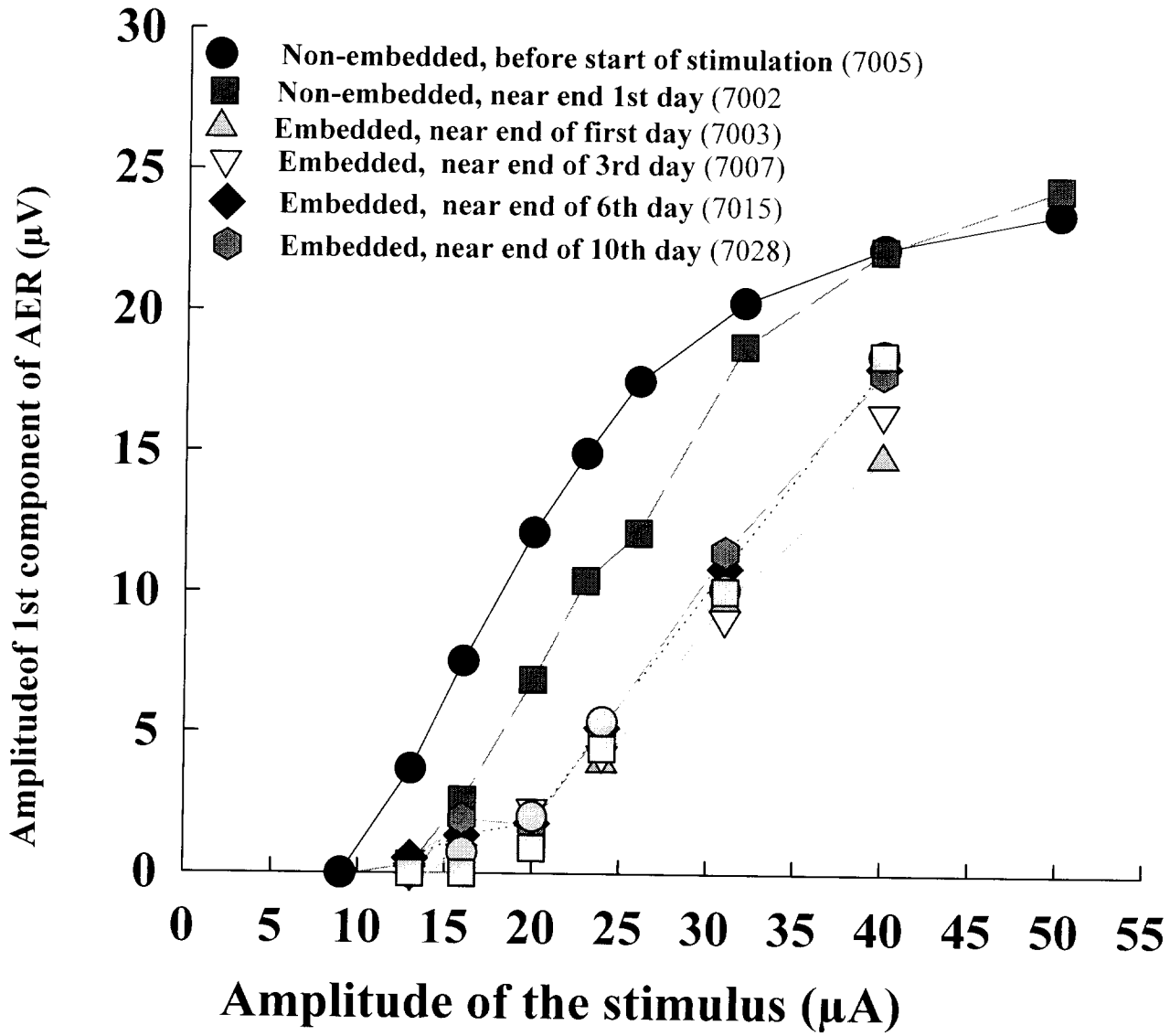
Figure 4

cat CN119

Electrodes 2,3,4 pulsed 7 hrs/day, 13-44 μA, 250 Hz, log-compressed artificial voice signal with 50% duty cycle

Stimulus pulse duration is 40 μs/phase

Embedded and non-embedded responses evoked from electrode #2



cn119t2a.spw

Figure 5

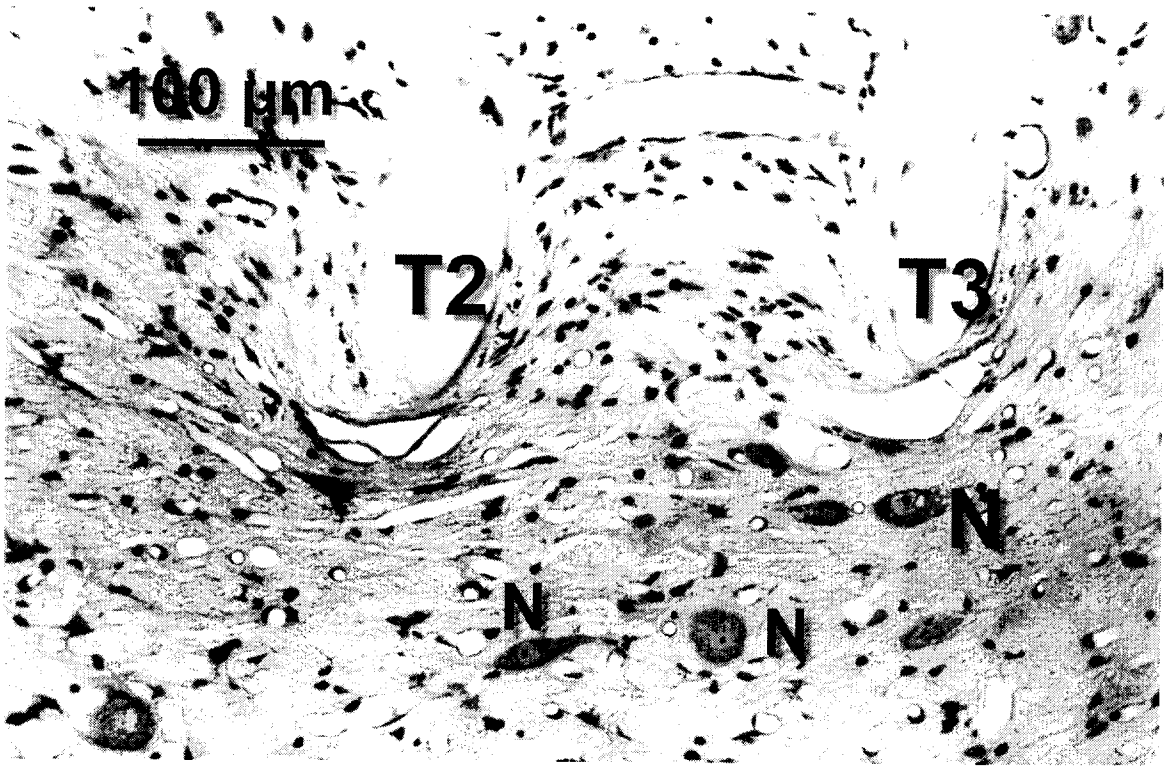
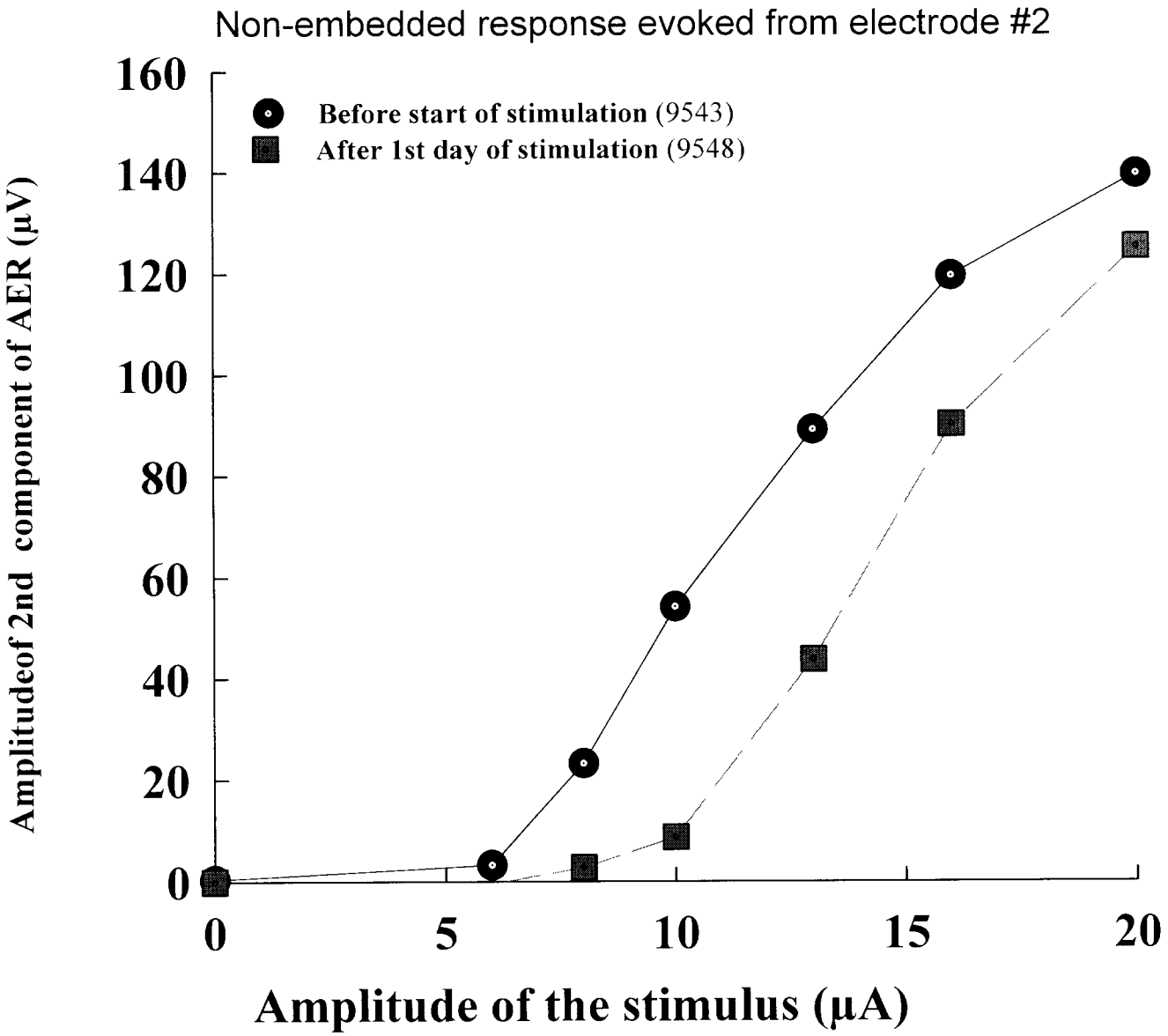


Figure 6

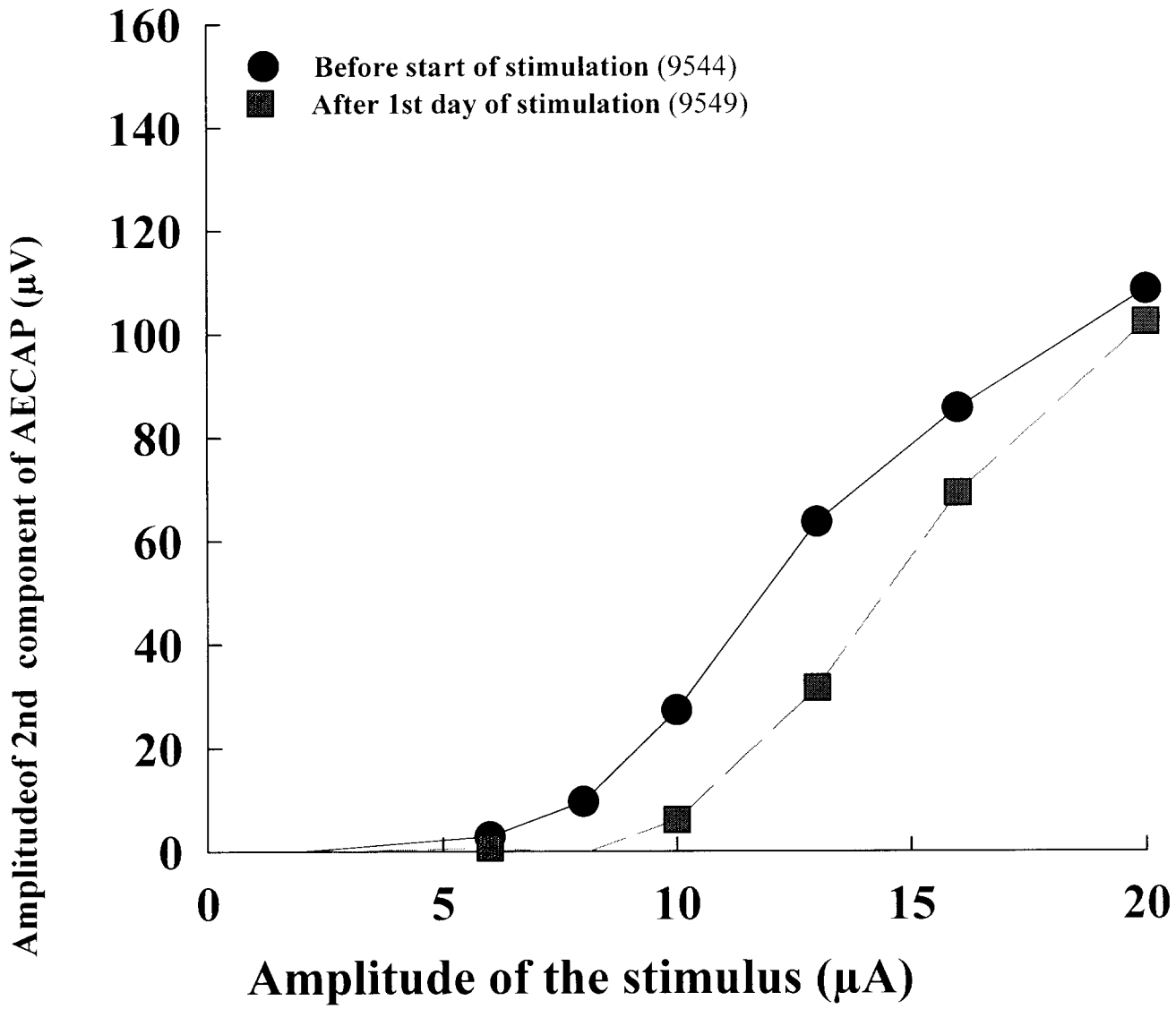
cat CN131
Electrodes 2,3 pulsed over range of 6-20 μA, 250 Hz, for 7 hrs/day, according to log-compressed artificial voice signal with 50% duty cycle

Stimulus pulse duration is 150 μs/phase



cn13112b.spw

Figure 7A



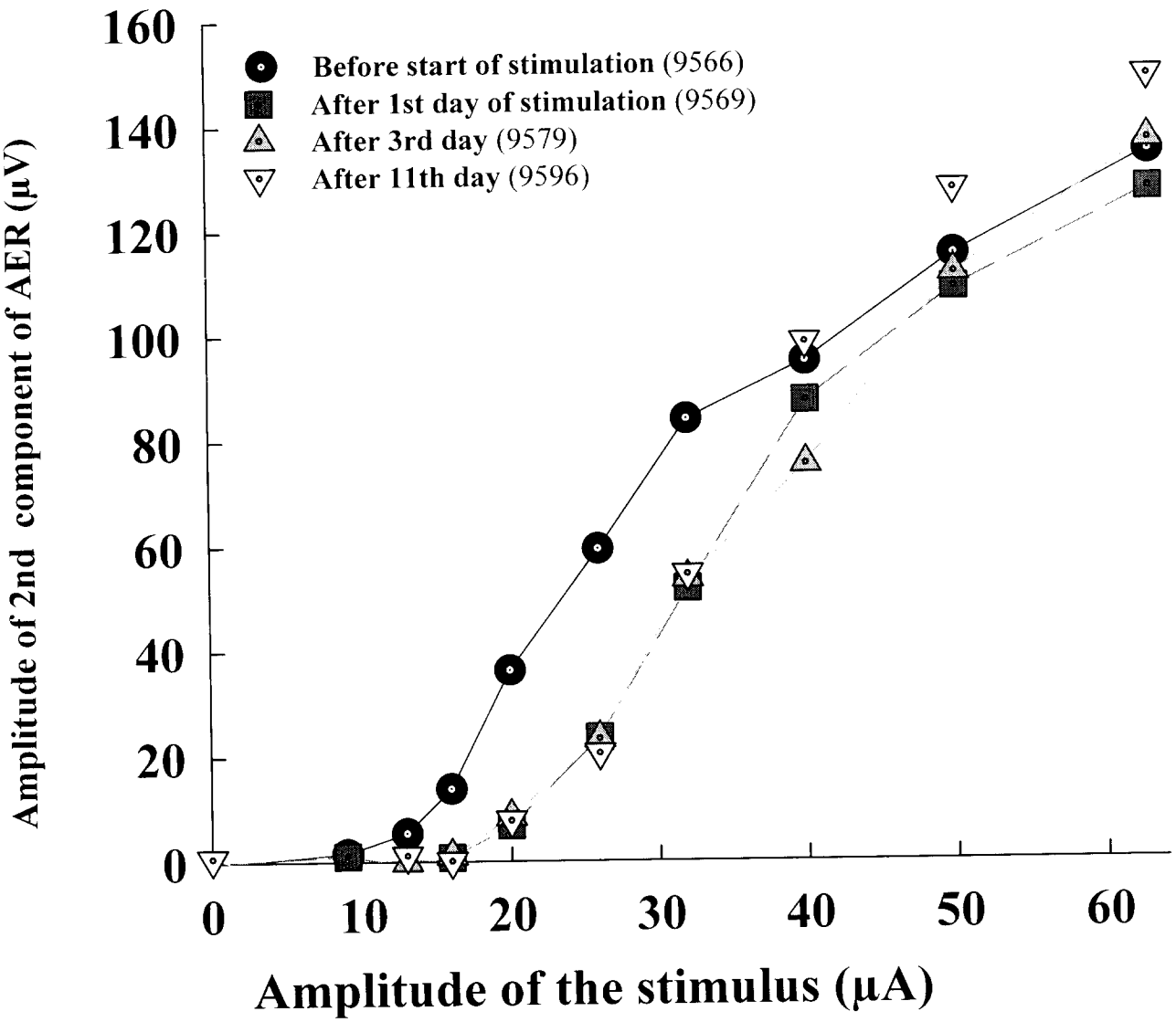
cn13113b.spw

Figure 7B

cat CN131
Electrodes 2,3 pulsed over the range of 14-48 µA, 250 Hz, 7 hrs/day, according to log-compressed artificial voice signal with 50% duty cycle

Stimulus pulse duration is 40 µs/phase

Non-embedded response evoked from electrode #2



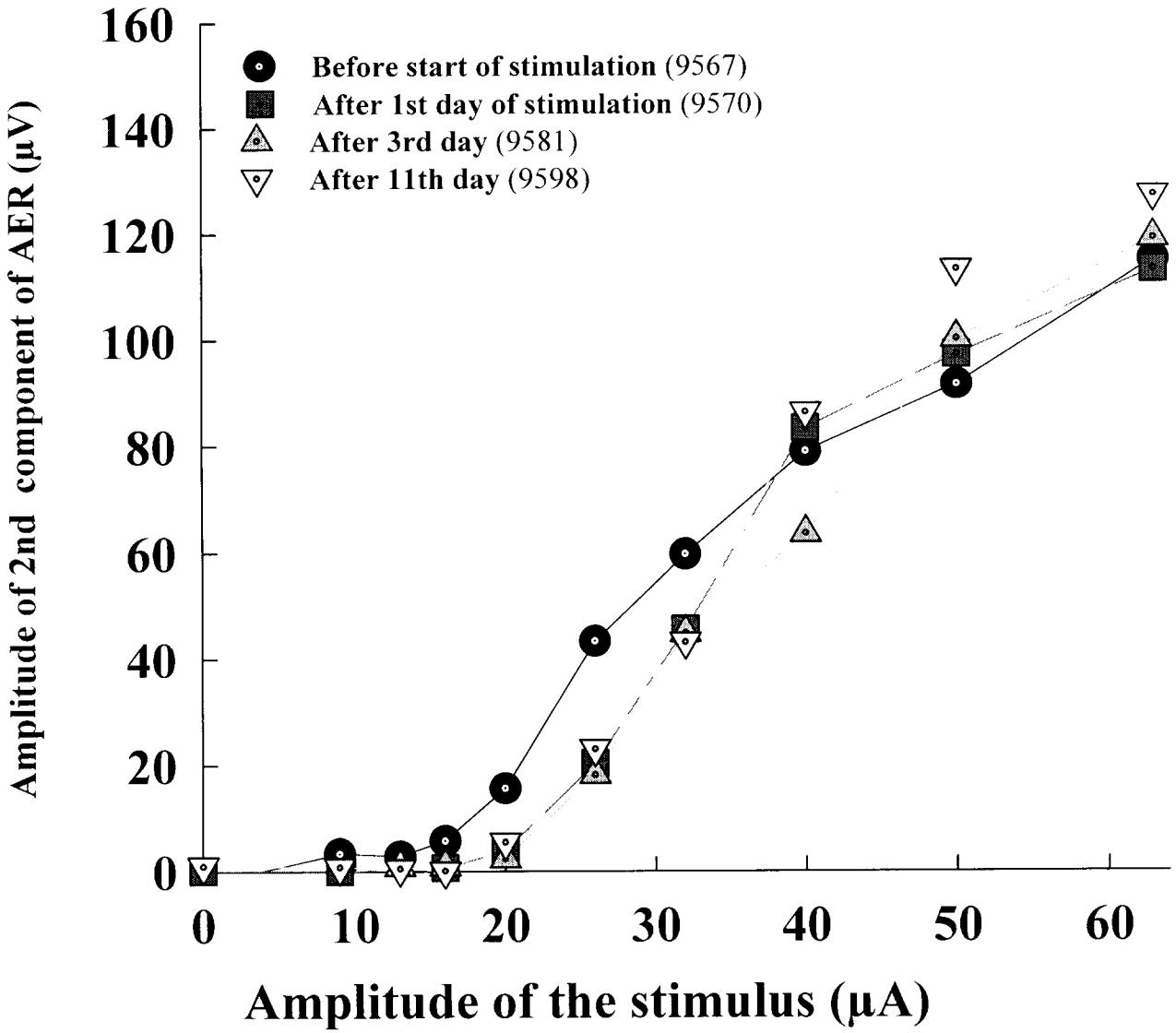
cn131o2b.spw

Figure 8A

cat CN131
Electrodes 2,3 pulsed over the range of 14-48 μA, 250 Hz, 7 hrs/day, according to log-compressed artificial voice with 50% duty cycle

Stimulus pulse duration is 40 μs/phase

Non-embedded response evoked from electrode #3



cn131o3b.spw

Figure 8B

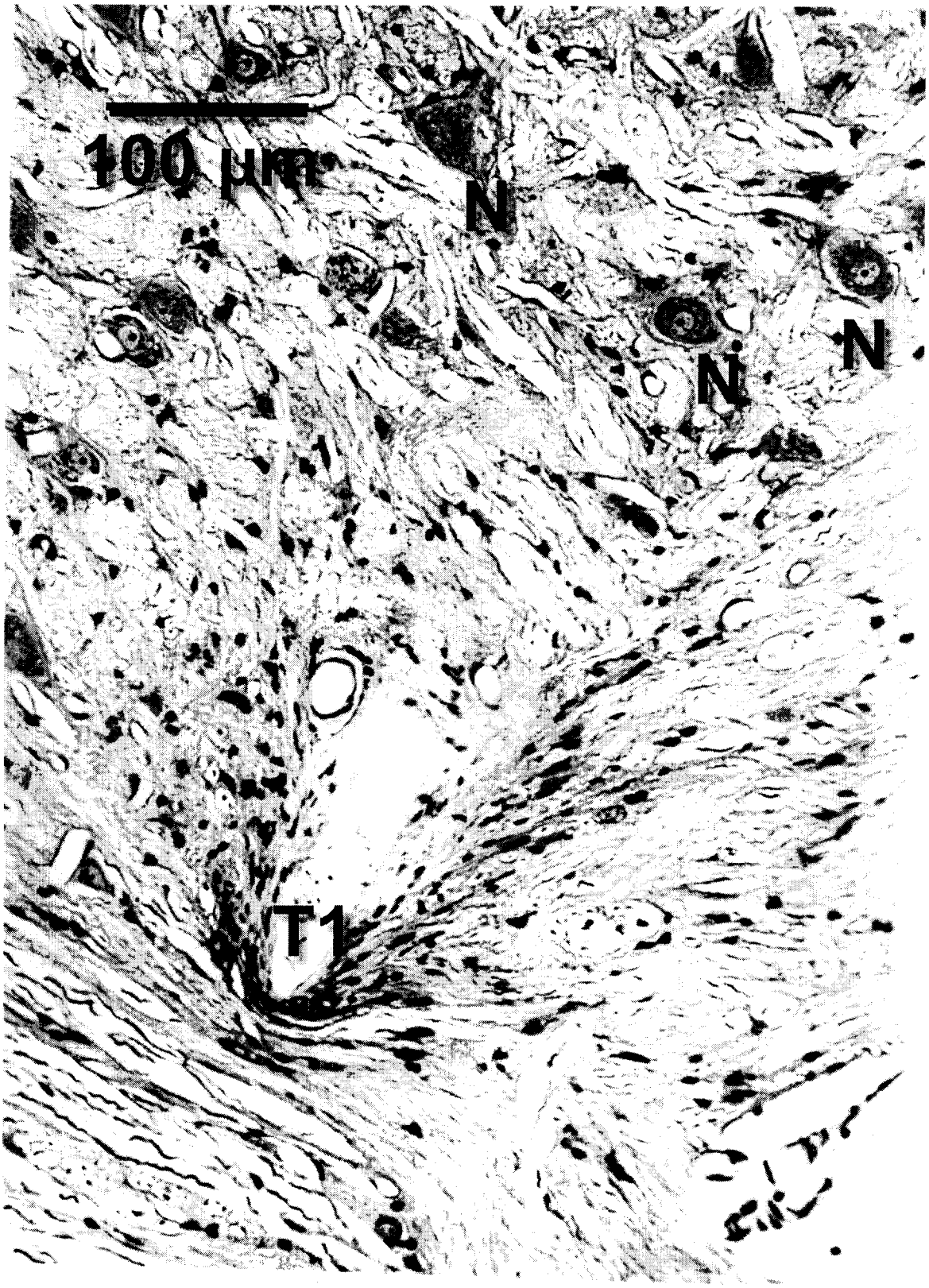


Figure 9A

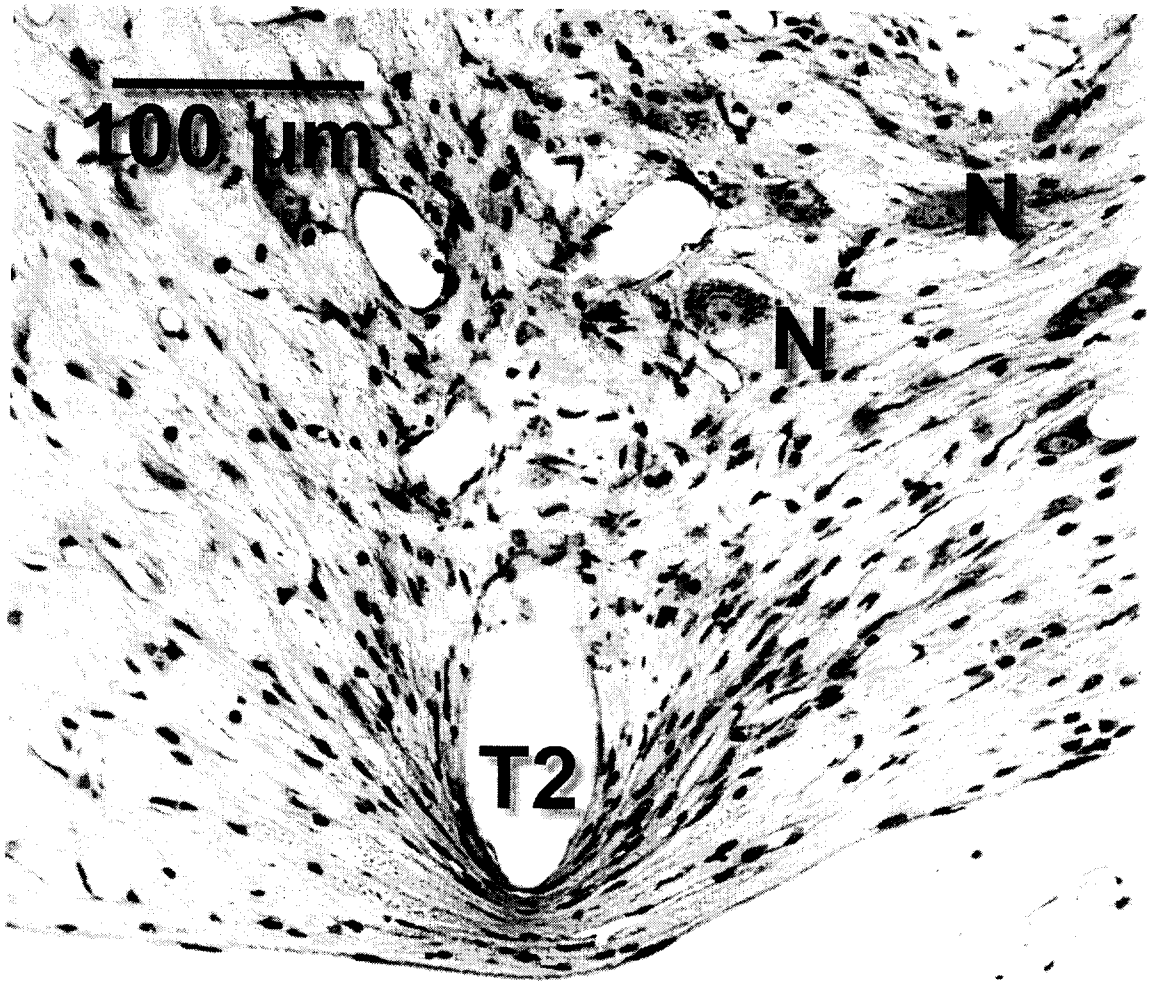


Figure 9B

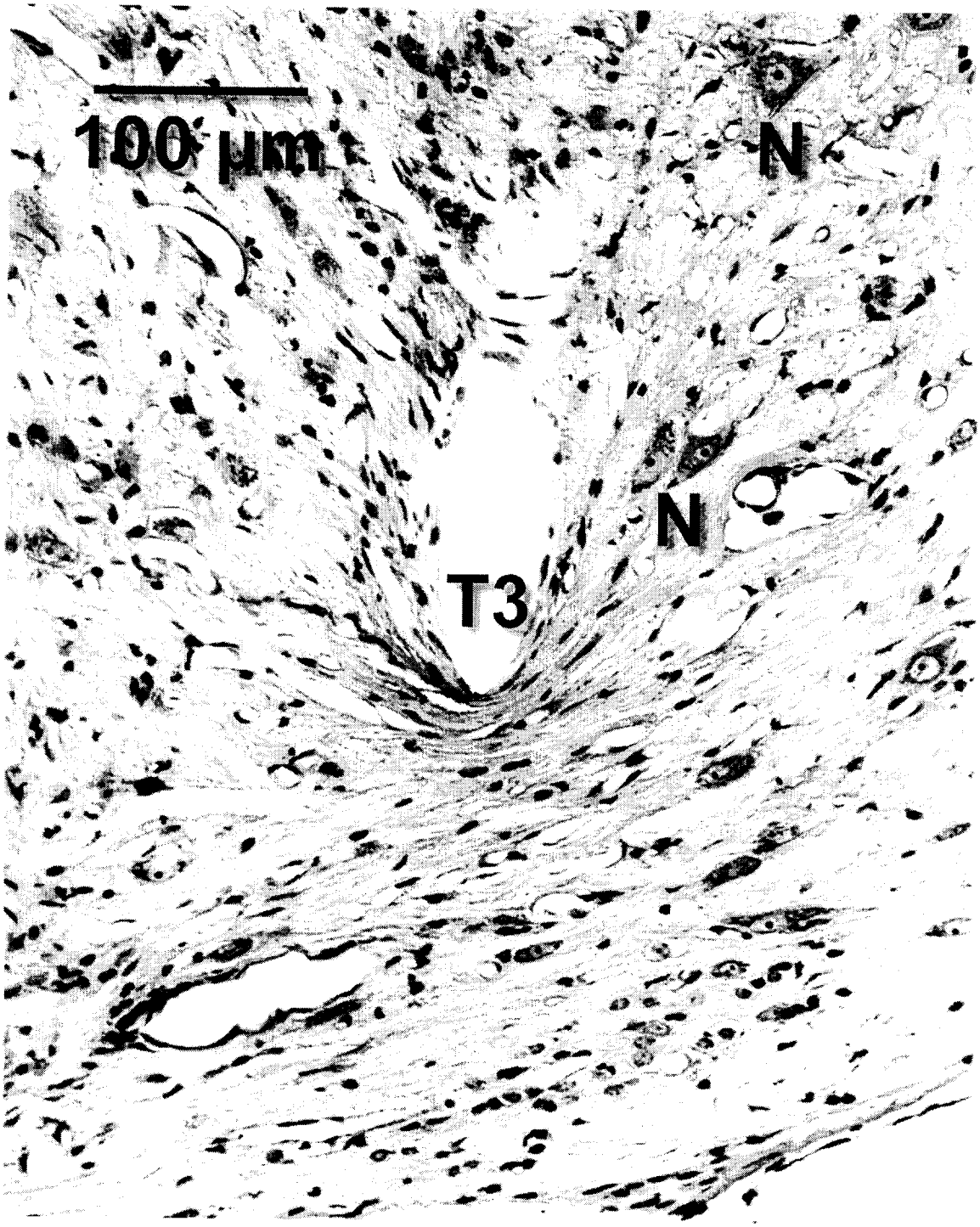
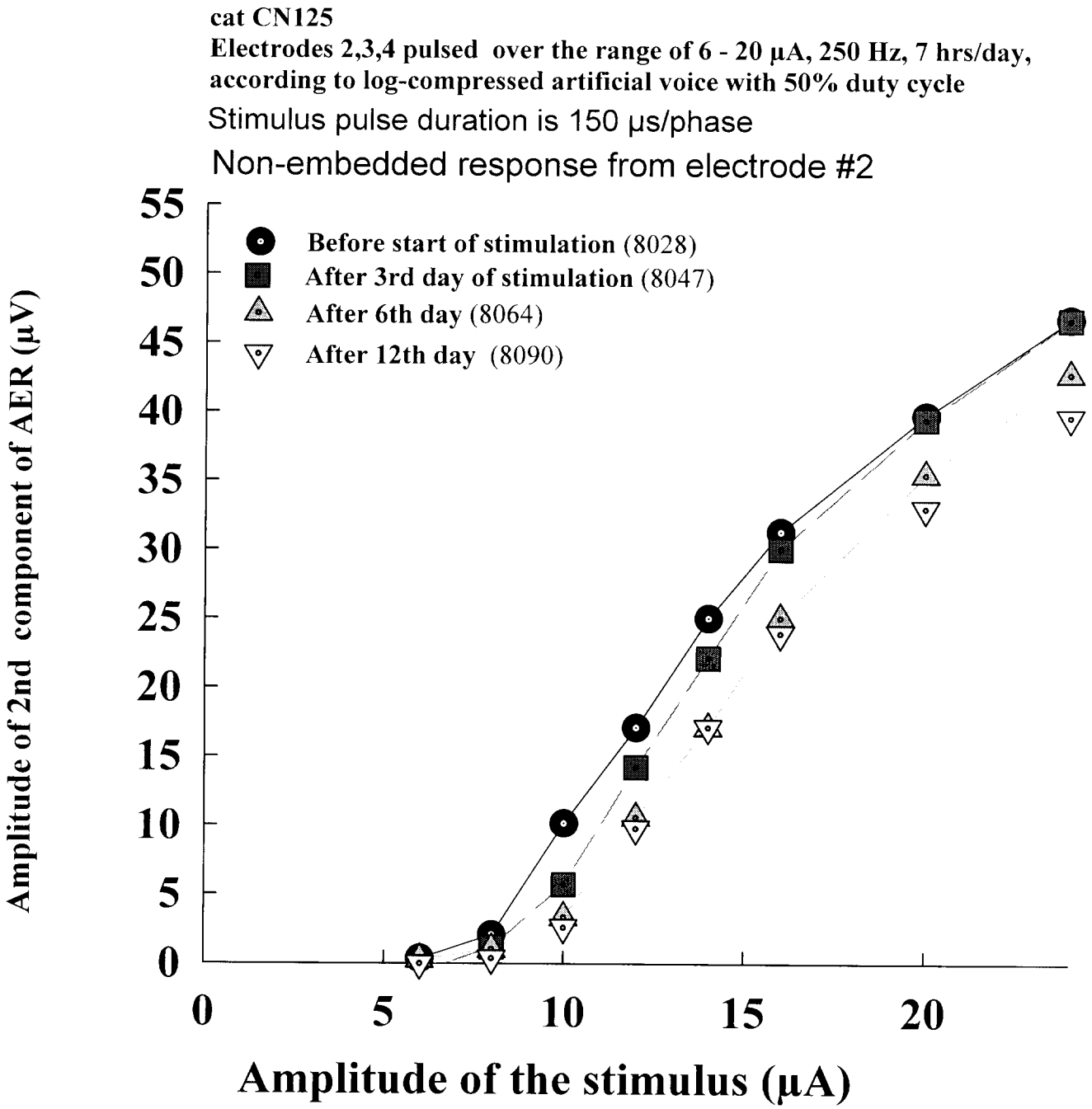


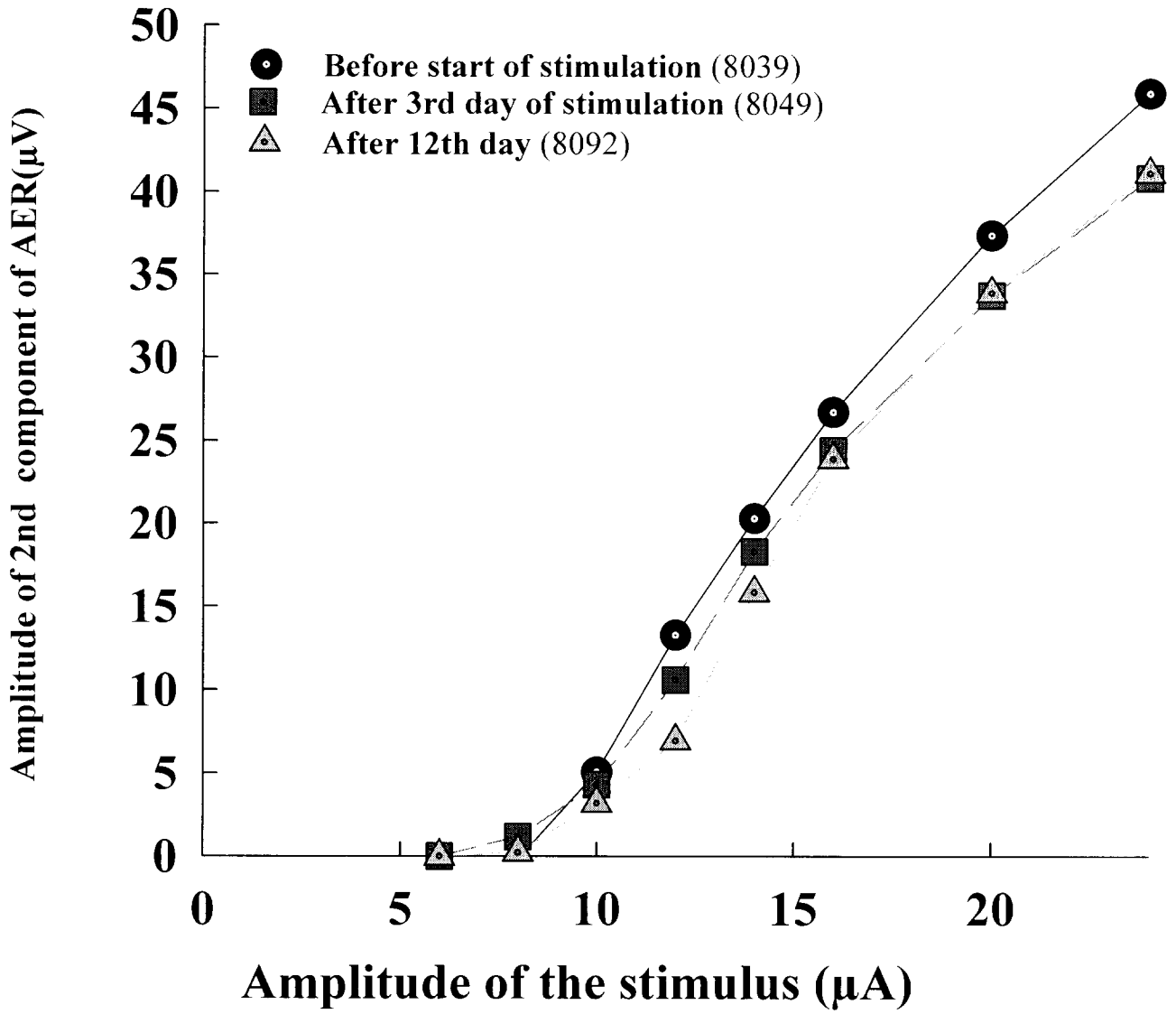
Figure 9C



cn125i2b.spg

Figure 10A

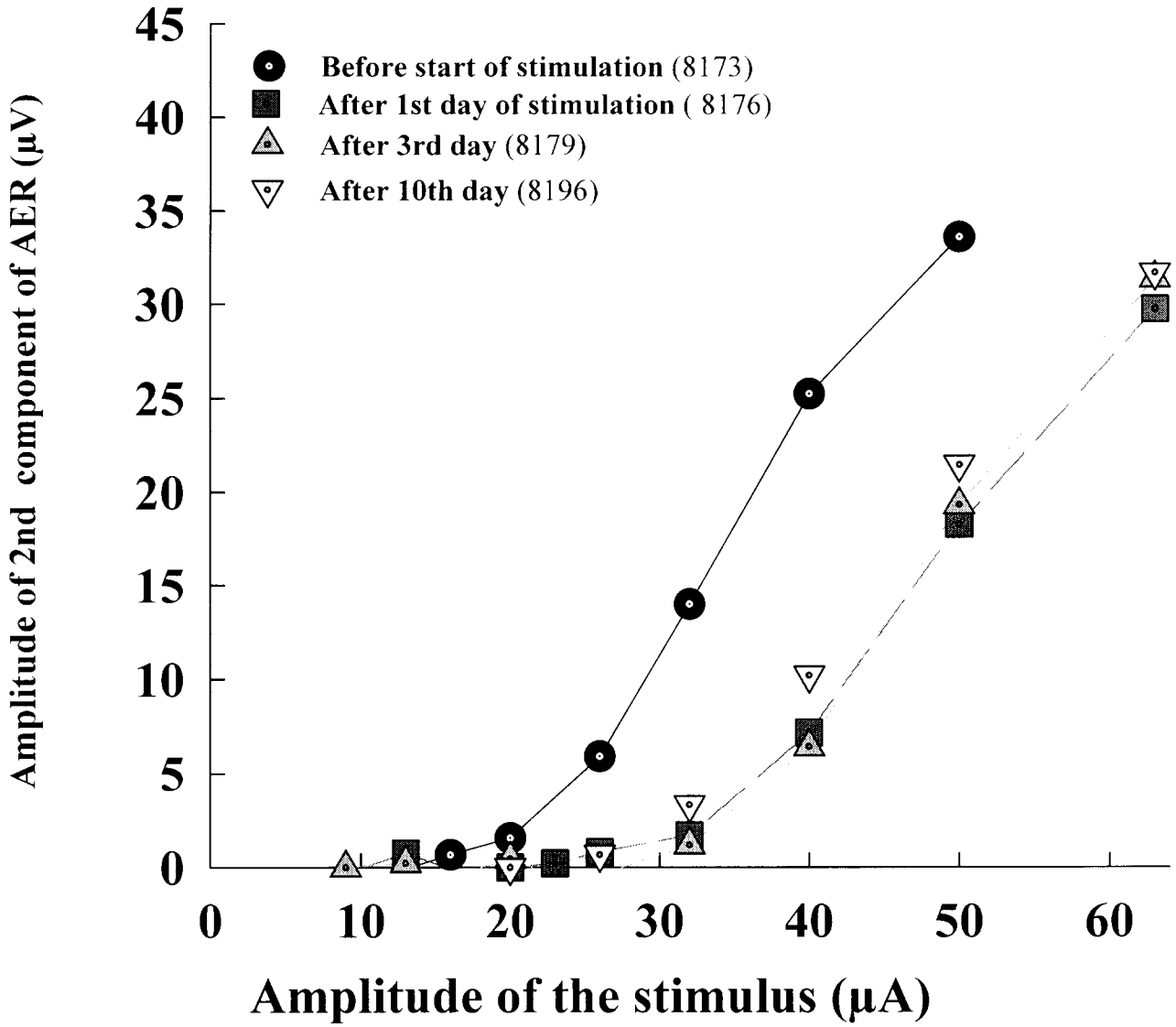
cat CN125
Electrodes 2,3,4 pulsed over the range of 6 - 20 µA, 250 Hz, 7 hrs/day, according to log-compressed artificial voice signal with 50% duty cycle
Stimulus pulse duration is 150 µs/phase
Non-embedded response from electrode #3



cn125h3b.spg

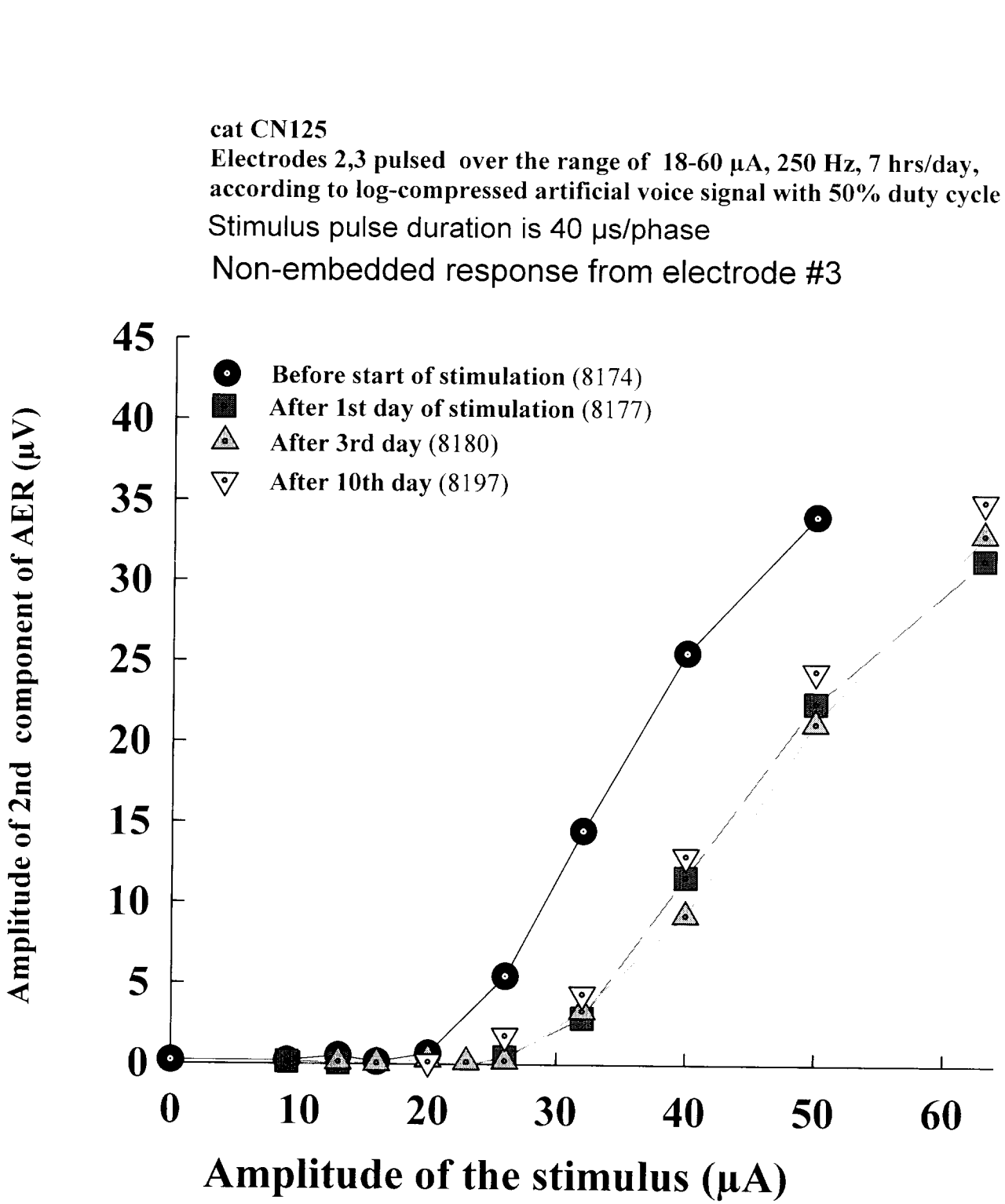
Figure 10B

cat CN125
Electrodes 2,3 pulsed over the range of 18-60 µA, 250 Hz, 7 hrs/day, according to log-compressed artificial voice signal with 50% duty cycle
Stimulus pulse duration is 40 µs/phase
Non-embedded response from electrode #2



cn125m2b.spw

Figure 11A



cn125m3b.spg

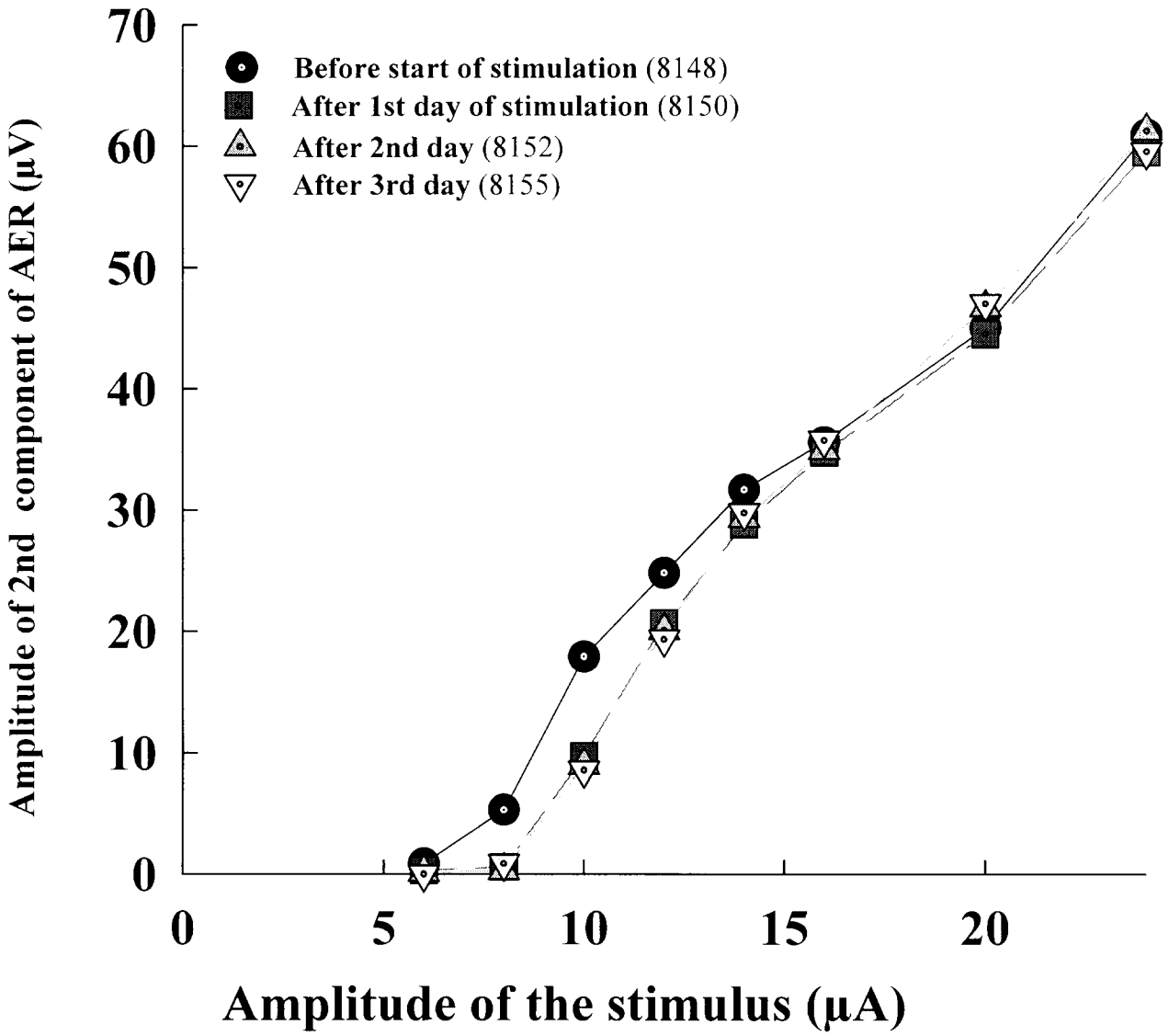
Figure 11B

Figure 12A

Figure 12B

Figure 12C

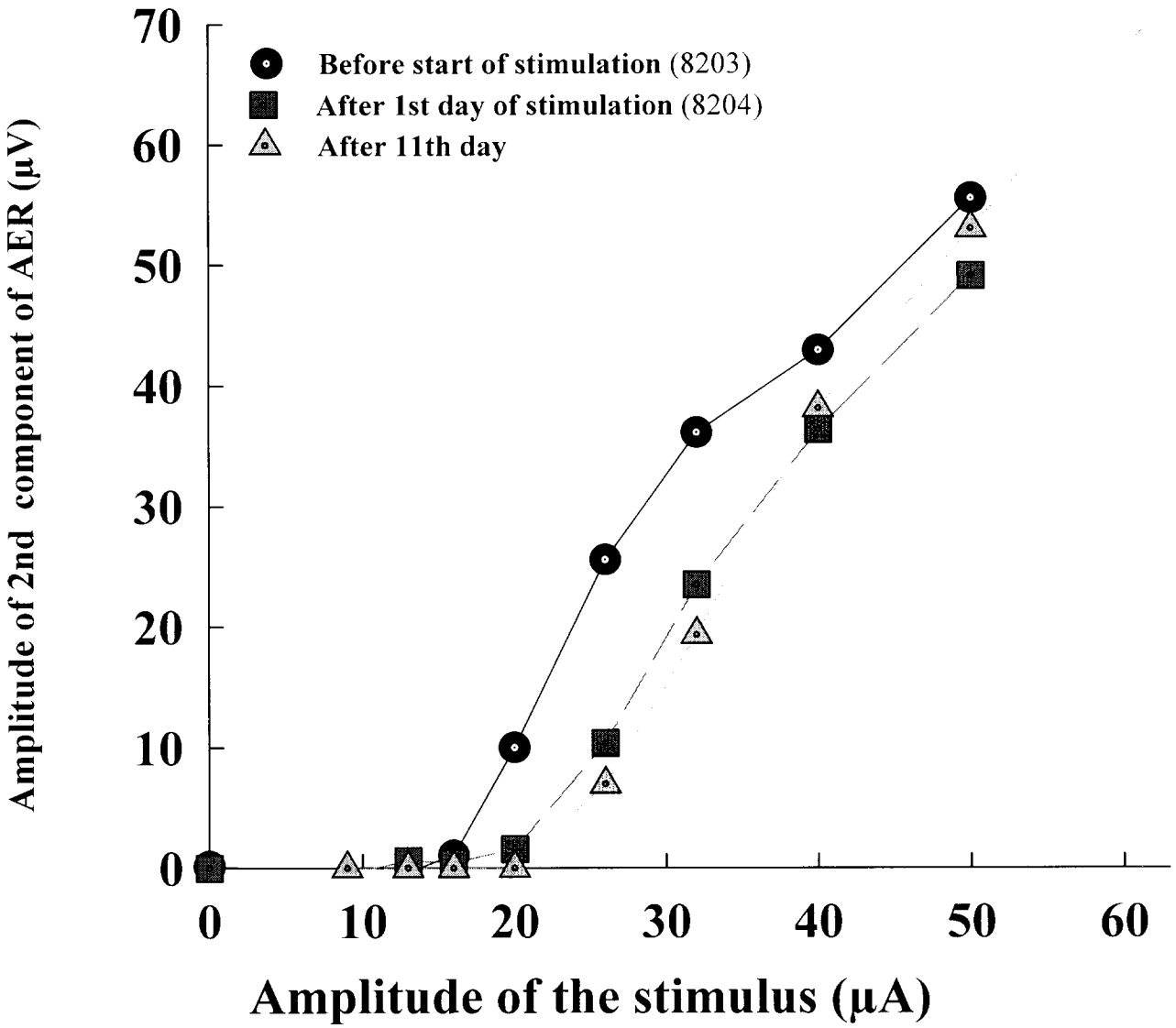
cat CN126
Electrodes 1,2,3 pulsed over the range of 6-20 μA, 250 Hz, 7 hrs/day, according to log-compressed artificial voice signal with 50% duty cycle
Stimulus pulse duration is 150 μs/phase
Non-embedded response from electrode #2



cn126f2c.spw

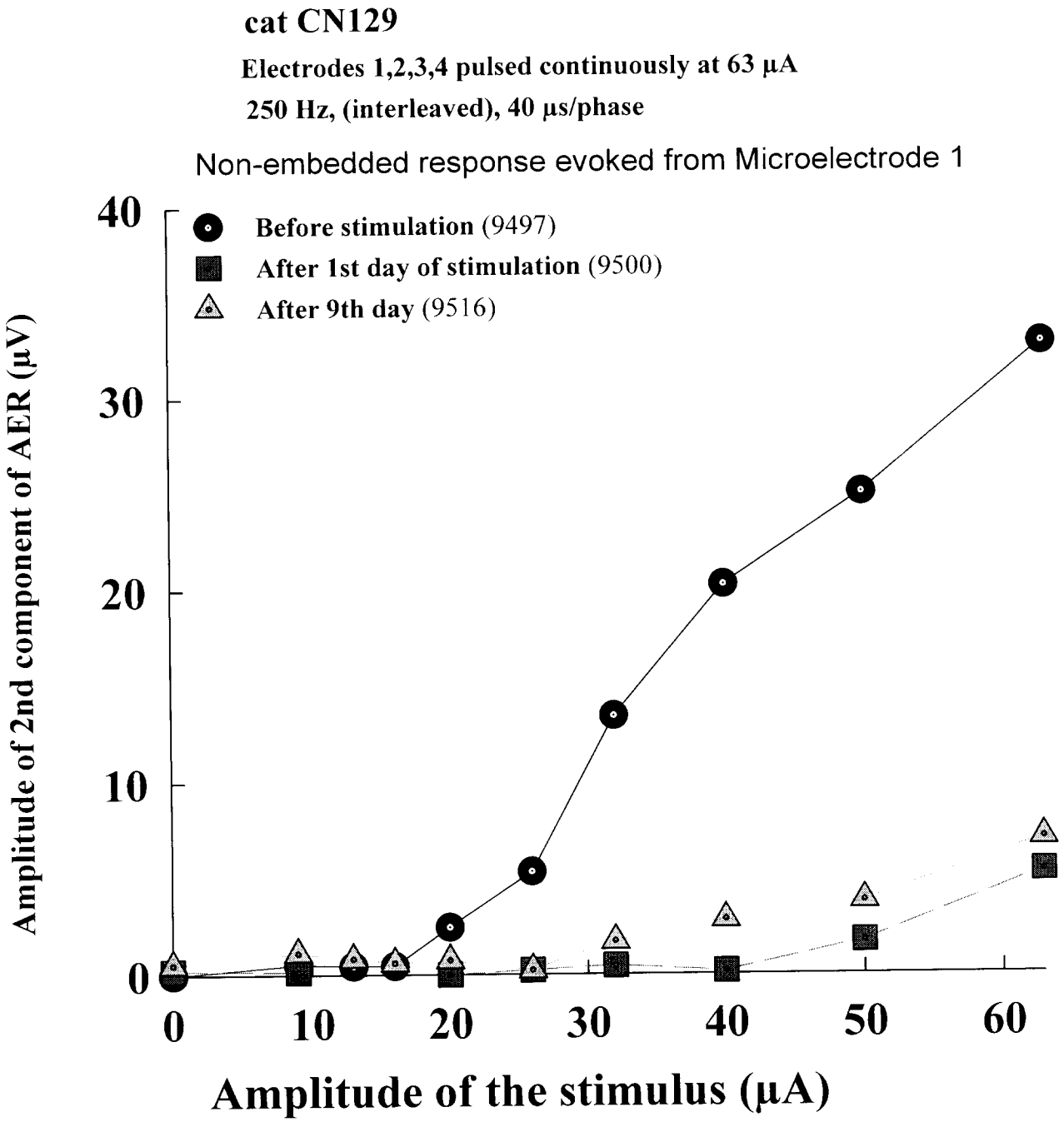
Figure 13

cat CN126
Electrodes 1,2,3 pulsed over the range of 18- 60 µA, 250 Hz, 7 hrs/day, according to log-compressed artificial voice signal with 50% duty cycle
Stimulus pulse duration is 40 µs/phase
Non-embedded response from electrode #2



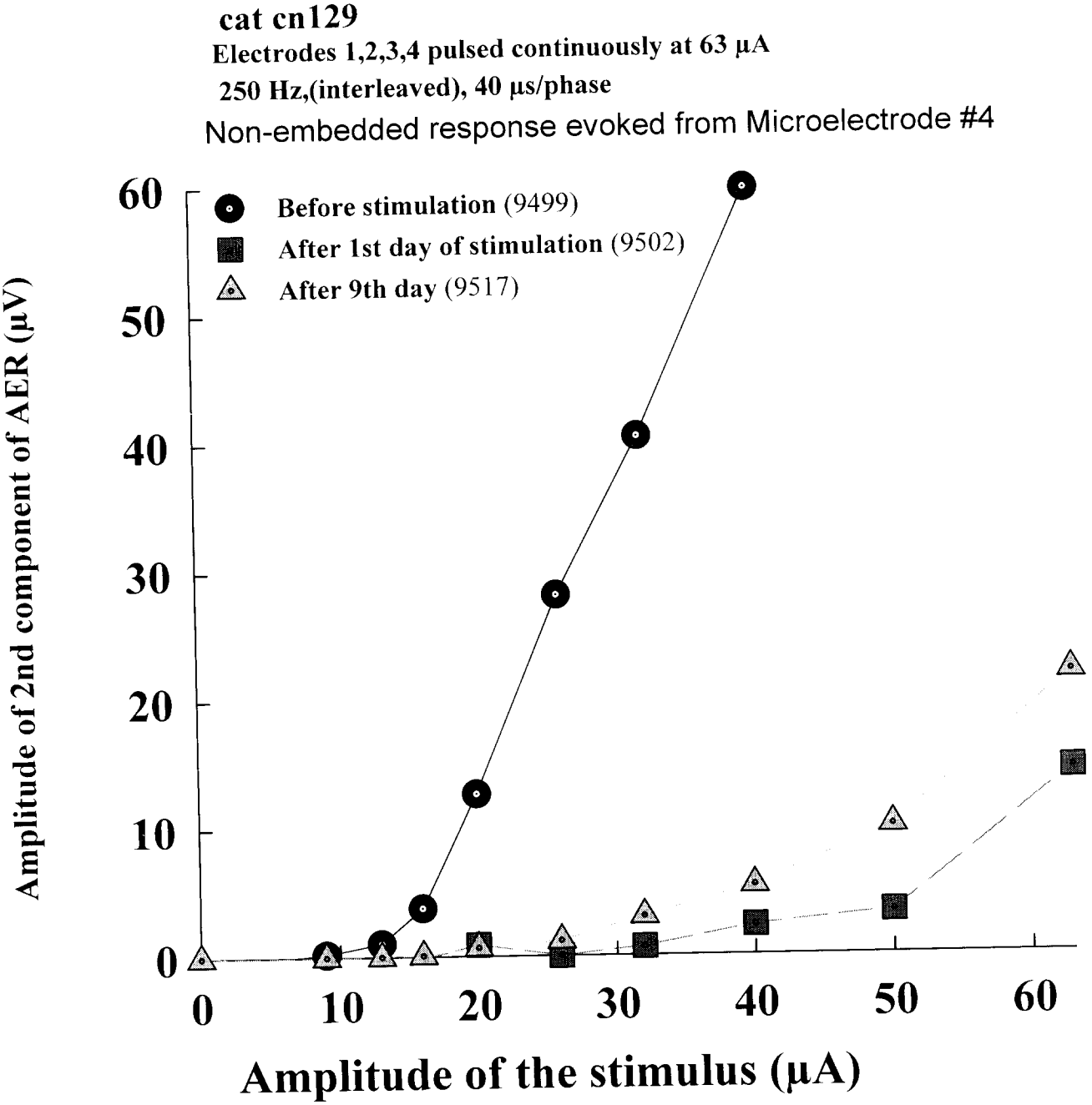
cn126k2d.spg

Figure 14



cn1291lc.spw

Figure 15A



cn129l4c.spw

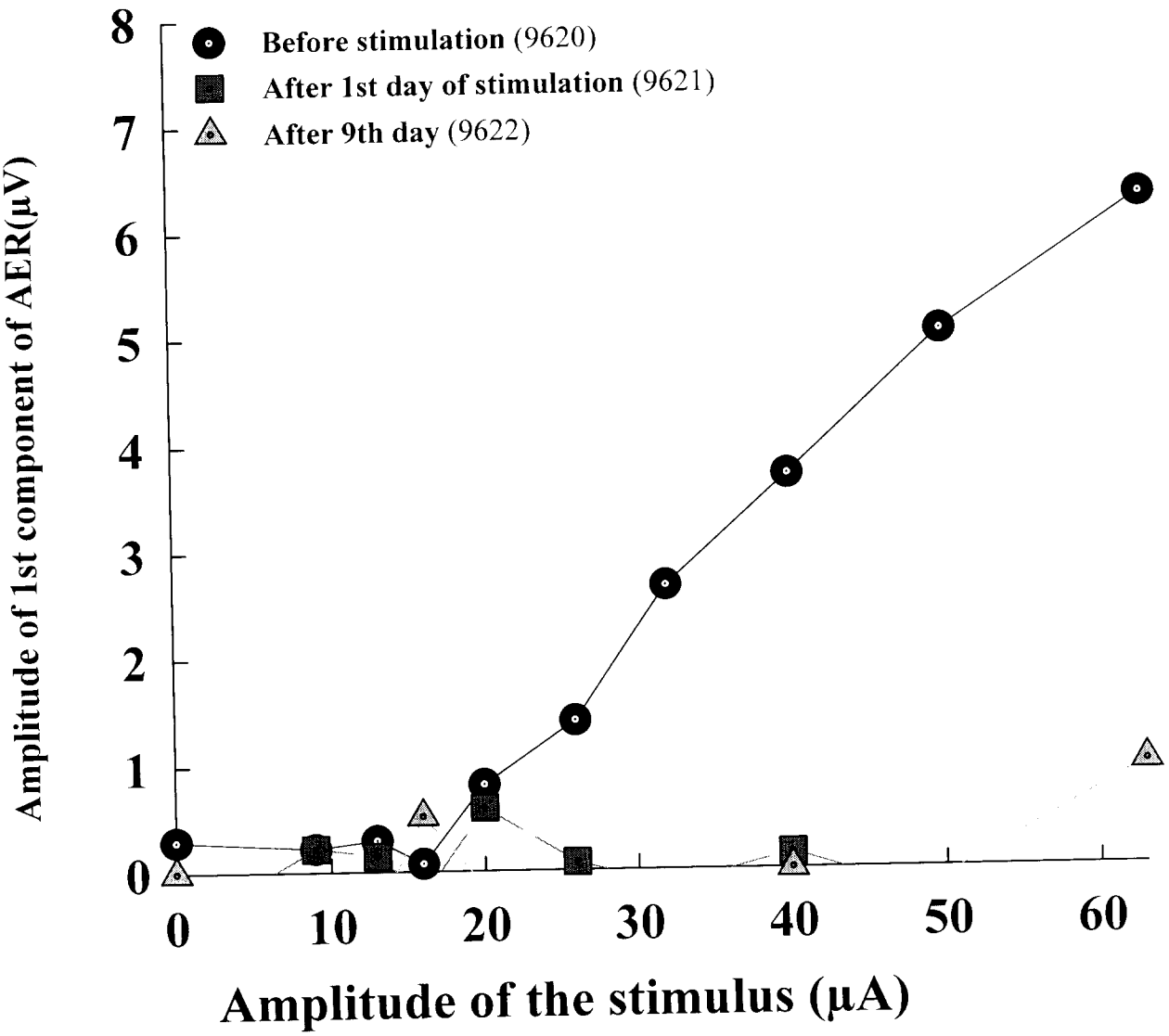
Figure 15B

cat cn129

Electrodes 1,2,3,4 pulsed continuously at 63 μA

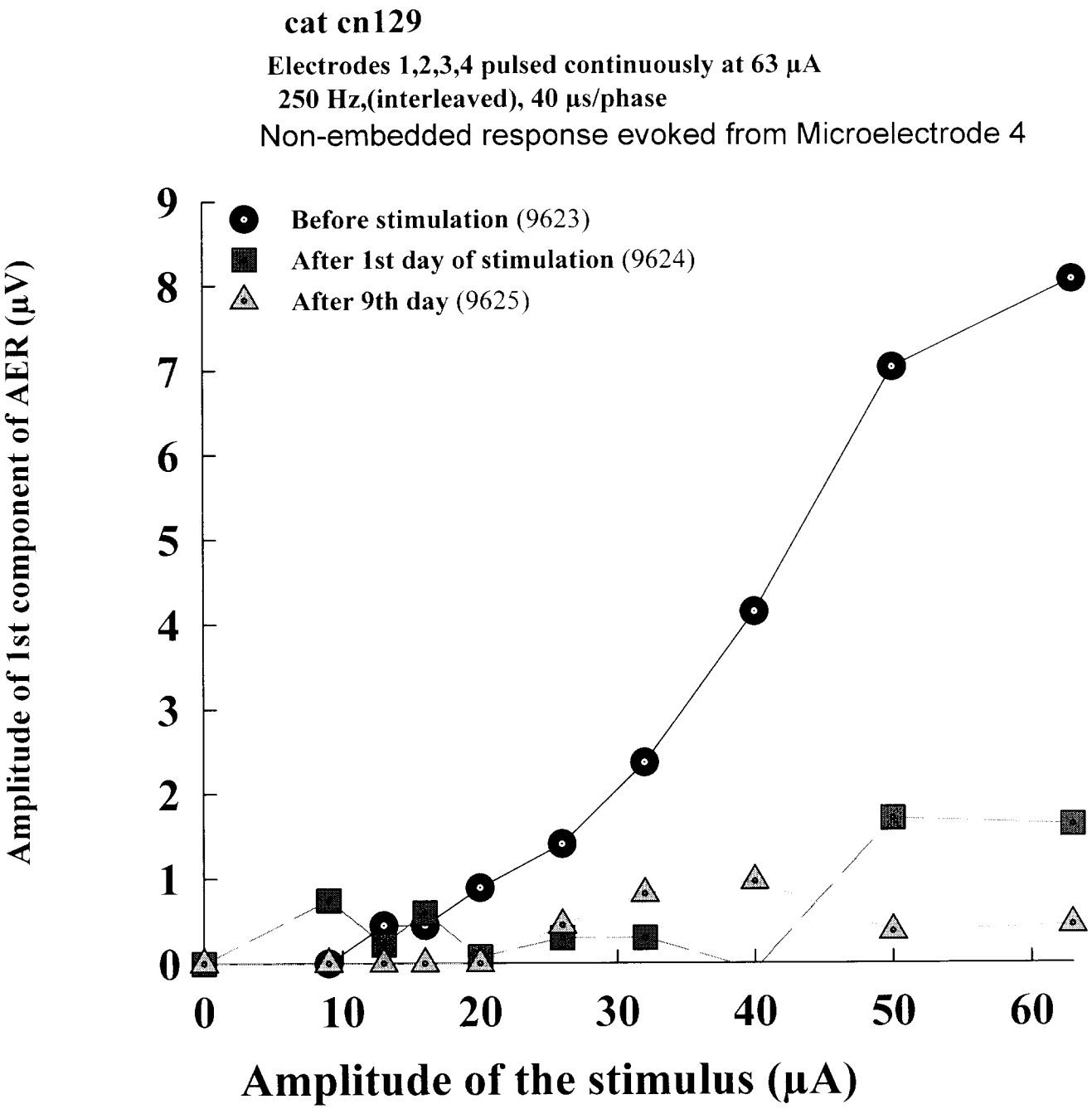
250 Hz,(interleaved), 40 μs/phase

Non-embedded response evoked from Microelectrode #1



cn129l1d.spw

Figure 15C



cn129l4d.spw

Figure 15D

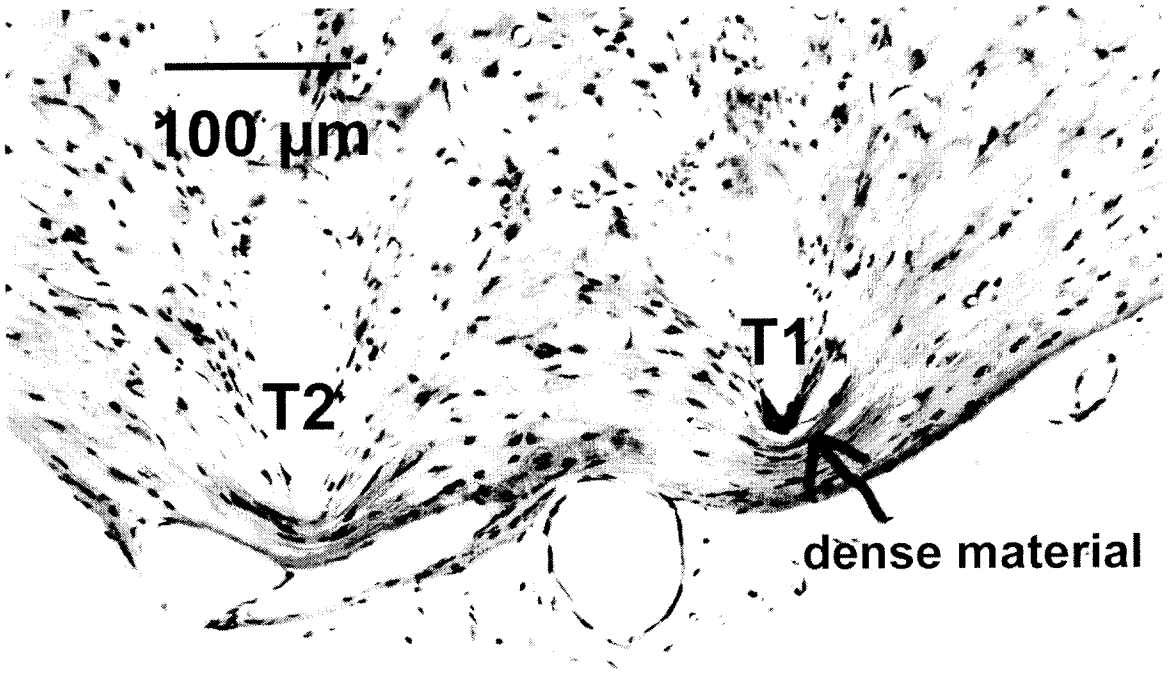


Figure 16A

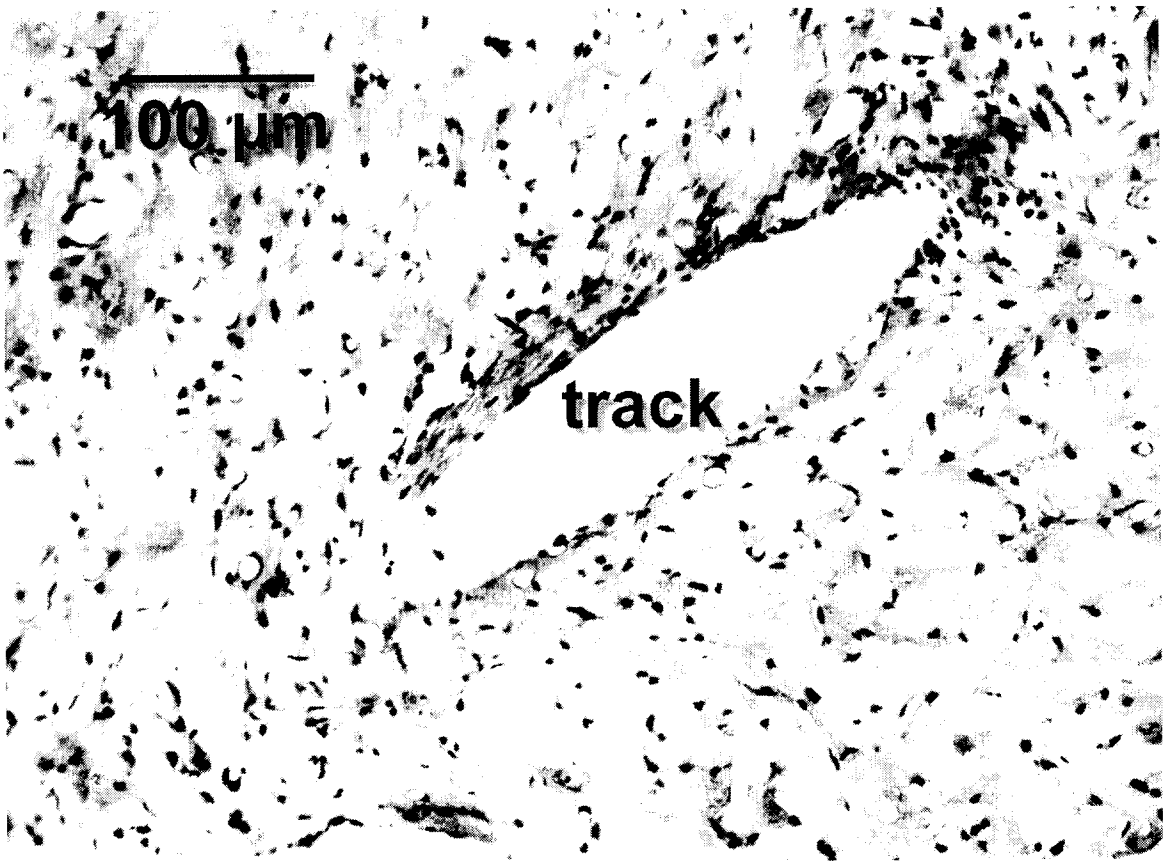
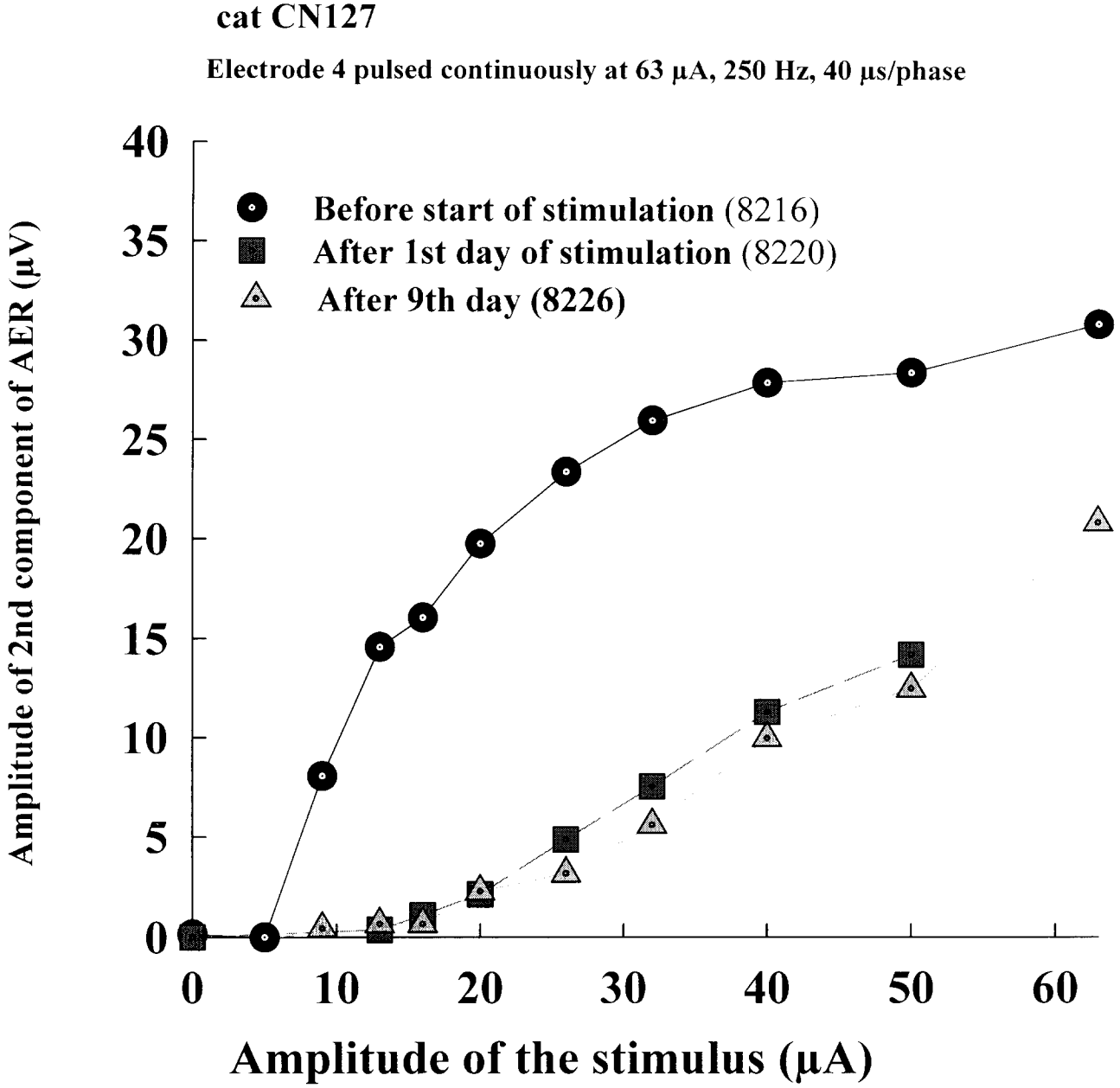


Figure 16B



cn127d.spw

Figure 17

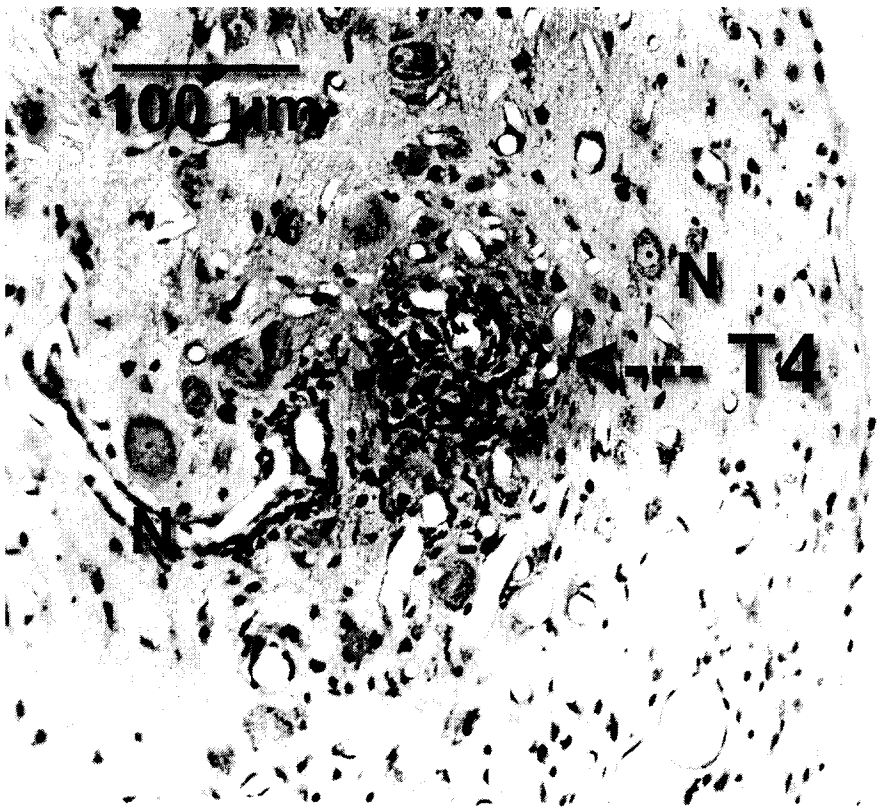


Figure 18A

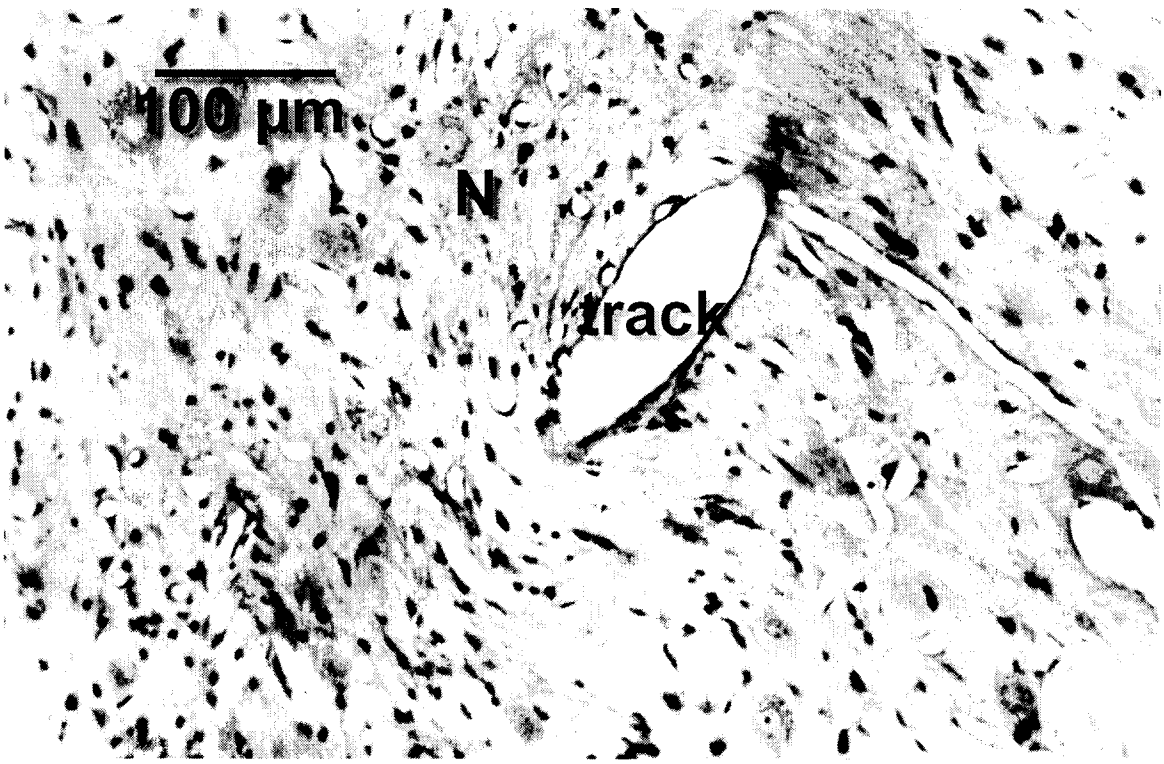


Figure 18B

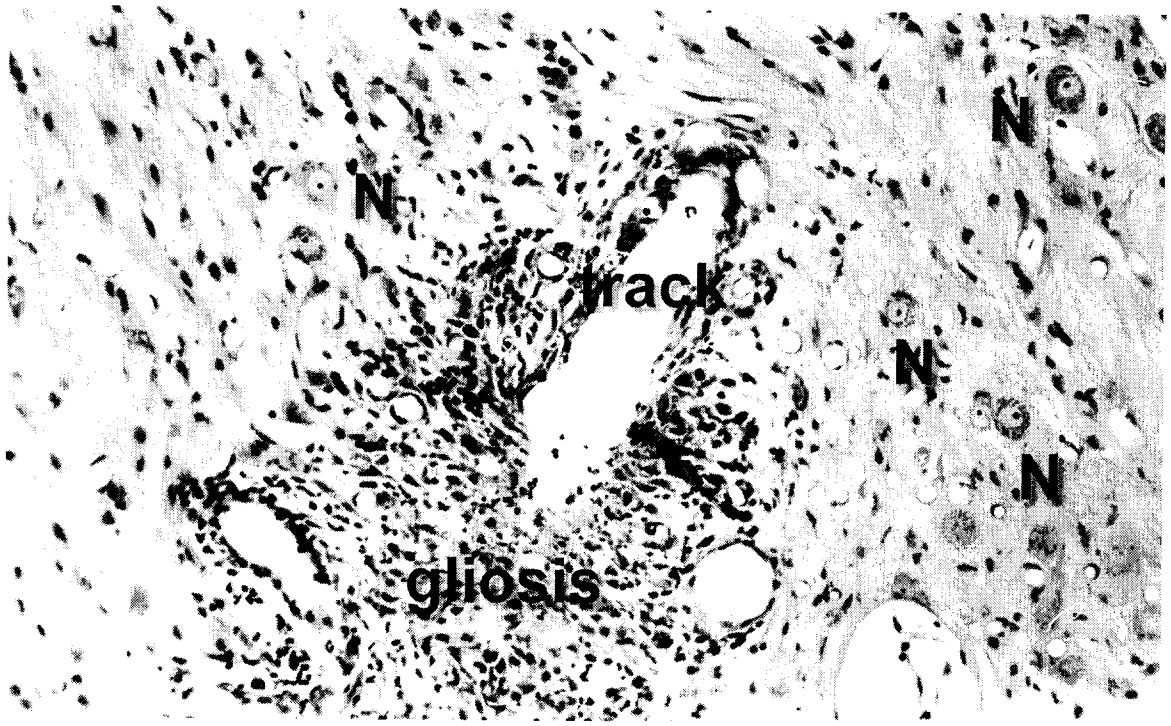
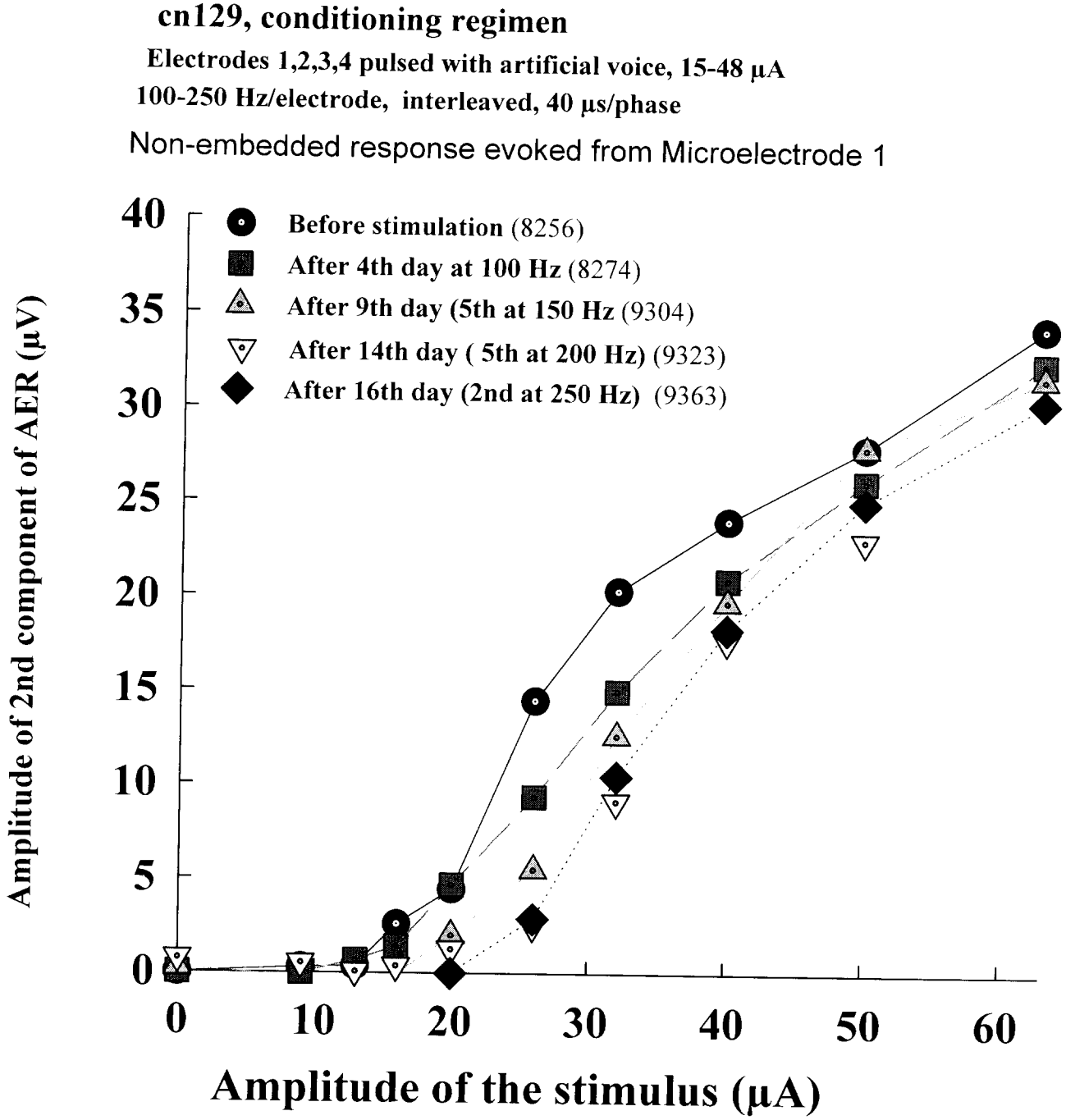


Figure 18C



cn129j1a.spw

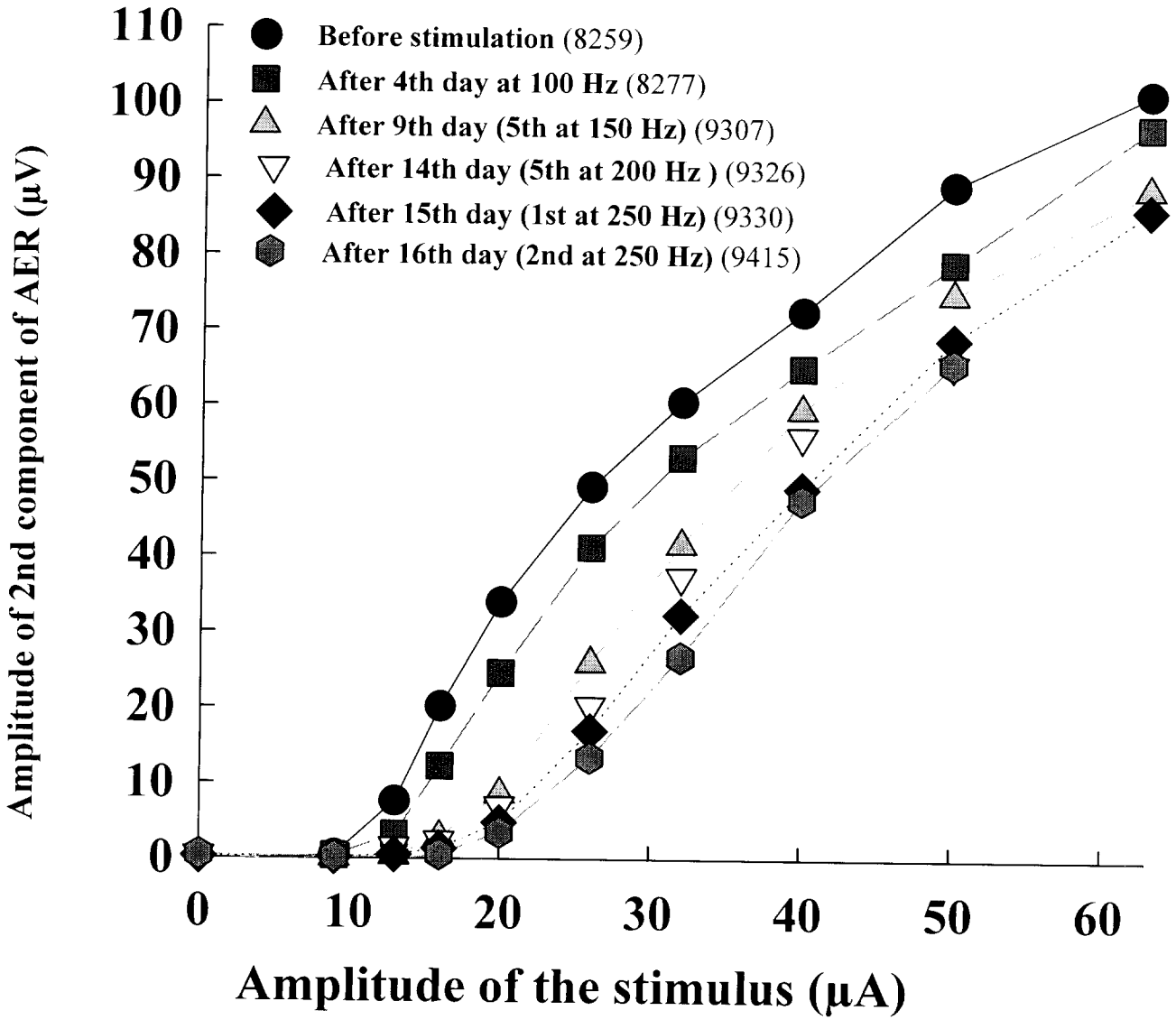
Figure 19A

cn129, conditioning regimen

Electrodes 1,2,3,4 pulsed with artificial voice, 15-48 μA

100-250 Hz/electrode, interleaved, 40 μs/phase

Non-embedded response evoked from Microelectrode 4



cn129j4a.spw

Figure 19B