## N-Form® 3D Microelectrode Array Chronic Recording Performance in 3 Cats (~10 Months)

The challenge of long duration 3D chronic recording has hindered neuroscience for decades. Many factors contribute to long duration chronic recording performance including reliable manufacture of electrodes [1], [2], insertion [3], proximity to neurons, and bodily response [4].

### Experiments:

Three N-Form microelectrode arrays were implanted in the auditory cortexes of three cats at a customer's lab between January of 2015 and December of 2015. The surgical insertion did not utilize the N-Form insertion system, as it was not yet available.

### Results:

N-Form 3D microelectrode arrays demonstrated long-duration chronic reliability and 3D recording performance across three awake restrained cats. The literature does not contain a 3D demonstration of the reliability, performance, and duration recorded in these long-term chronic experiments [4]–[9][1]. An overview of the performance of the N-Form 3D microelectrodes is shown below; the customer provided the raw data. Within each animal's 32 channel electrode, a significant number of sites recorded single units or potential multi-units for ~ 10 months. Each cat experiment was dedicated to the customer's goals and ended for reasons other than microelectrode performance. A more detailed summary of the data is in Appendices A, B, and C.

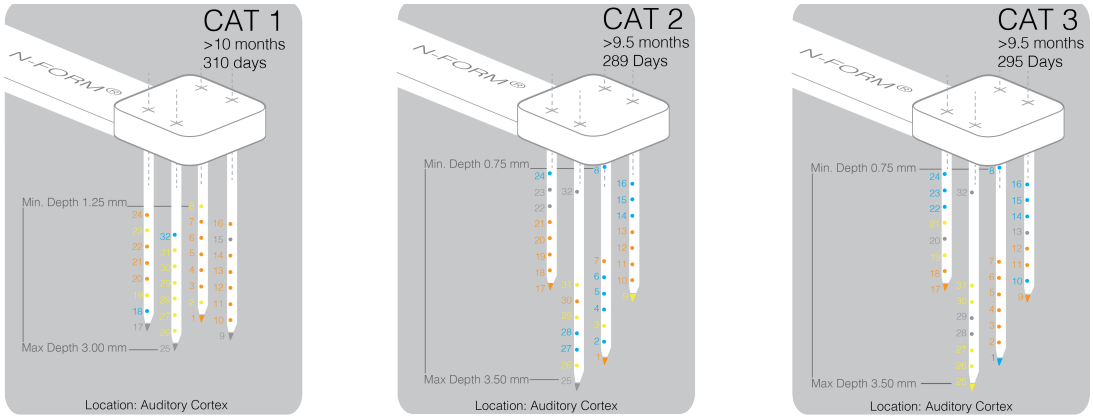


Figure 1: Overview of the performance of N-Form 3D microelectrodes across three cat experiments.
**Orange = single unit or potential multi-unit recording**
**Yellow = evidence of multi-unit recording**
**Blue = functioning site (based upon RMS noise levels)**
**Grey = a site of indeterminate functionality**

[1] Definitions: 3D electrode defined as an electrode with ≥ 3 shanks assembled within multiple planes and multiple recording sites along the side of each shank at significant distances from the tips of the shanks. Long-term chronic duration defined as ≥ 6 months. Reliability defined as a mean ≥ 87.5% of sites functioning across multiple 3D arrays and experiments. Performance defined as a mean ≥ 41% of chronic 3D arrays' sites producing single unit or multi-unit recordings across multiple experiments (range: 34% - 50%). Note: no assistive technology was used in any experiment (e.g., viral modification, bioactive gels, etc.).

### *In vivo* recording and channel functionality:

A customer recorded raw data from the three cats during experiments exploring the auditory system in 2015. The analysis of the N-Form was constrained by the protocol of this customer's experiments. Modular Bionics Inc. used the supplied raw data to calculate signal-to-noise ratio (SNR), inter spike interval (ISI), and peristimulus histogram (PSTH) plots. The reader is encouraged to review the underlying data for all channels within appendices A – C.

Spike selection criteria

Techniques categorizing waveforms as spikes vary widely in the literature; the data presented here are separated into two categories:

1. Conservative spike sorting.
2. Conventional spike sorting.

Signal-to-noise ratio (SNR) was defined as:

SNR = peak-to-peak amplitude of mean waveform / peak-to-peak amplitude of noise floor

**Peak-to-peak amplitude of noise floor was estimated as six times the root mean square (RMS) noise level** [10] (i.e., SNR greater than 1 means a spike projects above the noise floor and can be selected with a simple threshold). **The SNRs reported here are three times the magnitude of a SNR definition of only two times the RMS noise level** (e.g., [4], [6], [8]).

Spike detection was performed using an amplitude threshold for each channel. Features were extracted using wavelets and then grouped using a super-paramagnetic clustering algorithm [11]. Manual review of the resulting clusters' spike panels selected the most promising waveform shape. In some cases, more than one spike panel had a promising waveform (i.e., potential multiple single units on one channel); for simplicity, only the most promising waveform is presented here. Correlated noise was removed prior to sorting.

PSTHs and ISI histograms were generated from each cluster. PSTHs were reviewed to determine if a cluster had a relationship with the stimulus. The ISI histograms were used to evaluate the clusters' firing frequencies and their absolute and relative refractory periods. The PSTHs and ISI histograms were used to evaluate potential neural signals; some neural signals might have been composed of multi-unit activity (i.e., no observable refractory period) and / or might not have responded to the stimulus.

**1.Conservative spike sorting criteria to identify neural signals (Orange)** [10]**:**
**Note: Noise used for calculation of SNR = 6 x RMS of noise; resulting in a significantly conservative (i.e., lower) SNR (versus 2 x RMS of noise).**

- Waveform shape (average and raw waveforms)
- Average SNR = 2.74 ±1.36 (min: 1.12, max: 7.37)
- Minimum firing rate of 1 spike/s
- ISI evaluated as indicator of single unit activity
- PSTH evaluated as indicator of neural activity

**2. Conventional spike sorting criteria (Yellow)** (e.g., [4], [6], [8])**:**
**Note: Noise used for calculation of SNR = 6 x RMS of noise; resulting in a significantly conservative (i.e., lower) SNR (versus 2 x RMS of noise).**

- An SNR greater than 1
- A minimum firing rate of 0.2 spikes/s [5]
- ISI referenced
- Evaluated as background activity (PSTH not a criteria)

Determination of site functionality by comparison of RMS

Site impedance is a common parameter to assess site functionality. In the absence of impedance data, RMS noise level can indicate a site's impedance relative to other sites on an electrode [12]. To assess functionality, sites were grouped into two categories: sites with and without neural signals. The conservative spike sorting criteria were used to select sites with neural signals. Sites without neural signals were considered functional if their RMS noise level was within ±2 standard deviations of the mean of sites with neural signals. Sites outside this range were considered indeterminate.

## Brief Discussion of Results:

Sites along the sides of shanks within a 3D microelectrode array[1] have not previously demonstrated neural recordings over an ~10 month duration in the literature. The chronic performance of the custom configured N-Form sites described here demonstrated a new benchmark within three-dimensional chronic neural recording.

Note: within the literature, sites along shanks recording ≥10 month chronic durations required viral modification [5].

## Why did a subset of N-Form sites not produce single unit or multi-unit recordings?

Many factors contribute to long duration chronic recording performance including reliable manufacture of electrodes [1], [2], insertion [3], proximity to neurons, and bodily response [4].

- The N-Forms were delivered to the customer with functional sites. The electrodes might have been mishandled and damaged during inspection or surgery.

- The N-Forms used in these experiments did not utilize the N-Form insertion system, as it was not yet widely available. The use of an independent insertion system might have increased the damage to tissue during insertion.

- Sophisticated anatomical targeting equipment was not used within these experiments. The N-Forms might not have been implanted accurately, leading to proximal sites within tissue with fewer or no neurons. Any site might have been implanted in a region with little to no neural activity during the brief recording sessions.

- The N-Form implanted in Cat 1 contained 4 shanks of the same length, with sites positioned to be within the targeted auditory cortex. The N-Forms implanted in Cats 2 and 3 had varying shank lengths to compensate for an inaccurate depth of implantation. A subset of sites along the N-Form shanks implanted in Cats 2 and 3 were outside of the targeted auditory cortex (based upon estimation of the depth and size of the auditory cortex).

- The response of the CNS to foreign bodies varies among species and individual animals. Glial scarring and other response mechanisms might have varied significantly around each shank and / or electrode array. The inconsistency of this response might have led to significant increases in impedances and / or the distance between sites and nearby neurons.

## Bibliography


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**Appendix A**
**Cat 1**
Diagram of N-Form 3D electrode implanted in Cat 1 and a summary of data recorded from Cat 1 by the N-Form 3D microelectrode.

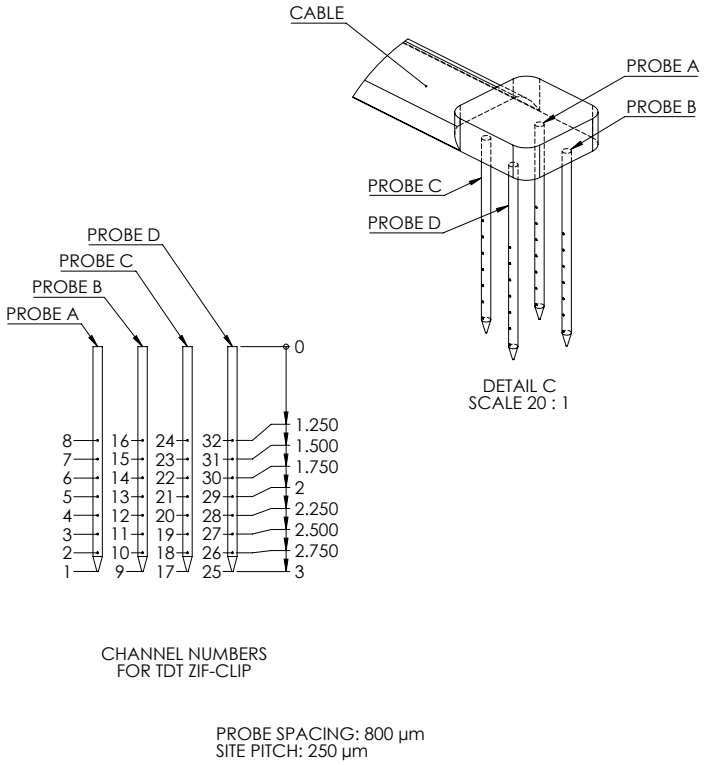


Figure A1: Diagram of N-Form electrode implanted in Cat 1

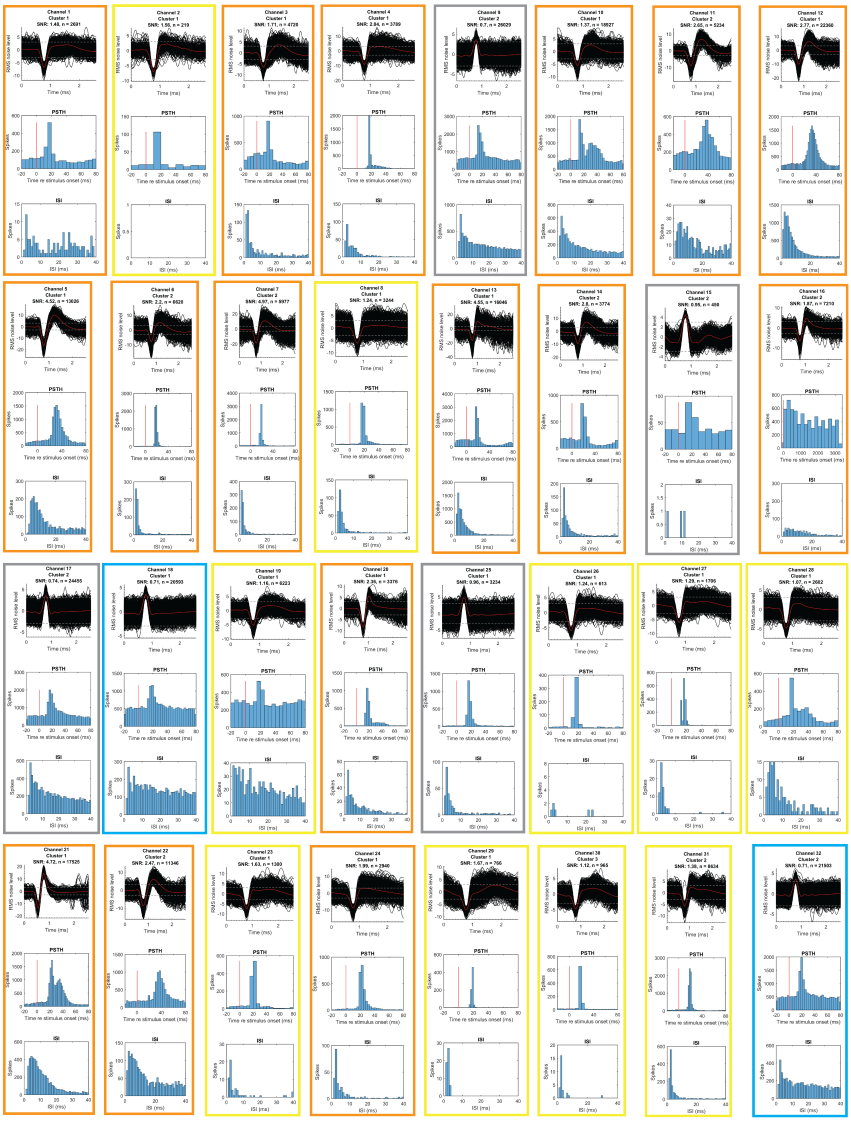

Figure A2: Summary of data recorded from Cat 1 by an N-Form 3D microelectrode. Orange border signifies a single unit or potential multi-unit recording, yellow border signifies evidence of multi-unit recording, blue border signifies a functioning site based upon RMS noise level, and grey border signifies a site of indeterminate functionality.

**Appendix B**
**Cat 2**
Diagram of N-Form 3D electrode implanted in Cat 2 and a summary of data recorded from Cat 2 by the N-Form 3D microelectrode.

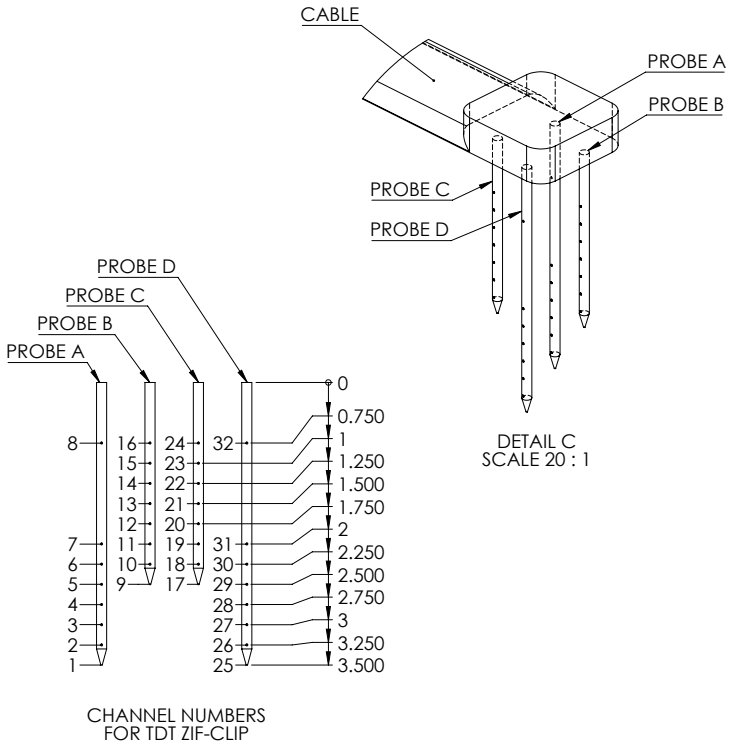


Figure B1: Diagram of N-Form electrode implanted in Cat 2

Figure B2: Summary of data recorded from Cat 2 by an N-Form 3D microelectrode. Orange border signifies a single unit or potential multi-unit recording, yellow border signifies evidence of multi-unit recording, blue border signifies a functioning site based upon RMS noise level, and grey border signifies a site of indeterminate functionality.

**Appendix C**
**Cat 3**
Diagram of N-Form 3D electrode implanted in Cat 3 and a summary of data recorded from Cat 3 by the N-Form 3D microelectrode.

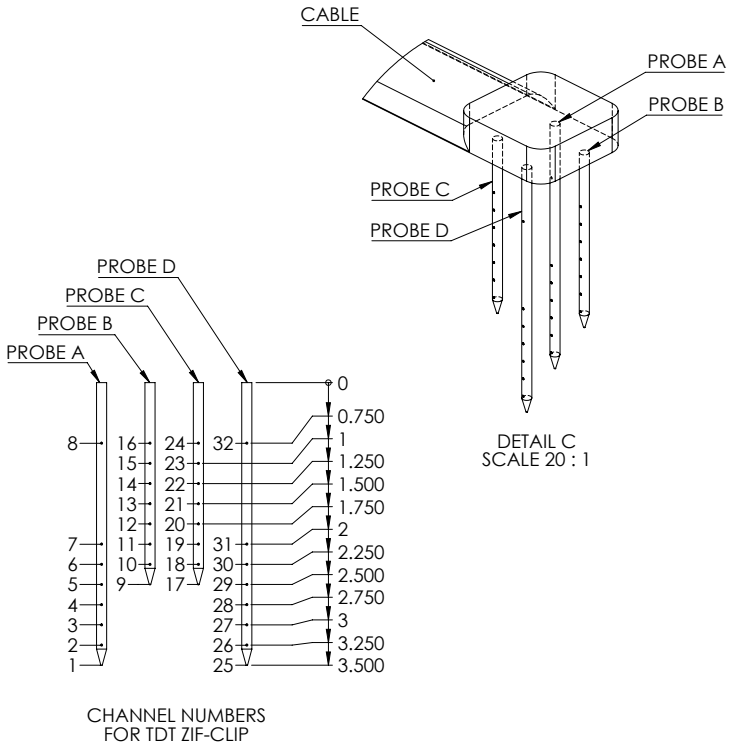


Figure C1: Diagram of N-Form electrode implanted in Cat 3

Figure C2: Summary of data recorded from Cat 3 by an N-Form 3D microelectrode. Orange border signifies a single unit or potential multi-unit recording, yellow border signifies evidence of multi-unit recording, blue border signifies a functioning site based upon RMS noise level, and grey border signifies a site of indeterminate functionality.