33rd Annual International Conference of the IEEE EMBS
Boston, Massachusetts USA, August 30 - September 3, 2011

A Waveform Independent Cell Identification Method to Study Long-term Variability of Spike Recordings

Ehsan Chah *Student Member, IEEE*, Vincent Hok, Shane M. O'Mara, Richard B. Reilly *Senior Member, IEEE*.

Abstract— **Extracellular potential recordings are important in neuroscience; however the variability of spike waveforms has not been extensively studied to date. This study examines the variability of spike recordings within and between sessions. Place cell recordings were used in order to identify the cells across successive sessions. Place cells allow neuron spike recordings to be identified across different sessions using place fields, which are independent of the cell's spike waveform. The results show that the distribution of the residues within a session does not follow a normal distribution, a t-distribution is more suitable. The results also show that the amplitude of the spikes can vary largely between successive sessions (up to 47%), this is an important factor to be considered in long term spike recording systems.**

I. Introduction

Long-term recording of extracellular potentials, spikes, is an important experimental method in neural engineering and neuroscience research. Understanding the variability of spikes has important applications in many areas such as spike sorting [1] as well as aiding the design of electrodes for chronic implantation [2].

It has been established that the distance between the electrode and the neurons plays an important part in determining the amplitude recorded [3]. It is also reported that spike waveforms vary [1] within recording sessions. The changes in spike waveforms can be attributed to two factors. The first factor is the background neural activity of other neighboring cells, the second factor is systematic change in the neuron's spike waveform. An example is seen in the case of spike burst where the spike amplitude decreases with successive spikes in the burst [1].

Fee et al [1] studied the variability of spikes recorded from layers 2/3 through layer 6 of the primary somatosensory vibrissa cortex of the rat. They examined the variability within a session in terms spike waveforms and spectral properties, and reported that spike variability does not follow a stationary Gaussian process. It was reported that half of the neurons recorded exhibited systematic changes as a function of interspike intervals. Lin et al [4] studied the stability of neural recordings of motor cortex neurons, recorded in the behaving monkey. The recording employed in the study included well isolated cells and spanned over 3 years. It was concluded that stability can last for long periods of time. However, the challenge of identifying neurons across successive sessions using the spike waveform was noted. Williams et al [5] implanted electrodes in the auditory cortex of guinea pigs. They examined the stability of one cell over a period of six weeks on a day to day basis. This cell was classified as a "stable unit". This corresponds to a cell which the waveform did not change from day to day, hence allowing the cell to be identified across different sessions. Principal Component Stability Tube was used to track waveform changes. Porada et al [6] examined the stability of neuron populations from the visual cortex of rabbits and monkeys. Stability was quantified by measuring the range of peak amplitudes, spike shape, spike frequency and the spike train autocorrelation histogram. The ability to record for periods up to 711 days was demonstrated. However it was difficult to establish whether spikes recorded across different sessions originated from the same neuron. Santhanam et al. [7] recorded from motor cortex of a monkey for a period over two days. The stability of spike waveforms during this period was examined. Neurons were identified across different five minute segments using the spike waveform. Neurons with spike waveforms that were distinct during the recordings were selected to ensure accurate tracking of waveform changes. Substantial variations up to 30% of the spike waveform were observed.

In studying variability of spike waveforms it is difficult to determine if a single cell is recorded over long period of time, hence some studies consider the "stable cells" [4]-[5] where the waveform is distinct and does not fluctuate largely. To overcome these challenges in this study we examined the variability of the spikes generated by place cells within and between recording sessions. Only place cell recordings were included in this study, in order to identify spikes of the neurons across consecutive sessions. The place field of these place cells provides a marker independent of the waveform, allowing objective tracking of waveforms across different sessions.

II. Methods

A. Place cells

Place cells have been first described in the rat

Manuscript received April 15, 2011.

E. C. is with the Trinity Centre for Bioengineering and Trinity College Institute of Neuroscience, Trinity College Dublin, Ireland (e-mail: chahe@tcd.ie).

V. H. is with the Trinity College Institute of Neuroscience, Trinity College Dublin, Ireland (e-mail: hokv@tcd.ie).

S. M. O. is with the Trinity College Institute of Neuroscience, Trinity College Dublin, Ireland (e-mail: smomara@tcd.ie).

R. B. R. is with the Trinity Centre for Bioengineering and Trinity College Institute of Neuroscience, Trinity College Dublin, Ireland (e-mail: reillyri@tcd.ie).

hippocampus [8]. These pyramidal cells emit complex spikes when the animal is in a specific location within a particular environment, and are thought to provide the animal with an internal sense of it's location in space [9]. Place cell activity can be visually ascertained by plotting the trajectory of the rodent in the environment, and superimposing the spike occurrences (Fig. 2A). The place field (the location where the cell is active) can be examined in more detail using firing frequency plots (Fig. 2B). Place cells generally have only one firing field in an environment of this size [10]. Objective measures can be used to characterize and identify place cells activity such as the spatial information content (SIC) [11]. This specificity index quantifies the amount of information (in bits) that a single spike conveys about the animal's location [12]. The SIC is calculated as follows:

$$SIC = \sum P_i \left(\frac{R_i}{R} \right) \log_2 \left(\frac{R_i}{R} \right), \qquad (1)$$

Where i is the bin index, P_i is the probability of occupancy of the bin, R_i is the mean firing rate of the cell in bin i, and R is the overall firing rate of the cell during the session. Spatial coherence is the z-transform of the correlation between the firing rate in each bin and the average firing rate of the eight surrounding bins. It quantifies the extent to which firing rate in a particular bin is predicted by the average rate of the eight surrounding bins. Thus, high positive values result if the rate for each bin could be better predicted given the firing frequency in its neighbors [13]. Average firing rate is the total number of spikes emitted during the session divided by the recording duration.

B. Surgery

Prior to the beginning of the experiment the Male Han Wistar rats underwent surgical implantation of drivable microwires for collecting multiple single neurons activity. The recording electrodes consisted of eight bundles of four platinum-iridium wires twisted together. Tetrodes were then mounted on a lightweight microdrive (Axona Ltd.), cut flat and implanted in the dorsal hippocampus (-3.8 to -4.2 AP, ±3.0 to ±3.6 ML and 1.2 to 1.5 mm dorsoventral to dura). The microdrives used here are built around a precision screw, machined to a pitch of 200 µm, which advances the electrodes in 25 µm steps. A spring tensions the screw and prevents it from moving spontaneously, therefore allowing recording of the same cell over several days. Behavioural, electrophysiological, and surgical procedures fulfilled the EU guidelines on protection of vertebrates used for experimentation (European Community Council Directive 86/609/EEC).

C. Spike recording

After a one week recovery, animals were connected, via a thirty-two channel headstage to the recording system which also allowed for animal position tracking. Signals were amplified and bandpass filtered between 380 Hz and 6 kHz for spike detection. In order to maximize spike separation, spikes were detected if their amplitude was at least three times noise level. Average number of spikes detection per recording session was ~7555 spikes. Spike waveforms were sampled at 48 kHz, with each spike represented by 50 samples corresponding to ~1ms. Candidate waveforms were then sorted off-line (average ~1361 spikes per cluster) using graphical cluster-cutting software (TINT, Axona Ltd.). Autocorrelation histograms were built for each cell and the whole cell was removed from analysis if the histogram revealed the existence of correlations within the first 2 ms (refractory period), inconsistent with good cell isolation. On average 43% of detected spikes were reliably sorted. Similar to others [14]-[15], place cells were selected for study if their spatial firing patterns were location-specific (spatial coherence ≥ 0.25; spatial information content ≥ 0.5 bits/AP) and robust (average firing rate ≥ 0.25Hz).

The recordings took place in a square arena (64 x 64 x 25 cm high) located in the center of a room with multiple cues available. Rats were food-deprived to 85% of their original weight. Rats were then placed in the open field and 20 mg food pellets were thrown in every 20 seconds to random locations; in this way, animals were in continuous locomotion, allowing for complete sampling of the environment. Each recording session lasted 20 minutes.

D. Within session variation

Similar to Fee et al [1], we defined the action potential residues as the difference between each spike waveform and the average waveform recorded for the each neuron. The distributions of these residues were then compared to normal and t-distributions. Probability plots were used to determine if either normal or t-distributions would fit the residues distributions. This comparison was carried out on each cell recording. Only the channel with the largest amplitude recorded was considered in this analysis, as this electrode is the closest to the neuron and records the maximum voltage deflections. Fig. 1 shows an example of spike recording using a tetrode (four channels), one of the channels (largest amplitude) captures the largest voltage deflection.

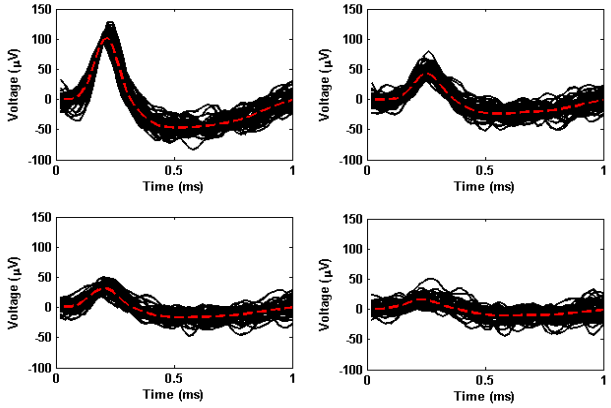

Fig. 1. Example of spike waveform recorded using a tetrode (four channels), the black solid lines represent the spikes and red dashed line represents the average waveform.

E. Between session variations

Place cells were identified across different sessions using

the Pearson correlation coefficient. The coefficient was calculated between the firing frequency plots (as described in Fig. 2B) of cells recorded from the same electrode across all recording sessions. Cells that yielded an average correlation coefficient higher than 0.8, were added to the group and assumed to be the same cell (computed between the cell and the members of the group). This procedure allows for the comparison of spike waveforms across different sessions while guaranteeing the sameness of the neuron being studied. Fig. 2 shows spatial firing frequency plots of a place cell tracked across several sessions where the average correlation coefficient between the sessions shown is 0.86.

The average amplitude was computed from each cell recording. The amplitude is defined as the peak-to-trough amplitude of the spike. Percentage change of the amplitude was computed and compared between successive sessions. The slope of these changes was also examined to test for systematic slow changes in the amplitude across the entire recording period of individual cells.

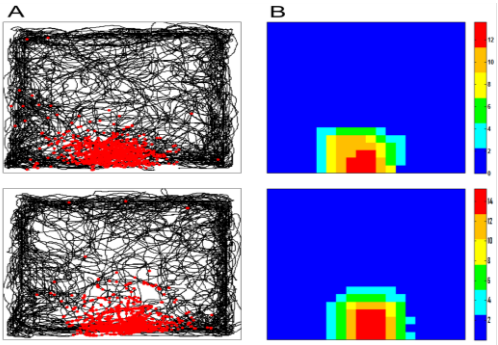

Fig. 2. Example of a place cell recorded in the hippocampal CA1 sub region (A) black line indicates the trajectory of the animal in the environment (square box). Red dots correspond to the location of the animal when the cell fired a spike (B) firing frequency plot of the place cell; the environment is divided by a set of squares (bins) (3x3 cm) and the number of spikes in each bin is divided by the time spent in that bin. The firing rate in each bin was smoothed using a 5 X 5 kernel, meaning that the firing rate for each bin was calculated as the average of the 5 X 5 bin square centred on that bin. Each row corresponds to a session. When Comparing the place field of a place cell across two successive sessions, the similarity between the place fields allows for objective and simple identification of the cell across these sessions. The average correlation between the sessions is 0.86.

III. Data Set

Data set recordings were obtained from four rats. A total of 438 cell recordings were included in examining the within session variability in this study. From these recordings a total of 19 cells were identified across different sessions using their respective place fields. The cells were tracked up to a maximum of 46 days. Cells that were tracked for more than four sessions were only included in examination of the between sessions variability.

IV. Results

A. Within session variation

We examined the distribution of the residues of the spikes recorded from the largest amplitude channel. We found that in 77% of these recordings the residues followed a t-distribution. By contrast in a low percentage (5%) of the cases the residues followed a normal distribution. Fig. 3 shows two examples of the residues distribution, where t-distribution can be used to approximate the residues distribution (Fig. 3A), Fig. 3C shows a rare case where normal distribution can be used to model the residues distribution.

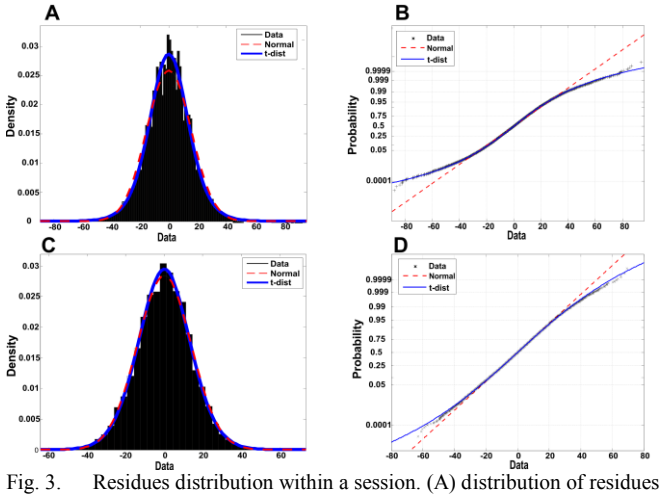

Fig. 3. Residues distribution within a session. (A) distribution of residues where t-distribution fits the data better than normal distribution, (B) corresponding probability plot(C) rare case where both distribution are similar. (D) Corresponding probability plot for the data represented in C.

B. Between session variation

An examination of the average amplitude percentage change, between successive sessions was carried out using the entire cell population in this study (Fig. 4). It is evident that the change in amplitude is random varying around zero. A t-test showed that mean percentage change of the amplitude is not significantly different from zero.

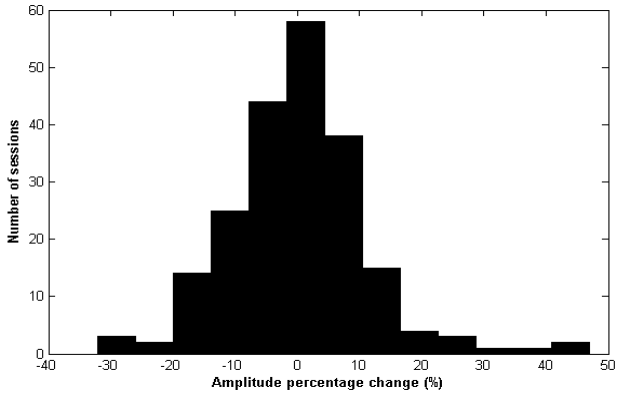

Fig. 4. Histogram of the average amplitude percentage change between successive sessions for the entire cell population recorded in this study. The average is 0.02 (not significantly different from zero) standard deviation is 11.32. The range of the values is between (-32, 47).

A linear function was fitted to the percentage change of the cells' average amplitude, across all the sessions where the cell was tracked successfully, in order that slow systematic changes across the entire recording period of the cell can be examined (as shown in Fig. 5 where slope is

-1.19). By calculating the slope, it is possible to quantify the slow changes in average amplitude. The slope for all cells tracked range between 5.03 and -7, average -0.78, standard deviation 2.46.

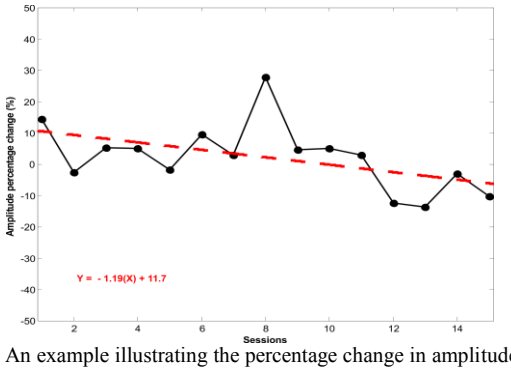

Fig. 5. An example illustrating the percentage change in amplitude across several sessions for a particular cell. The X-axis represents the number of sessions where the cell was successfully sorted and identified. Y-axis represents the percentage change in amplitude between successive sessions. The solid line with circular markers represents the amplitude percentage change between the successive sessions. The dashed line is a linear line fitted through the data.

V. Discussion

In this study we examined the variability of spike waveforms between and within sessions. The place field of the place cells was chosen as a marker to identify the cells across successive recording sessions. This criterion is independent of the spike waveform. Hence the results obtained are not affected by large variation in the spike waveform. The results show that the distribution of the residues do not follow a normal distribution in line with previous findings [1]. A t-distribution fits the residues in the majority of cases. This has important implication for spike sorting and analysis as many studies assume a Gaussian variability of spike waveforms.

The results show that amplitude average variation can be up to ~47%. This variation is large and it has important implications for long term neural recording systems. For example, in automatic spike sorting methods it cannot be assumed that spike waveforms are stationary for long periods of time. Santhanam et al. [7] recorded, using an accelerometer, rapid head movement along with neural recordings. It was shown that in some cases large amplitude variation of spikes was as a result of vigorous head movement. Although surgical procedures were followed to prevent the electrode from moving spontaneously, large amplitude variations observed in this study may also be explained by movement of the electrode. In this study, a systematic decrease and increase in amplitude was observed, this indicates the amplitude change is not due deterioration in electrode. This is expected as the cells were recorded for a maximum of 46 days. It has been reported that "stable recordings" for periods longer than 6 months can exist [4][6], where the recording period in this study is relatively short. There are two possible mechanisms for the slow changes; slow drift of electrode through the tissue, which explains an increase or decrease in average amplitude as the electrode moves closer or further away from the cell body. The second mechanism is the brain tissue response to the electrode where the gliosis pushes the cells away from the electrode [16]. This study demonstrates the need for improved electrode implantation procedures to reduce the effects of electrode movements after implantation, and also the need for flexible spike analysis to account for changes in spike waveform.

References

[1] M. S. Fee, P. P. Mitra, and D. Kleinfeld, "Variability of extracellular spike waveforms of cortical neurons," J Neurophysiol, vol. 76, pp. 3823-33, Dec 1996.

[2] M. D. Linderman, V. Gilja, G. Santhanam, A. Afshar, S. Ryu, T. H. Meng, and K. V. Shenoy, "Neural Recording Stability of Chronic Electrode Arrays in Freely Behaving Primates," in Engineering in Medicine and Biology Society, 2006. EMBS '06. 28th Annual International Conference of the IEEE, 2006, pp. 4387-4391.

[3] D. A. Henze, Z. Borhegyi, J. Csicsvari, A. Mamiya, K. D. Harris, and G. Buzsaki, "Intracellular features predicted by extracellular recordings in the hippocampus in vivo," J Neurophysiol, vol. 84, pp. 390-400, Jul 2000.

[4] W. S. Lin, S. H. Tillery, and H. Jiping, "Stability of the chronic multichannel recording neuron signals," in Engineering in Medicine and Biology Society, 2003. Proceedings of the 25th Annual International Conference of the IEEE, 2003, pp. 2193-2196 Vol.3.

[5] J. C. Williams, R. L. Rennaker, and D. R. Kipke, "Stability of chronic multichannel neural recordings: Implications for a long-term neural interface," Neurocomputing, vol. 26-27, pp. 1069-1076, 1999.

[6] I. Porada, I. Bondar, W. B. Spatz, and J. Kruger, "Rabbit and monkey visual cortex: more than a year of recording with up to 64 microelectrodes," J Neurosci Methods, vol. 95, pp. 13-28, Jan 31 2000.

[7] G. Santhanam, M. D. Linderman, V. Gilja, A. Afshar, S. I. Ryu, T. H. Meng, and K. V. Shenoy, "HermesB: a continuous neural recording system for freely behaving primates," IEEE Trans Biomed Eng, vol. 54, pp. 2037-50, Nov 2007.

[8] J. O'Keefe and J. Dostrovsky, "The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat," Brain Research, vol. 34, pp. 171-175, 1971.

[9] E. I. Moser, E. Kropff, and M. B. Moser, "Place cells, grid cells, and the brain's spatial representation system," Annu Rev Neurosci, vol. 31, pp. 69-89, 2008.

[10] A. A. Fenton, H. Y. Kao, S. A. Neymotin, A. Olypher, Y. Vayntrub, W. W. Lytton, and N. Ludvig, "Unmasking the CA1 ensemble place code by exposures to small and large environments: more place cells and multiple, irregularly arranged, and expanded place fields in the larger space," J Neurosci, vol. 28, pp. 11250-62, Oct 29 2008.

[11] W. E. Skaggs, B. L. McNaughton, and K. M. Gothard, "An Information-Theoretic Approach to Deciphering the Hippocampal Code," presented at the Advances in Neural Information Processing Systems 5, [NIPS Conference], 1993.

[12] E. J. Markus, C. A. Barnes, B. L. McNaughton, V. L. Gladden, and W. E. Skaggs, "Spatial information content and reliability of hippocampal CA1 neurons: effects of visual input," Hippocampus, vol. 4, pp. 410-21, Aug 1994.

[13] R. U. Muller and J. L. Kubie, "The firing of hippocampal place cells predicts the future position of freely moving rats," J Neurosci, vol. 9, pp. 4101-10, Dec 1989.

[14] A. A. Fenton, W. W. Lytton, J. M. Barry, P.-P. Lenck-Santini, L. E. Zinyuk, S. Kubík, J. Bures, B. Poucet, R. U. Muller, and A. V. Olypher, "Attention-like modulation of hippocampus place cell discharge," J Neurosci, vol. 30, pp. 4613-4625, Mar 2010.

[15] J. K. Leutgeb, S. Leutgeb, M.-B. Moser, and E. I. Moser, "Pattern separation in the dentate gyrus and CA3 of the hippocampus," Science, vol. 315, pp. 961-966, Feb 2007.

[16] V. S. Polikov, P. A. Tresco, and W. M. Reichert, "Response of brain tissue to chronically implanted neural electrodes," J Neurosci Methods, vol. 148, pp. 1-18, Oct 15 2005.