THE HIGH FREQUENCY DISCHARGE OF PALLIDAL NEURONS DISRUPTS THE INTERPRETATION OF PALLIDAL CORRELATION FUNCTIONS

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1. INTRODUCTION

A fundamental tool in electrophysiological research is correlation. The autocorrelation function, which describes the probability that a neuron will emit a spike as a function of the time elapsed from another firing of a spike by that neuron, is an important step in the understanding of the cell's intrinsic firing pattern (Perkel et al. 1967). Calculation of the cross-correlation function, on the other hand, is a major tool for examining interaction between multiple cells. The cross-correlation function describes the probability that a neuron will emit a spike as a function of the time elapsed from the firing of a spike by a second neuron (Perkel et al. 1967). Both of these methods have been used extensively to assess firing characteristics and functional connectivity of neurons within the basal ganglia (Groves et al. 1978; Stern et al. 1998), and specifically within the pallidum (Nini et al. 1995; Raz et al. 2000). The results obtained from these methods drive our understanding of the firing properties of the pallidum in health and disease.

However, several newly discovered mathematical artifacts (Bar-Gad et al. 2001a; Bar-Gad et al. 2001b) emphasize the problems associated with using these functions to conclude the neuronal behavior. The artifacts distort the results and mask the underlying physiological phenomena, to some degree, in all brain areas. However, the artifacts were discovered during research involving the pallidum since the high firing rate of the pallidal neurons makes the correlation functions of the firing in this nucleus especially prone to the artifacts. The combination of a relatively long refractory period with the unusually high firing rate causes the autocorrelation to falsely signal an oscillatory or bursty function. In addition to this effect, the combination of the firing rate with the misidentification of multiple spikes discharged by multiple cells (all recorded on the same electrode) causes them to appear correlated in their activity. Only a full

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understanding of these artifacts and estimation of their effect on the data will enable a correct study of the firing characteristics of pallidal neurons.

2. METHODS

Two vervet monkeys (Monkey C and S, *Cercopithecus aethiops aethiops*, female, weight 3-3.5 Kg) were trained to perform a visuomotor task. After training, a Cilux recording chamber was attached to the skull to allow recording of the simultaneous activity of pallidal neurons. Details of the task, surgery and data recording methods are similar to those given elsewhere (Raz et al. 2000). The monkeys' care and surgical procedures were in accordance with The NIH Guide for the Care and Use of Laboratory Animals (1996), and with the Hebrew university guidelines for the use and care of laboratory animals in research, supervised by the institutional animal care and use committee.

The electrode output was sorted and classified in real time by a template-matching algorithm (MSD 3.21, Alpha-Omega Engineering, Nazareth, Israel). The electrode output was also band-pass filtered (Monkey C: 300-6000Hz, Monkey S: 1-6000Hz, 4 pole Butterworth filter, MCP-plus 2.8) and continuously sampled at 24 KHz (AlphaMap 5.0). The continuous sampling of the electrode output was further subjected to off-line spike sorting procedure (AlphaSort 3.8). This algorithm is based on principal component analysis of the spike pattern. We applied two sets of principal components, a default one based on cortical recording (Abeles and Goldstein 1977) and a set of principal components that were created by a library of well-isolated pallidal spikes recorded by the same setup. The sorting was verified by the existence of refectory period in the ISI, and by the stability of the firing rate of the cells. Each sorting was verified by at least two experimenters. All further analysis of the spike timing is performed using 1ms bins. For the autocorrelation studies, only cells recorded for at least 600 seconds were taken. The cross-correlation studies used only pairs from the same electrode with at least 600 seconds in which both cells fired. Auto and cross correlation functions were calculated only for cells satisfying the stability and isolation criteria and were normalized to the firing rate (Abeles 1982).

The artifacts were tested using simulated neurons that reflect the core characteristics of the physiological neurons. The simulated neurons were modeled as a realization of a renewal process featuring reduced initial probability (simulating the refractory period) followed by a constant probability. A renewal process is defined as effected only from the last spike and not from any event prior to it, i.e., all sub-threshold phenomena are being reset by the last action potential. This specific renewal process has been previously addressed as a Poisson process with a refractory period (MacGregor 1987). In the model, cells have a constant firing probability (p) for each time bin (Dt). However, after a spike occurs the neuron enters a refractory period (of length τ_r bins) in which its probability of firing is smaller than the steady-state probability. The refractory period was simulated as an exponential function of the firing probability $p_t = k^{(t_r+1-t)} \cdot p \quad t \le t_r$, k=0.5. The firing probability description of the neuron is equivalent to its hazard function, the probability of firing at any offset given that no other firing has happened since time zero. The values of p and t_r were typical values for the globus pallidus: $0.05 \le p \le 0.25$, $4ms \le t_r \le 8ms$ (DeLong 1971). The length of the simulation was in the range of the duration of the electrophysiological recordings (1000 seconds).

3. RESULTS

Autocorrelation functions calculated for neurons in the GPe and GPi display a very characteristic shape on the short time scale (Fig 1a). The shape of the function is characterized by several stages: a refectory period with low correlations, followed by a period of increased correlation, which drops down, sometimes in an oscillatory manner, to the steady state correlation. Traditionally, cells with such autocorrelation graphs were viewed as neurons with a tendency for burst creation due to the peak in the autocorrelation (Abeles 1982; Perkel et al. 1967; Rodieck et al. 1962). This bursting assumption was based on the notion that the peak results from a short-term increase in the firing probability of the neurons. However, modeling very simple simulated cells shows that this characteristic shape does not result from a period increase in firing probability (i.e. bursting). The simulated neurons' firing probability is described by a refractory period characterized by a lowered firing probability (p_t) followed by a fixed firing probability (p). The autocorrelation of these simulated neurons resembles greatly the shape of the experimentally recorded neurons (Fig 1b). In a very simplistic neuron with a zero probability of firing the peak's value is $l_{peak}=p/Dt$ while the value at steady state is $l_{¥}=p/[Dt*(1+p*t_r)]$. The value of the peak is slightly lower and the value of the steady state is slightly higher in case of a non-absolute refractory period. A complete analysis of the autocorrelation function shape in the general case appears elsewhere (Bar-Gad et al. 2001a).

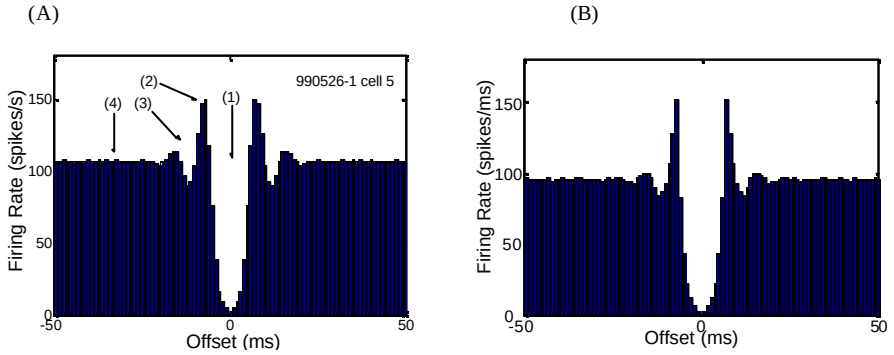

Figure 1: (A) Typical shape of the short-term autocorrelation function of pallidal neurons. The marked phases of the function are: (1) Refractory period phase (2) Elevated correlation phase (3) Oscillatory correlation phase (4) Steady state phase. (B) Simulated neuron with a refractory period (t_r) of *6ms* and a fixed firing probability (p) *0.18* following the refractory period.

This short-term effect is reflected in the autocorrelation function of neurons recorded in all brain areas. However, the size of this artifact (as described by the difference between the peak and the steady state) varies greatly with the length of the refractory period and the firing rate of the cells (Table 1). Not only does the absolute size of the artifact increase but also its relative ratio to the firing rate. The phenomenon, which is evident clearly in pallidal cells that have a high firing rate, is extremely small in other

brain areas with lower firing rates such as the cortex and usually disappears within the noise.

	λ_∞ (Hz)	t_r (ms)	$\Delta\lambda$ (Hz)	$\Delta\lambda/\lambda_\infty$ (%)
Globus Pallidus	60	6	33.75	56.25
STN	25	4	2.78	11.11
Cortex	5	2	0.05	1.01

Table 1: Typical values of firing rate $(l_¥)$ and refractory period (t_r) for different brain areas and their effect on the expected difference of the peak from the steady state (Dl) and on the ratio of this difference to the firing rate $(Dl/l_¥)$.

Pallidal neurons recorded using different electrodes during the same session display a flat cross-correlation function depicting non-correlated behavior (Fig 2a) similarly to previously shown results (Nini et al. 1995; Raz et al. 2000). However, the cross-correlation of neurons recorded by the same electrode in the pallidum reflect the following phenomenon: around time difference zero there is a very short period of low correlation resulting from the inability to identify spikes originating from multiple sources occurring at the same time or within a very short time difference. This effect is well known and occurs to some extent in all online and offline sorting methods (Lewicki 1998). However, the surprising result is that this period is surrounded by a short-term positive peak in the correlation (Fig 2b). This peak exists when the spikes are sorted using various online and offline sorting methods. This type of correlation has traditionally been interpreted as a result of functional connectivity (Eggermont 1990) between the neurons. However, computational simulation of independent neuron pairs sheds new light on the phenomenon. Two simulated neurons were created using a refractory period followed by fixed firing rate, independently from each other. A short period of the firing sequence (Fig 2c) of both cells is shown. Due to the independence in the creation of the sequences, the resulting cross-correlation function is flat (Fig 2d). A simple simulation of the spike sorting mechanism removes some of the spikes that occur in close temporal proximity. The removal is dependent on $S_{i,j}(t)$ which describes the probability of a spike removal of a spike if there is another spike at offset t (Fig 2e). The resulting cross-correlation of the two sequences reflects the obvious lowered correlation around zero offset, and also display a short-term positive peak around that time (Fig 2f).

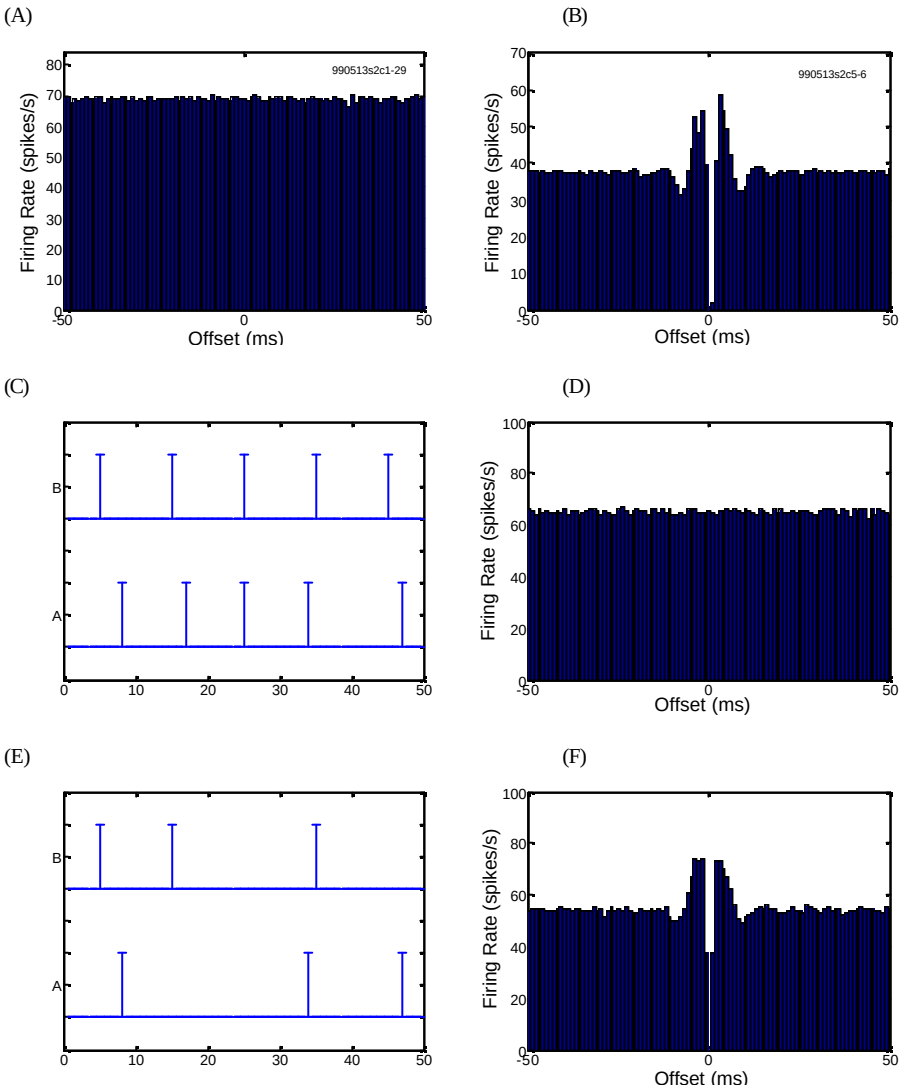

Figure 2: (A) Typical cross-correlation of two neurons recorded on different electrodes in the pallidum (B) Typical cross-correlation of two pallidal neurons recorded on the same electrode (C) Simulated spike trains of two cells without removal of spikes occurring at the same bin, simulating recording from different electrodes (D) Cross-correlation of simulated cells shown in *(C)* (E) Simulated spike trains after removal of spikes occurring within a short temporal difference, simulating recording from the same electrode (F) Cross-correlation of simulated cells shown in *(E).* The parameters used in the figure are:
Δt=1ms, τ_1=6ms, p_1=0.15, τ_2=8ms, p_2=0.12, $S_{m,n}=S_{n,m}$=[0.5 1 0.5]

The described artifact occurs during recording of multiple cells from a single electrode throughout the brain. However, the relative size of the effect depends on two

parameters: the effectiveness of the sorting methods in separating temporally close spikes and the firing rate of the two neurons. The high rate of the pallidal neuron firing makes them extremely vulnerable to the artifact relative to other brain areas (Table 2). A complete analysis of the size of the effect appears elsewhere (Bar-Gad et al. 2001b).

	Observed Firing rate (spikes/s)	Original Firing rate (spikes/s)	$\Delta\lambda$ (Hz)	$\Delta\lambda/\lambda_\infty$ (%)
Globus Pallidus	60	78.5	42.6	71.1
STN	25	27.2	4.6	18.6
Cortex	5	5.1	0.2	3.1

Table 2: The size of the short-term peak $(D\,l)$ caused by the shadowing effect and its ration relative to the firing rate $(D\,l/l_{¥})$ differs according to the characteristics of the brain area (for the same experimental setup for the spike detection process), the shadowing parameters are typical to those measured in the experimental setup: α=2, $S_{m,n}$=$S_{n,m}$=[0.25 0.75 1 0.75 0.25]. The observed firing rate is lower than the original (real) rate due to the unidentified overlapping spikes.

4. DISCUSSION

The misleading peak in the autocorrelation function can be understood by examining the firing probability of the neuron and its reflection in the autocorrelation function. Immediately following the refractory period the chances of the cell being in another refractory period are very small therefore the probability is the probability (p) reflected in the hazard function of the cell. On the other hand, at larger temporal offsets, the chance of additional spikes occurring increases and with it the chance that the neuron be in a state of a refractory period. This causes the value of the autocorrelation function to decrease below (p). The exact value that the autocorrelation function assumes at large offset is dependent upon the firing probability and the refractory period's length and shape. Moreover, manipulations causing a change in rate (for example lesion or pharmacological treatment) cause a change in the size of the phenomenon that might be interpreted as an effect on the pattern of the spike train (Bergman et al. 1994) instead of being properly interpreted as an epiphenomenon of rate changes. The artifact can be compensated analytically by calculation of the hazard function of the neuron's firing and reconstruction of the compensated autocorrelation function (Bar-Gad et al. 2001a).

The logic behind the positive correlations resulting from the "shadowing" or misidentification of neural firing of multiple spikes recorded by a single electrode lies once again with the effect of the refractory period. On an average the correlation function is lowered due to synchronous firing that is not identified. However, during the refractory period, the chance of another spike is lower so the chances of "shadowing" decrease leading to higher correlation level. Due to the removal of spikes during the "shadowing" period, the autocorrelation functions of the two cells are reflected in the cross-correlation much like the phenomenon occurring in functionally connected neurons (Eggermont 1990). The reflection of the autocorrelation can be seen by the smaller short-term troughs surrounding the cross-correlation peaks. These troughs reflect the autocorrelation peaks that were described previously. The reflection can also be seen on longer time scales as a

trough extending 500-3000 milliseconds reflecting the long term peaks in the autocorrelation function which are due to the pauses in activity characteristic to pallidal neurons (DeLong 1971). The situation is further complicated, since any systematic misidentification of spikes will cause such correlation. An example of this is a neuron whose spikes are too small to be identified but causes a distortion in the spike shape of a bigger spiked cell thereby decreasing its identification. This "invisible" cell will also cause the cross-correlation function of the identified cells to appear correlated. The artifact caused by the shadowing period has to be handled by a combination of two methods. The first of which is reducing the misidentification of spikes occurring in close proximity by any of a large number of (mainly offline) spike sorting techniques (Lewicki 1998). However, since none of the current methods is able to achieve the same reliability in identifying overlapping spikes as it has with non-overlapping ones, it must be complemented by the second method. The second method is based on assessing the "shadowing" effect by analyzing the autocorrelation shape and the "shadowing" period. The result of such an analysis is an estimation of the size and shape of the peak (Bar-Gad et al. 2001b).

The described artifacts occur during the analysis of electrophysiological data from all brain areas. However, their effect was probably overlooked due to their negligible size resulting from the low firing rate. The firing characteristics of the pallidal neurons and especially their high firing rates make them extremely prone to such artifacts. In the pallidum, the mathematical and technical artifact associated with the correlation functions might obscure any phenomena resulting from the physiological characteristics of the single neurons or of the complete network. Only a full understanding of these artifacts and their consideration and compensation can lead to a reliable analysis of the behavior of the pallidal neurons and network.

5. ACKNOWLEDGEMENTS

This study was supported in part by the Israeli Academy of Science and the US-Israel Bi-national Science Foundation. We thank Gali Havazelet-Heimer, Joshua A. Goldberg & Sharon Maraton for sharing their data with us.

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