Stochastic Neural Firing Properties in Neurons of a Cerebellar Control System

Henrik Jörntell, Jonas Dürango, and Rolf Johansson

Abstract—The cerebellar system for the voluntary control of arm-hand movements involves a large number of different neuron types. These neurons are located both inside the cerebellum but also in extracerebellar brain structures, which provide processed motor and sensory information to the cerebellum. The various different neuron types have different morphology, receive different types, number and patterns of synaptic inputs and have different firing rates and kinetics. However, a common trait for all neurons is that their firing properties have clear stochastic components, which is evident in recordings from the neurons recorded in brain preparations when all synaptic inputs is removed (in vitro). Here, we take advantage of a unique, comprehensive database of the various neuron types present within the cerebellar arm-hand control system, recorded from the brain in vivo, to provide a comparative description of their spike firing patterns. Although the inter-spike intervals for most cell types in this system can be described by a simple type of distribution characteristic for stochastic neurons, it is only for a few exceptional cases that the consecutive inter-spike intervals are independent of each other. We conclude that the spike patterns of these neurons may be the result of multi-factorial sources of variability that include the patterns in the various synaptic inputs that neurons receive in vivo and the inherent stochasticity of spike generation.

I. INTRODUCTION

The function of the brain is the result of the functions laid down in its neuronal circuitry components. The function of a local neuronal circuitry, in turn, depends on the precise pattern of synaptic interconnections between the involved neurons and the pattern of the information which the individual neurons receive via their synapses. The information received via the synapses will be transformed into a pattern of spike output by each individual neuron, and this transform function will also be an integral part of the function of the neuronal circuitry. However, since neuron types differ substantially with respect to the internal electrical properties as well as the number and weights of their synaptic inputs, they will also feature very different transform functions (or input-output maps). It follows that an understanding of the function of the brain in different tasks requires an understanding of the transform functions of the constituent neurons.

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Figure 1. Overview of cerebellar control system and neuronal morphologies. (A) Simplified neuronal circuitry diagram of the cerebellar control system for voluntary movements and its associated circuitry in the motor cortex, brainstem and spinal cord. Signals always travel from the neuron soma towards the synaptic terminal. The travel direction of the signals is indicated at a few places in the circuitry diagram. Conventions: Neurons are depicted as large circles (exception: the Purkinje cell, for which the typical morphology is sketched). Synaptic junctions between neurons are depicted with triangles (exception: Climbing fiber synapse, for which the typical morphology is sketched). Empty elements are excitatory (i.e., they have excitatory effects on the neurons which they contact), filled elements are inhibitory (i.e., they have inhibitory effects on the neurons which they contact). For simplicity, some local interneuron types are not indicated in this diagram. DCN = deep cerebellar nucleus. (B) Examples of morphologically identified neurons, which were recorded and stained with neurobiotin. This figure show three different neuron types in the cerebellar cortex and illustrates the extreme variability in morphologies and sizes between different neuron types. pf=parallel fiber (see (A)).

Neuron-to-neuron communication in the brain is mediated by small, fast electrical signals (neuronal spikes). Each neuron samples inputs from a high number of incoming (afferent) neuronal connections. These inputs are mediated via synaptic junctions, in which electrochemical transduction converts the incoming neuronal spike to a synaptic potential. The synaptic potential can be either excitatory or inhibitory in the targeted neuron depending on which transmitter substance the synapse contains, which in turn is specific for each afferent neuron type. The function of the neuron is to summate these excitatory and inhibitory synaptic potentials and, based on the result of the summation at the level of the initial segment of the axon, generate a spike output [1]. The intensity of the spike output will depend on the membrane potential in a linear fashion (integrate-and-fire elements). At least during engagement in a control task, the neurons of the
involved circuitry components typically have a resting discharge or a so called spontaneous firing. The effect of the incoming synaptic information is a temporary increase or a depression of this firing rate, and possibly its pattern.

However, different neuron types have very different spontaneous firing properties. This can be partly explained by that different neuron types can differ substantially with respect to morphology and size (Fig. 1B). The morphology will affect the electrical properties of the neuron since these depend on the membrane surface area (the neuronal membrane functions as a capacitor), the total membrane resistance (determined by the number of ion channels per membrane surface area) and possible electrical compartmentalization of the neuron (believed to occur in the largest neurons as a result of the limited axial conductance along the long axons of the neurons’ dendrites, for example). The electrical properties of the neurons will affect the rate at which they can integrate synaptic potentials since activation of a synapse results in a current injection of a given time course. But the spike generator at the initial segment of the axon is primarily affected by the membrane potential, and the membrane potential change generated by the synaptic current will naturally be affected by these inherent electrical properties. An additional explanation is that some neurons, in particular some of the largest ones, may not work as simple, linear integrate-and-fire elements, but are endowed with special membrane conductances that are activated to various different degrees depending on the membrane potential and that confer non-linear properties to the spike generation mechanism of the neuron [11].

On top of these basic aspects of neuronal function is added the fact that the spike generator of the initial segment is not completely predictable. The spike is generated at the initial segment through a electrochemical process, i.e., local voltage changes drives a conformational change in a membrane protein which, in turn, results in the opening of a pore which is selectively permeable for sodium. Local influx of sodium will then rapidly excite the membrane further, which results in the opening of additional voltage-gated sodium channels and further membrane potential excitation or polarization. This positive feedback mechanism results in a peak polarization, or spike, which is achieved when no additional sodium can enter the cell (which typically occurs when the spike has an amplitude of about +100 mV). This process initiates and peaks extremely fast (within less then 1 ms) but at least its initial phase, which may depend on the electrochemical activation of just a few sodium channel molecules, is inevitably subject to Brownian noise and therefore exhibits stochastic variations [10, 18].

The aim of the present paper is to provide an overview of the firing properties of a variety of neuron types in the brain in vivo. Our system of interest is a subsystem of the cerebellum devoted to the control of voluntary movements of the arm and hand [5, 12, 1, 3]. Apart from the relevant neurons within the cerebellum, this system includes additional neurons in the motor cortex, cuneate nucleus, lateral reticular neurons, spinal interneurons and alpha-motoneurons (Fig. 1A). We will test the hypothesis that the spike patterns recorded in these neurons in the brain at rest are not completely random. At least in some cases, this hypothesis can be discarded, which suggests a stochastic spike pattern. These patterns follow a gamma distribution or a log-logistic distribution [10].

Figure 2. Sample recording of spike activity from an identified cerebellar stellate cell. (A) Raw trace showing spikes recorded from a well isolated unit. (B) 10 superimposed spikes in expanded raw traces illustrate that all spikes were from one and the same unit. (C) The recorded neuron was stained with neurobiotin and histologically processed to obtain a morphological identification. This neuron was a cerebellar stellate cell, an inhibitory interneuron that innervates the Purkinje cells of the cerebellar cortex. (D) Frequency histogram of the inter-spike intervals recorded from this neuron.

II. METHODS

In order to be able to record neuronal activity in an intact neuronal circuitry and in a normal chemical environment, neuron recordings in the in-vivo preparation was a requirement. A trade-off in order to be able to record activity also from the smallest neuron types was to have the animals in an acute preparation. In the acute in vivo preparation, the brain and its connection with skin, muscles and joints are left intact but the animals immobilized to minimize tissue movement, thereby allowing a recording microelectrode to come close enough to a single neuron to be able to pick up and isolate the small extracellular electric signals generated by its spikes. Our preparation was a decerebrated preparation, in which the cerebral cortex and thalamus are disconnected from the rest of the central nervous system, which is an alternative to the other form of acute preparation, the anesthetized preparation. For our purpose, a decerebrated preparation is preferable over the anesthetized preparation because all known anesthetic drugs has been shown to interfere both with synaptic function but also
directly with the ion channels contributing to spike generation. For the recordings made here, the experimental animal was the cat, in which the neuron types of the cerebellar control system have been particularly well characterized and defined [3].

Neuronal recordings were obtained with the use of tungsten-in-glass microelectrodes, fabricated in-house, with very fine tips (exposed tip 2-7 μm) or patch clamp electrodes used to obtain loose-patch cell-attached extracellular recordings. The type of neuron recorded from was identified on the basis of anatomical location of the recording site and some basic physiological criteria and in some cases on basis of direct morphological identification when the neuron recorded from was stained with neurobiotin and histologically processed in postmortem examination (Fig. 2). All raw recording data was digitized at 25-100 kHz. Spike times were identified in custom-made software which identified all recorded spikes of a given unit and returned the spike times with a resolution of 0.1 [ms]. The data was then given as a series of inter-spike intervals with the 0.1 [ms] resolution. All methods and details of this preparation are described in previous publications [4, 13-15, 2].

When analyzing spontaneous neuronal activity, stationary conditions on the data are often imposed. Therefore, from each recording, the longest stationary segment of inter-spike intervals were isolated. This is not trivial task, as many properties of the underlying process might be time varying, such as mean, variance, correlation, etc. A minimal requirement for stationarity is a time-invariant mean [10]. Thus, in order to test this, the data was divided into subsets of ten consecutive inter-spike intervals each. Under the assumption of stationarity, each individual inter-spike interval $\tau_i$, $i=1,...$, can be considered identically and independently distributed with $E[\tau_i] = \mu$ and $V[\tau_i] = \Sigma^2$, where the constants $m$ and $\Sigma$ were estimated from the entire dataset. According to the central limit theorem, each of the subset means would qualify as observations of the approximate Gaussian distribution

$$N(m, \Sigma/\sqrt{10}).$$

From this distribution, confidence intervals were derived as

$$m \pm k \cdot s/\sqrt{10},$$

where $k$ is the preselected level of significance (i.e., $k=1.96$ signifies a 95% confidence interval)—e.g., for a 85% confidence interval, 85% of the subset means should end up inside the limits set up by the confidence interval. All data analyzed in this paper fulfilled this criterion and were considered stationary.

For each cell recording, properties estimated were the underlying distribution (as characterized by the inter-spike intervals histogram and empirical cumulative distribution function, Fig. 3), autocorrelation and the first order conditional mean. Various different standard distributions (gamma, with probability density functions (pdf)

$$f_\gamma(x; A, B) = \frac{x^{A-1}e^{-x/B}}{B^A\Gamma(A)}, x > 0$$

and the log-logistic distribution with pdf

$$f_\log(x; \mu, \sigma) = \frac{e^{\ln(x) - \mu}/\sigma}{\alpha(1 + e^{(\ln(x) - \mu)/\sigma})^2}, x > 0$$

were fitted to the data and evaluated using the Kolmogorov-Smirnov test where the null hypothesis that data is drawn from a pre-specified distribution was tested against the alternative hypothesis [17]. In many cases we found that the null hypothesis could not be rejected (Fig. 3), making the pre-specified distribution a plausible model for the inter-spike intervals recorded. If the goal of the analysis is to give a plausible process model capable of recreating the properties of the cell recordings, the dependence structure of the inter-spike intervals must be examined. A popular way to model spontaneous neuronal activity is the use of homogeneous renewal processes. In such processes each inter-spike interval is independently drawn from an underlying distribution. Thus, if one could provide a good estimate of this distribution every property of the process would be captured. In cases where dependency between inter-spike intervals can be shown, a renewal process model will not suffice and a more complex model structure must be employed. Here we examined the dependency structure by estimating the autocorrelation function and conditional mean of the inter-spike intervals. When estimating the conditional length for inter-spike interval $\tau_{i+1, | \tau_i}$, conditioned on the length of $\tau_i$, i.e., $E[\tau_{i+1} | \tau_i]$, we binned the data in bins of varying length, depending on the dataset at hand, and conditioned on all intervals within each bin [10]. Confidence intervals for the estimate were derived under a renewal process assumption, so that if the estimated conditional mean differed too much from the conditional mean of a renewal process (which is simply the mean inter-spike length of the data), we could reject the hypothesis that a renewal process accurately could model the data. To complement the dependency analysis, we also estimated the autocorrelation function of the inter-spike intervals. Finally, to investigate neural response variability [6], Fano factors $F_x$ were computed for sample size $n_x$ of data sets of several neuron classes ($n_{DCN}=1000$, $n_{LRN}=603$, $n_{CF}=303$, $n_{B/IN}=1139$, $n_{Go}=1307$, $n_{Gr}=248$, $n_{P}=84986$ where subscript denotes the neuron class). From the empirical distributions of the various neuron classes, information bit rates and entropies were calculated [7].

III. RESULTS

After a clear-cut isolation of the spiking activity of a single neuron was achieved (Fig. 2), we recorded its activity over 2-60 mins to allow a statistical analysis of its inter-spike intervals. The data set analyzed consisted of granule cells (N=4), inferior olivary neurons/climbing fibers (N=3), Purkinje cells (N=10), cells of the lateral reticular nucleus...
(LRN) (N=4), cells of the deep cerebellar nucleus (DCN) (N=7), interneurons of the cerebellar cortex (N=3), Golgi cells of the cerebellar cortex (N=7) and cells of the cuneate nucleus (N=4). In 72% of the neurons, we found that either a gamma distribution or a log-logistic distribution could account for the recorded series of inter-spike intervals, with P-values between 0.17-0.98. Climbing fibers (or inferior olivary neurons) were consistently fitted to a gamma distribution (A=2.1-2.2; B=147-195) whereas Golgi cells were consistently fitted with a log-logistic distribution (µ=4.0-4.8; σ=0.19-0.30). For other cell types, the distribution was either a gamma or a log-logistic distribution (Fig. 3), or the statistical analysis indicated that neither type of distribution could be fitted to the recorded data (Fig. 4).

Figure 3. Example of a neuron for which the null hypothesis could not be rejected. The neuron was located in the lateral reticular nucleus (LRN). The red trace shows the distribution of the inter-spike intervals of the recorded data whereas the black trace shows the fit obtained with a gamma distribution.

Figure 4. Example of a neuron for which the null hypothesis, that the data comes from a logistic distribution, could be rejected (p<0.05). This neuron was recorded in the deep cerebellar nucleus (DCN). The red trace shows the distribution of the inter-spike intervals of the recorded data (here displayed with a 1 ms binwidth in the histogram) whereas the black line represents the fitted log-logistic distribution.

In order to more extensively characterize the process generating the inter-spike intervals, the dependency between intervals must be examined. As previously mentioned, in the case of completely independent inter-spike intervals a renewal process arises. This is the simplest form of process to describe spike trains, as individual inter-spike intervals can be seen as drawn independently from the underlying distribution. In 20% of our cells we found no significant serial dependency between intervals, making a renewal process a sufficient model of spike generation. This result was seemingly randomly distributed among the cell types, except for climbing fibers in which all neurons could be classified to the renewal process category.

Figure 5. Example of recorded Purkinje cell for which the analysis indicated that consecutive inter-spike intervals tended to display dependency. Display for the top two figures as in Fig. 4 (p=0.48 for the fit to a log-logistic distribution). For the two bottom figures, the autocorrelation shows a clear positive correlation between adjacent inter-spike intervals (dashed lines serving as confidence intervals for zero-valued correlations) while the first-order conditional mean (estimated in bins of 4 ms) exhibits increasing values before settling at this value. The dashed lines serve as confidence intervals for the conditional mean under a renewal process assumption, which is clearly violated here.

Figure 6. Examples of other neuron types for which the specified distributions could be fitted to the recorded data. (A) Climbing fiber/inferior olivary neuron. (B) Cerebellar molecular layer interneuron. (C) Golgi cell. (D) Granule cell.
When dependencies between inter-spike intervals are present, a simple renewal process will not suffice for describing the data. Dependency was examined using an estimate of the first order conditional mean of inter-spike intervals in bins of varying interval length. If there were no dependencies between consecutive inter-spike intervals, the conditional mean would simply equal the expected value of the inter-spike interval distribution, as in the renewal process. Conversely, in cases of dependencies a higher-complexity model had to be assumed. During our investigations we found that for non-renewal spike trains the conditional mean increased for inter-spike intervals shorter than the mean interval length, while the conditional mean for inter-spike intervals longer than the mean interval showed no dependency on the previous interval length (Fig. 5). In these cases, it would mean that a short inter-spike interval leads to an increased probability of a consecutive short interval. Interestingly, this was the case for all Purkinje cells (Fig. 5B). Fano factors $F_{\text{DCN}}=0.34-0.73$, $n_{\text{LRN}}=0.34$, $F_{\text{CF}}=0.47-0.70$, $F_{\text{BRN}}=1.01-1.05$, $F_{\text{Go}}=0.34-0.63$, $n_{\text{Gr}}=1.54-3.02$, $F_{\text{P}}=0.44-0.78$, clearly exhibiting high values for granule cells and basket cells and interneurons. Whereas the Purkinje cells and Golgi cells exhibited the highest entropy value ranges—[5.00, 7.36], [4.20, 5.59], respectively—the DCN and interneurons exhibited the highest bit rates [306, 367], [1282] [bits/s], respectively.

Synaptic plasticity is generally considered the most important mechanism for adaptation in the CNS [13, 14]. The experiment compared the spike responses evoked from a skin area which were originally activating the interneuron (Fig. 7) with the spike responses evoked from a skin area, not originally activating the interneuron, but was induced by applying a stimulation known to change the synaptic weights for specific inputs to this type of neuron (Fig. 8). A series of spike intervals could be classified as a renewal process only in a limited number of cases. Our results therefore suggest that it is only the spike intervals recorded in climbing fibers/inferior olivary neurons that can be consistently classified as a gamma distribution in which the consecutive spike intervals seem to be completely independent. The absence of independence between consecutive inter-spike intervals, which was recorded for all other neuron types, suggests that there may be other determinants of the spike firing properties than the spike generator of the respective neuron only. In the brain in vivo, the neurons recorded from are part of an extensive network and receive a consistent bombardment of background synaptic inputs. Although also the other neurons, from which the synaptic inputs are received, are stochastic elements, it cannot be excluded that the recurrent connections of the network tend to create patterns in these synaptic patterns, which in turn would create patterns in the spike firing of the recorded neuron. A second potential contributor to spike patterning is the intrinsic electrical properties of the individual neurons. Here, we include all the reactive conductances that may impose highly nonlinear membrane potential responses to synaptic inputs. Such reactive conductances are well known to be present in the
dendrites in some of the largest neurons of the brain [11]. In particular, the Purkinje cell dendrites are richly endowed with such conductances [16, 8]. Although these issues need to be investigated further, against this background, it is interesting to note that the Purkinje cell was the only type of neuron which showed a consistent pattern in their autocorrelation (Fig. 5B). An information-theoretic issue is the information rate associated with spike patterns and Shannon entropy offers a quantitative characterization of the information rate [7]. The information bit-rate results agree with the assessment that the climbing fibers and the Golgi cells provide the slowest response. Whereas the granule cells and interneurons provide the fastest response in physiologic conditions, these neurons appear as relatively silent in the experimental conditions of our measurements though exhibiting high Fano factors. Whereas the Purkinje cells and the DCN exhibit a fairly high information rate, the LRN have variable responses which prompts a cautious interpretation. During our investigations, we found that several sets of inter-spike intervals from different cells exhibited constant conditional mean and zero-valued autocorrelations for time lags different from zero. These could well be approximated with a renewal process with inter-spike interval distribution modeled as different gamma and log-logistic distributions. Other recordings showed a positive decaying autocorrelation and non-constant conditional mean that increased for inter-spike intervals roughly shorter than the mean interval length, while settling at the mean inter-spike interval length for further increasing interval lengths. This would imply increased probability of a short inter-spike interval followed by another short, while the same cannot be said for longer intervals. The cerebellar control system, which we have studied (consisting of both cerebellar and non-cerebellar parts of the central nervous system) is structured as a network of neuronal units. In order to understand the functionality of this control system, it is necessary to also understand how these neurons encode and transmit information. Whereas no functional prediction beyond the stochastic firing properties was made, the system is responsible for voluntary arm movement control of the arm. Understanding the spike firing properties of the neurons is essential for understanding the function of the circuitry. Since the statistical analysis becomes more reliable with large datasets, i.e., very long recordings of neuronal spike firing, we have chosen to illustrate this encoding when the neurons are at rest, i.e. they do not receive high levels of synaptic inputs. We found that all constituent neurons, when recorded at rest, have a spike output that to a large extent can be characterized as random. This randomness is attributable to the voltage-sensitive ion channels which generate the spikes [6] and, perhaps, information coding. Naturally, the spike output is not all random since each neuron exhibits a constant average firing frequency— i.e., the condition of stationarity. When the neurons receive synaptic excitation, this average firing frequency increases, but the encoding mechanism would be expected to remain the same.

V. REFERENCES

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