Oscillations in subthalamic nucleus measured by multi electrode arrays

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Abstract— **The subthalamic nucleus (STN) of the basal ganglia, is involved in the generation of Parkinsonian symptoms and forms one of the main targets for Deep Brain Stimulation (DBS). Effective frequencies of DBS are around 130 Hz. The effect of such stimuli in the STN is largely unknown but has been hypothesized to result in neuronal block, interrupting the pathophysiological oscillatory behavior which is observed in the Parkinsionian basal ganglia. Modelling studies suggest that synchronized oscillation at tremor (4-8 Hz) or beta (14-30 Hz) frequencies may occur. To study synchronicity of the STN in detail, we record action-potential activity from rat brain slices using multi electrode arrays (MEAs). These arrays consist of 60 recording sites and thus allow the study of spatio-temporal activity patterns. Here we show the characteristics of spike trains which we recorded in the STN.**

Keywords— **Parkinson's disease, multi electrode arrays, brain slices, oscillatory activity**

I. Introduction

The origin of the three cardinal symptoms of Parkinson's Disease (tremor, rigidity and bradykinesia) is speculated to lie in the basal ganglia. Specifically, it is in the substantia nigra pars compacta of the basal ganglia that deterioration of dopaminergic neurons occurs. The loss of subsequent dopaminergic innervation to the basal ganglia, the striatum and also the motor cortex ultimately leads to PD symptoms. From a number of studies it has been shown that oscillatory activity in the lower frequency bands (<30 Hz) increase in PD [1-3]. The application of the precursor of dopamine (L-dopamine or levodopa) decreases oscillatory power in the lower frequency bands, and increases power in the gamma frequency bands (50-90 Hz) [1]. It has been suggested that, next to an increase in oscillatory behaviour, also an increase in synchronicity between neuronal oscillations may occur [4, 5]. Once established, such synchronicity may self-perpetuate through mediation by the T-type calcium current. This membrane current causes cells to depolarize after prolonged inhibition, leading to bursts of action potentials when released from inhibition. The possibility of such a mechanism in the basal ganglia has been demonstrated using organotypic culture preparations [4].

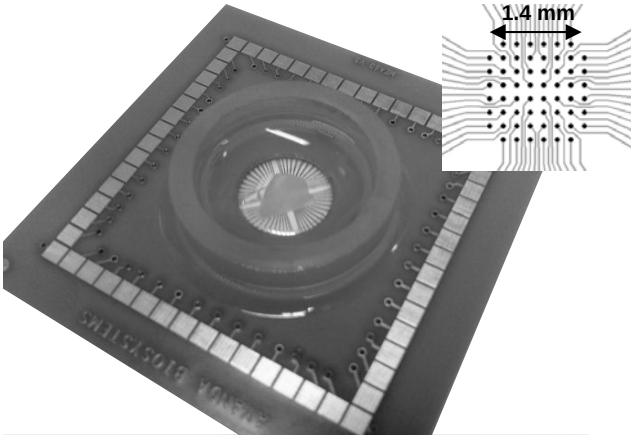

Fig. 1 A multi electrode array. The round culture chamber has an inner diameter of 2.4 mm. The 60 electrodes were spaced 200 µm apart, in an 8 by 8 grid (see inset). The electrodes shape is conical, with the tip protruding 50-70 µm from the glass surface.

It is our goal to elucidate the extent of oscillatory behaviour in the basal ganglia. We first concentrate on the subthalamic nucleus (STN), since it is the most effective target for suppression of most PD symptoms by Deep Brain Stimulation (DBS). The clinical effects mimic those of ablation, even though it has been shown that DBS increases activity in the target nucleus [6]. Therefore, more complex interactions within the basal ganglia, due to DBS are likely to occur. In-vivo, the location of the STN prohibits the use of

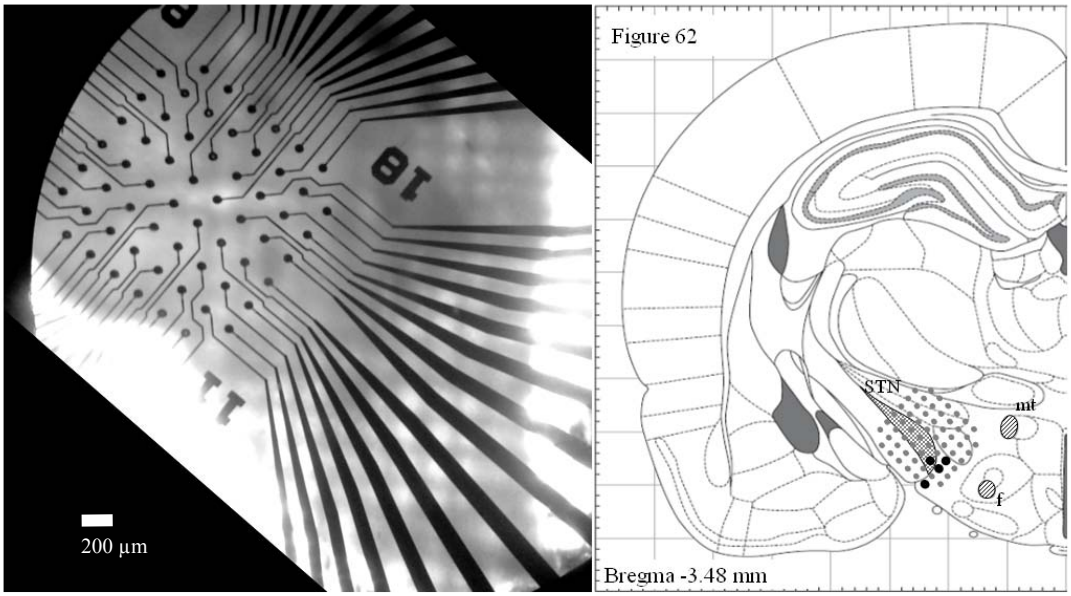

Fig. 2. (Left panel) Microscope image of a coronal slice placed on a multi-electrode array. (Right panel) Corresponding plate (62, Paxinos & Watson 2007) with MEA overlay. The mamillothalamic tract (mt) and fornix (f) are visible also in the microscope image. The STN is highlighted, as well as the electrodes which recorded activity.

EEG or EMG to record activity, while micro-electrode recordings suffer from a limited number of electrodes and thus provide a poor spatio-temporal resolution. The usage of brain slices as an alternative is therefore appropriate to study the underlying mechanisms of the oscillatory network behaviour in Parkinson's disease and the effect of electrical stimulation. However, such experiments have mostly been conducted using patch clamp techniques, making it impractical to record from more than two neurons simultaneously. We use a multi electrode array with a grid of 60 micro electrodes in combination with brain slices of the STN to observe and induce neuronal spiking patterns. The results presented pertain to the spiking characteristics of single neurons.

II. Methods

A. Slice preparation

Coronal brain slices (300 μm) from 16 to 52 day-old Wistar rat were cut on a Vibratome (Leica VT1000) in an ice-cold cutting medium containing artificial cerebro spinal fluid (aCSF) with additional 1.25 mM $MgSO_4$ and 1 mM ascorbic acid. Our aCSF consisted of (in mM): 124 NaCl, 3.3 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 2.5 $CaCl_2$, 20 $NaHCO_3$ and 10 glucose. Solutions were aerated with carbogen (95% O_2/5% CO_2). Rats were anaesthetized using Isoflurane before decapitation.

B. Recording setup

Coronal slices were compared with a rat brain atlas (Paxinos & Watson, 6th edition, Elsevier) using a dissecting microscope and the corresponding plate number (corresponding to rostro-caudal distance from bregma) was noted. Markers for comparison were 1) shape and position of fornix, mamillothalamic tract and third ventricle 2) the presence of CA3 of the hippocampus and optical tract. The STN was identified as a gray structure superior to the cerebral peduncle (or internal capsule) and inferior to the zona incerta and the nigrostriatal bundle. Slices and aCSF were transferred to 3D-

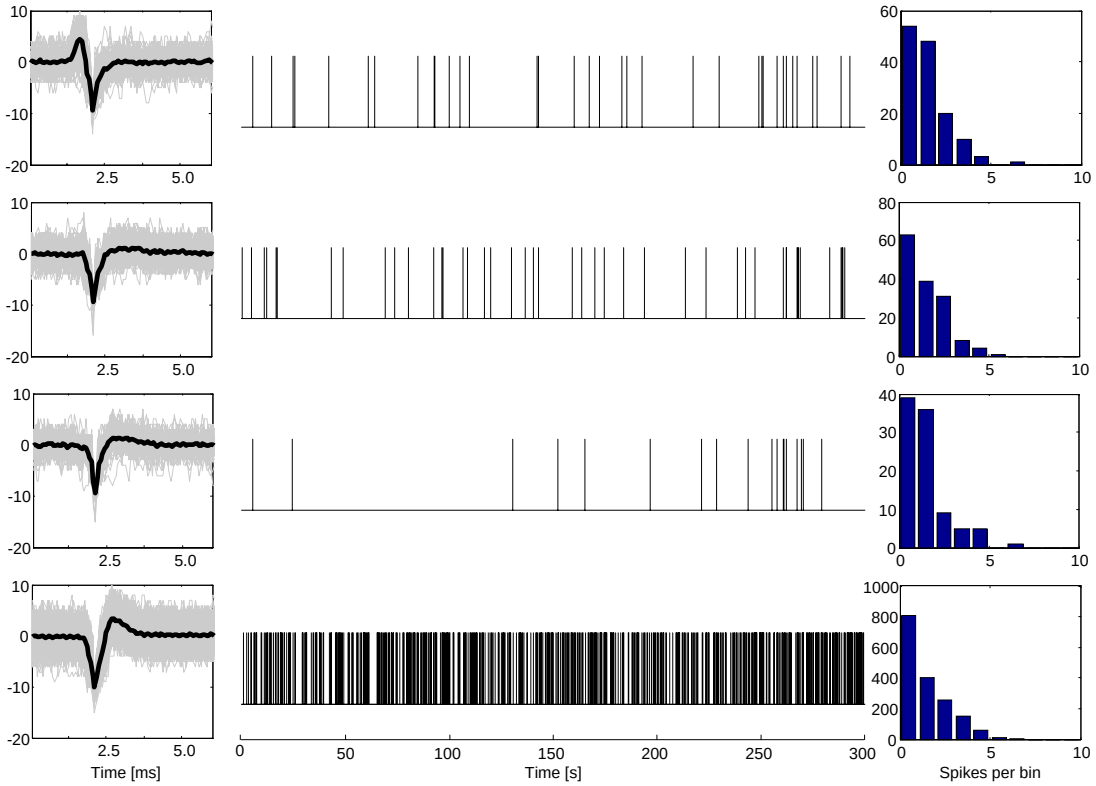

Fig 3. (Left panels) Waveforms of detected action potentials overlaid in gray, mean waveform in black. Peaks are aligned to maximum deflection, vertical axis is in µV. (Middle panels) Raster plot of 300 s recording. Each vertical line represents an action potential. (Right Panels) Density histograms of the four time series. Bars denote the occurrences of bins with a certain number of spikes.

multi electrode arrays (3D-MEA; Ayanda biosystems, Lausanne, Switzerland; fig. 1), and signals were amplified, bandpass filtered (10Hz-10kHz) and digitized at a rate of 16 kHz per channel using a measurement system by MCS (MultiChannelSystems GmbH, Reutlingen, Germany). Slices were kept in place by a nylon mesh glued onto a silver ring lowered into the chamber by a micromanipulator and were perfused with aerated aCSF at a rate of ~3 ml/min. Signals were visualized by a custom-made LabView (National Instruments, Austin Texas) program and threshold crossings exceeding 5 times the RMS noise value (typically 2 µV) were stored. Pictures of MEA and slice were taken during recording through a Nikon (Tokyo, Japan) Diaphot-TMD inverted microscope.

C. Data analysis

Offline analysis was done in MatLab (The Mathworks, Natick, Massachusetts). By semi-automatically comparing pictures with slides, we calculated the coordinates of the MEA electrodes. Only recordings of electrodes within the STN were used for further analysis. Spike trains were classified as random, regular or bursty, based on an algorithm introduced by Kaneoke & Vitek [7]. First, we computed the density histogram. To this end, a spike train was again discretized with a binwidth equal to the mean interspike interval. The number of spikes per bin was noted. The number of spikes per bin appears on the horizontal axis of the density histogram, and the number of times that a bin with this number of spikes was observed is on the vertical axis. We then checked whether this distribution was

significantly different from a Poisson distribution with a mean of 1 by χ-squared test ($p<0.05$). When this was not the case (i.e. the spike train was not considered 'random'), we checked whether the distribution was closer to a normal distribution with a mean of 0.6 ('regular'), or whether it was closer to a Poisson distribution with a mean of 0.8 ('bursty').

III. Results

An example of our mapping of the MEA with pictures taken during measurement can be seen in figure 2. Here, a slice located approximately 3.48 mm dorsal to Bregma (Paxinos & Watson). This corresponds to the rostral part of the STN, which is (a.o.) innervated by the motor cortex. The highlighted electrode within the STN is calculated at 2.3 mm lateral from midline and 8.6 mm ventral to Bregma. The recording lasted for 15 minutes. Action potentials from the 4 electrodes close to STN are shown in figure 3. The average firing rates of the four neurons were 0.1, 0.1, 0.06 and 1.2 Hz, respectively. Note that the neuron located within in the STN (the 4th neuron, also in figure 3) is the most active. Neurons 2 and 4 were classified as 'bursty', neuron 1 was termed 'random' and neuron 3 was labeled 'regular'. On further inspection, many spikes were part of *doubles* (two spikes in close succession) and bursts of longer duration were rare.

IV. Discussion

Despite the changes in firing rate, the method used was able to discriminate several spiking patterns. The bursty neuron was found in the part of the STN which is innervated by the motor cortex [8]. More data will be added to facilitate a meaningful comparison between our findings and literature.

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