Network Activity Patterns in the Subthalamic Nucleus of the Rat

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In Parkinson's Disease, pacemaker-like stimulation of the Subthalamic Nucleus (STN) at a frequency near 130 Hz can suppress symptoms like tremor, rigidity and bradykinesia. However, the mechanism by which this so-called Deep Brain Stimulation (DBS) exerts its effect is unknown. In order to increase our understanding of the network in which the STN is involved (the basal ganglia, among others involved in motor tasks), we are now measuring neuronal activity in brain slices containing STN using 3D-MEAs. 3D-MEAs offer the possibility to measure a large number of sites simultaneously in slices which retain *in-vivo* topography and connectivity. This approach is not yet used within Parkinson's Disease research and may answer crucial questions about the (patho-) physiology of the STN and surrounding network.

1 Introduction

The symptoms of Parkinson's disease (a.o. muscle rigidity, tremor, bradykinesia) can be suppressed by electrical stimulation of the basal ganglia [1]. The most common target nucleus of this so called Deep Brain Stimulation (DBS) is the subthalamic nucleus (STN). The mechanism(s) responsible for the clinical improvements through DBS are not yet elucidated. Based on organotypic culture studies, many models incorporate synchronous oscillatory activity between STN and external segment of the globus pallidus (GPe) [2]. This, in turn, would disrupt the functioning of the basal ganglia output structures (thalamus, globus pallidus internal internus (GPi); see figure 1). To date, such oscillations have not been observed *in-vivo*. Increasing complexity of models of the basal ganglia involve changes not only in firing rate, but also in oscillatory behavior and synchronous firing [3].

The location of the STN necessitates invasive methods in order to measure activity *in-vivo*. As such, activity is mostly measured at a single site and it is difficult to relate the activity in one nucleus to activity in another nucleus. As part of the BrainGain project, we study rat midbrain slices by means of multi electrode arrays. We will describe results on the relationships between firing patterns of neurons throughout the STN during spontaneous activity. In addition, activity may be evoked by pulses on an electrode, either from the array or from a separate microelectrode. Spatio-temporal responses to single pulses and high-frequency trains of pulses are studied.

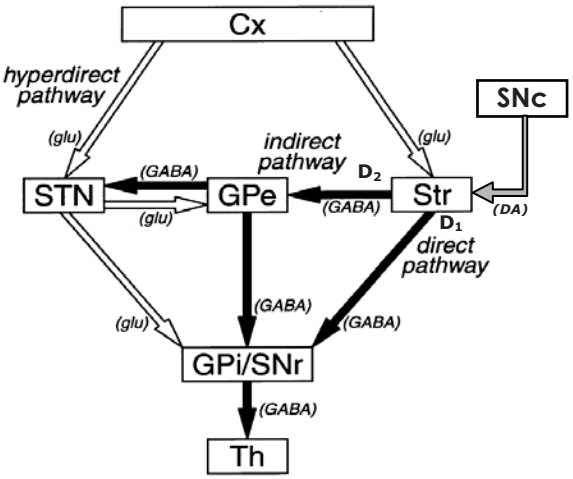

Fig. 1. Schematic of the main connectivity in the Basal Ganglia. The effect of dopaminergic innervation (DA) of the striatum (Str) depends on the receptor type: D_1-dopamine receptors are excitatory, while D_2 receptors are inhibitory. Other neurotransmitters are always excitatory (Glutamate, Glu) or inihitory (gamma-amino butyric acid, GABA). Cx=cortex, SNc/r=substantia nigra pars compacta/reticulata. Adapted from Nambu et al. [4]

2 Methods

2.1 Slice preparation

Coronal brain slices (300 μm) from 16-52 day-old Wistar rats were cut on a Vibratome (Leica VT1000) in an ice-cold cutting medium containing artificial cerebro-spinal fluid (aCSF) with additional $MgSO_4$ and ascorbic acid. Solutions were aerated with carbogen. Rats were anaesthetized using Isoflurane before decapitation.

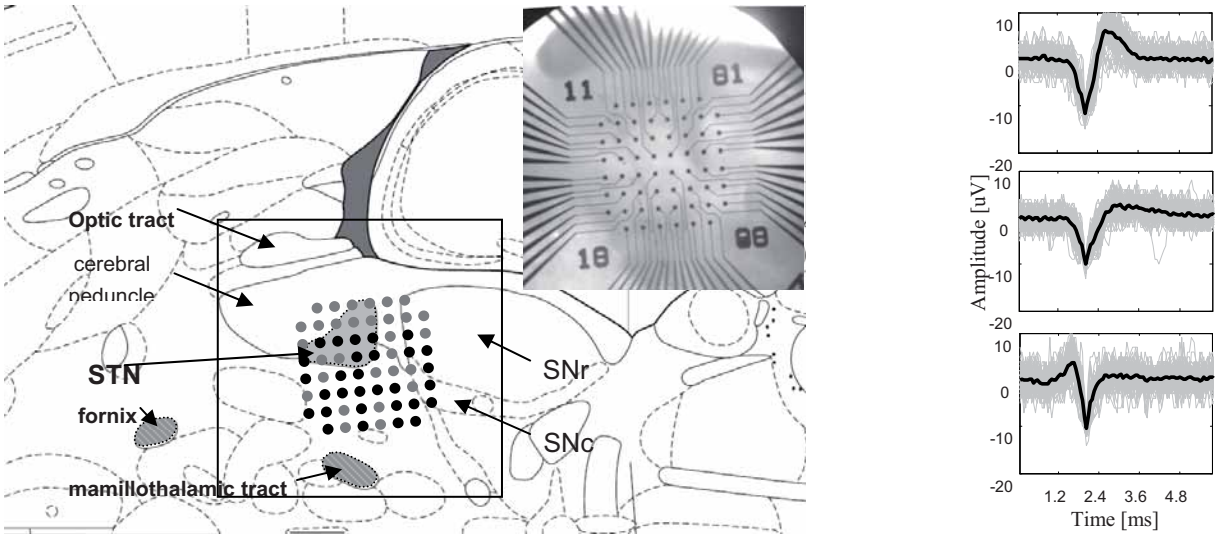

Fig. 2. (left) A MEA superimposed on the corresponding figure of the brain atlas (187, Paxinos & Watson, 2007). Some of the structures used for reference are indicated. Active electrodes are drawn in black. The inset show a picture of the slice on the MEA. The optic tract, cerebral peduncle and mamillothalamic tract appear dark. (Right) Three examples of action-potential wave forms.

2.2 Recording setup

Slices and aCSF were transferred to 3D-multi electrode arrays (3D-MEA; Ayanda biosystems), and signals were amplified, bandpass filtered (10 Hz-10 kHz) and digitized using a setup by MultiChannelSystems. Slices were kept in place by a nylon mesh glued onto a silver ring, lowered into the chamber by a micromanipulator and perfused with aerated aCSF at a rate of ~3 ml/min. Signals were visualized by a custom-made LabView program and threshold crossings exceeding 5 times the RMS noise value (typically 2 to 3 μV) were stored. Measurements were carried out at room temperature.

3 Results

We were able to record action-potential activity from many electrodes located within the midbrain (figure 2). We compared the location of the slice to the brain atlas (Paxinos and Watson, 2007) to identify which electrodes we recorded from were located in STN. Electrodes corresponding to STN, SNr, SNc or PLH (peduncular part of lateral hypothalamus, medial to STN and CP in figure 2) are sometimes active, while electrodes in the cerebral peduncle generally are not. The rate of firing responds to bath application of L-glutamine, potassium and magnesium thus proving their biological origin.

4 Conclusions

Recordings with multi electrode arrays can contribute to solving important questions in Parkinson disease research and also to improve our understanding of other neurological disorders. Specialized multi-electrode arrays can be designed for a specific target, thus increasing the applicability even further.

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References

[1] Lozano, A.M. (2001), Deep brain stimulation for Parkinson's disease. *Parkinsonism Relat Disord*, 7(3), 199-203.

[2] Plenz, D., Kital, S.T. (1999), A basal ganglia pacemaker formed by the subthalamic nucleus and external globus pallidus. *Nature*, 400(6745), 677-682.

[3] Cagnan, H., Meijer, H.G., van Gils, S.A., Krupa, M., Heida, T., Rudolph, M., Wadman, W.J., Martens, H.C. (2009), Frequency-selectivity of a thalamocortical relay neuron during Parkinson's disease and deep brain stimulation: a computational study. *Eur J Neurosci* 30(7), 1306-1317.

[4] Nambu, A., Tokuno, H.,Takada, M.. (2002), Functional significance of the cortico-subthalamo-pallidal 'hyperdirect' pathway. *Neurosci Res*, 43(2), 111-117.