Due to the NENS Stipends I was able to go for a 6 months training to Israel to study at the Bar Ilan university in Ramat Gan. The initial goals of my training stay were:

1) Learn to make electrodes in order to preform chronic recording in freely moving transgenic huntington rat
2) Learn how to analyze electrophysiological data
3) Bring this knowledge back home in order to improve/ change our current electrophysiology work

To start of with, unfortunately, I was not able to complete the first goal of my training stay. Even though I learned the methodology of building electrodes (see fig), due to several unforeseen reasons I was not able to import the transgenic Huntington rats. Since learning the techniques was as important as my project I preceded my training stay. This led to the formation of a manuscript, in which I was able to implement the learned methods. The manuscript has been submitted towards Frontiers in Neuroscience and is currently under reviewing.

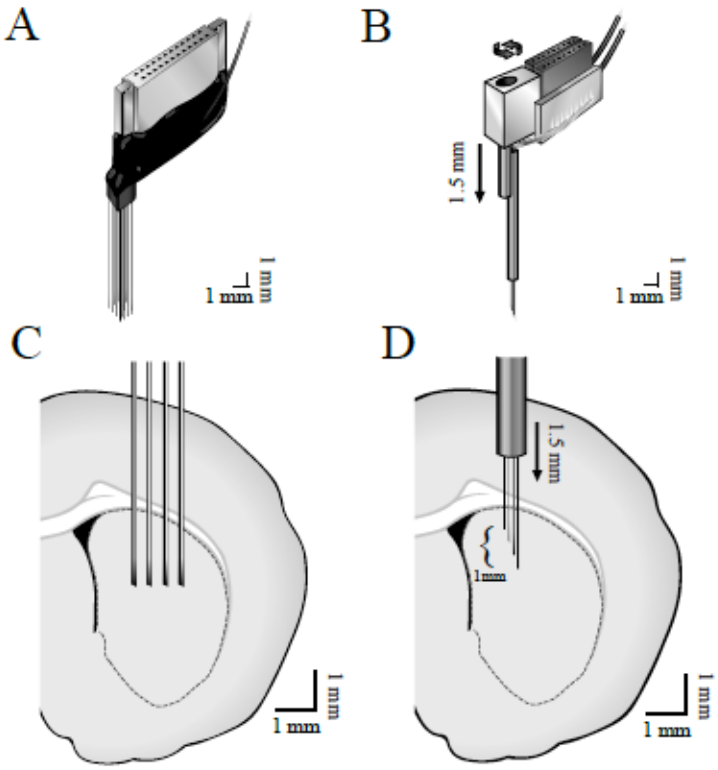
A short introduction of the manuscript:

The striatum is the main input structure of the basal ganglia, integrating input from the cerebral cortex and the thalamus, which is modulated by midbrain dopaminergic input. Dopamine modulators, including agonists and antagonists, are widely used to relieve motor and psychiatric symptoms in a variety of pathological conditions. Haloperidol, a dopamine D2 antagonist, is commonly used in multiple psychiatric conditions and motor abnormalities. In the study we investigated the effects of haloperidol on the activity of three major striatal subpopulations: medium spiny projection neurons (MSNs), fast spiking interneurons (FSIs) and tonically active neurons (TANs). Here fore, we implanted multi-wire electrode arrays in the rat dorsal striatum and recorded the activity of multiple single units in freely moving animals before and after systemic haloperidol injection. Haloperidol decreased the firing rate of FSIs and MSNs while increasing their tendency to fire in an oscillatory manner in the high voltage spindle (HVS) frequency range of 7-9 Hz. Haloperidol led to an increased firing rate of TANs but did not affect their non-oscillatory firing pattern and typical correlated firing activity. Our results suggest that dopamine plays a key role in tuning both single unit activity and the interactions within and between different subpopulations in the striatum in a differential manner. These findings highlight the heterogeneous striatal effects of tonic dopamine regulation via D2 receptors, which potentially enable the treatment of diverse pathological states associated with basal ganglia dysfunction.

Short description of the electrodes I have learned to make:

During my training stay I have learned to make 2 different microelectrode arrays formed by fine microwires in order to chronically record the extracellular activity of populations of individual neurons in behaving animals (rats). Presently, several laboratories around the world make their own recording electrodes and manufacture them differently. In the lab of Izhar Bar-Gad I have learned to make a non-moveable and a moveable 16-microwire array (see fig). The basic of both arrays are insulated metallic conductors with a diameter of 45 or 25 µm. The custom made non-movable array is a 4X4 45 µm diameter isonel-coated tungsten microwire linear array and the movable array contains a bundle of 16 25-µm-diameter formvar coated nichrome microwires. When making the arrays it is important that the microwires are as straight and stiff as possible. Following, before implantation the wires of the electrodes need to be cut. In the current lab we choose to cut the wires sharp. The angle cut lowers the impedance of the electrodes, which can reduce the ability to record from well-isolated single neurons. However, by using somewhat sharp tips the penetration through the brain is much easier and less traumatic. Since brain tissue is reactive to damage, minimizing the damage by using a sharp tip, lowers the chance of scar tissue at the recording site. In addition to cutting the electrode tips, the tips can be left bare, or they can be electroplated in order to reduce the impedance of the electrodes. Only the wires of 25 µm moveable array where electroplated since their initial impedance was exceptionally high.

For precise building details I would like to refer to the book: Methods for Neural Ensemble Recordings edited by Miguel AL Nicolelis 2008 Frontiers in Neuroscience.



The surgical procedure I learned entailed the following:

Two different strains of animals were operated: Long Evans and Sprague Dawley rats varying between 250-450 grams. The operation started after an intra-peritoneal injection of a mixture of ketamine HCl (100 mg/kg) and xylazine HCl (10mg/kg) and an intra-muscular injection of atropine (0.1 mg). Anesthesia during the operation was maintained using isoflourane and supplementary injections of ketamine. The animals' scalp was shaved and cleaned, and anesthetic cream (2.5% lidocaine and 2.5% prilocaine) was applied into their ears to prevent discomfort during head fixation. The rats were placed in a stereotactic frame (Stoelting Co., Wood Dale, IL, USA) and their head was fixed. The scalp was sterilized and lidocaine (10 mg/ml) was locally injected into the skin around the incision location. A central incision was made and the skull was cleaned. The head was then straightened by equalizing bregma-lambda height. Coordinates of the craniotomies were marked and holes were drilled. The dura was removed and 16 electrode arrays were slowly lowered into the striatum and were grounded and fixed to the skull by screws and dental cement.

Experimental procedures learned and data preprocessing and analysis:

After the implantation of the electrodes experimental sessions began at least 7 days post operation to allow full recovery of the animals. The animals were placed in the recording chamber, where they could move freely, and were connected to the recording system. The signal was continuously recorded throughout the session. The analog signal was amplified (*200), band-pass filtered (0.5-10000 Hz, 4 pole Butterworth filter) and digitized at a sampling rate of 44 KHz (Alpha-Lab SNR, Alpha-Omega Engineering, Nazareth, Israel). The digitized continuous raw signal was acquired to allow offline sorting and analysis. Sessions initiated with recordings in the naïve state that was followed by recording of neuronal activity during and after the subcutaneous haloperidol (0.05 mg) injection.

Spike sorting of multiple single units from the continuous raw signal was performed offline (Offline Sorter v2.8.8, Plexon, Dallas, TX). Learning how to work with this program broadened my knowledge on neuronal signals. With the Offline sorting software it is possible to align a certain spike shape and filter it from all recorded signals. Furthermore, using a continuous signal instead of the more common segmented data makes it possible to obtain long overlapping windows (1.5-2 ms in our data) surrounding each spike, thereby increasing the fidelity and quality of the sorting. In the lab I learned how to classify the different neurons and the importance of offline sorting.

Moreover I followed a course in signal-data-analysis. Izhar Bar-Gad gave this course at the Bar Ilan University. With the acquired knowledge I was able to analyze the obtained data and interpreted sufficiently in order to write an scientific article.

Collaboration and implementation of learned techniques

My stay at the Bar Ilan University in Ramat Gan, Israel, under the supervision of Izhar

Bar-Gad, has resulted in a good collaboration between both labs and maybe future implementation of the learned techniques. Future collaborations will concentrate on electrophysiological data analysis in Tourette patients. Izhar Bar-Gad and his PhD students have great experience in analyzing electrophysiological data recorded in a monkey model of Tourette. Currently in Maastricht several Tourette patients have been and will be operated with DBS electrodes. By combining our knowledge, we want to analyze the intra-operative data to acquire more knowledge on the disease mechanism of Tourette.

My personal experience

I would like to describe my 6 months at the Bar Ilan University in Ramat Gan, Israel, as a valuable period within my PhD. During this training stay I have been taught how to obtain and analyses electrophysiological information. Thereby I have improved my understanding of the brain and the mechanisms within.
By working in a different lab, in a different country, under different supervision I have broadened my personal ability to carry out research. Moreover it has expanded the network of my home lab and my personal network.

Final manuscript:

Zeef *et al.,* Haloperidol-induced changes in neuronal activity in the striatum of the freely moving rat. Frontiers in Neuroscience *In press*