(Final report)

“BASAL GANGLIA AND THE CONTROL OF LOCOMOTION”

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The basal ganglia is a subcortical network of nuclei whose dysfunction is associated with numerous prevalent pathologies such as movement disorders (Parkinson and Huntington disease, Tourette syndrome) and cognitive disorders (addiction, obsession). Despite an impressive body of experimental, clinical and theoretical works, the exact function of the basal ganglia is still highly debated. The understanding and treatment of basal ganglia dysfunctions require a better understanding of its normal function. Furthering this understanding was the general aim of this project.

The main objective of this project was to understand how neuronal populations in the basal ganglia contribute to the control of locomotion and the execution of habits. Reaching this objective implied to optimize two challenging technical aspects. One the one hand we needed to establish a behavioral paradigm in which rats would learn to repetitively perform a motor skill. Importantly for our question, this paradigm had to allow an exhaustive and precise quantification of the kinematics of the movements performed during skill execution. We therefore designed a running task in a treadmill. In short, to obtain reward and optimize their efforts, rats were trained to control their running speed in a timely, precise, and stereotyped manner (**Fig. 1**). This original behavior paradigm combines two main advantages. First, it focuses on a simple and natural motor command which is well known to be under the control of the basal ganglia: the locomotion. Second, a treadmill allow the use of lateral cameras and sensors (like accelerometers) that can fully capture the dynamics of locomotion over several seconds. On the other hand,we needed to use electrophysiological tools capable of recording simultaneously the spiking activity of ensemble of striatal neurons. Using low impedance custom-made tetrodes array and Neuralynx NLX9 microdrive we succeeded in simultaneously recording up to 80 striatal neurons in a freely moving animal. (**Fig. 2**)

The main results derived from those major technical efforts are as follow.
Rats were trained to run on a treadmill that can be turned off by the animals entrance in a stop area (**Fig. 1a**). Importantly, to turn the treadmill off, animals had to run outside the stop area for a duration longer than a fixed time interval (7 s for rats 1-2 and 5 s for rat 3) since the starting of the treadmill (**Fig. 1b**, correct trials, rewarded by sucrose solution). Entering the stop area before the fixed time interval triggered a warning sound and forced the rats to run for 20 s (**Fig. 1b**, incorrect trials, not rewarded). Naive animals instinctively rushed forward when the treadmill was turned on, which resulted in early stop area entrances and a high proportion of non-rewarded 20 s long trials (**Fig. 1c,** upper panels). Consequently, to perform correct trials (i.e., to stop the treadmill, run less and receive rewards), animals had to control their running speed in a timely manner. In a long trial-and-error process (**Supplementary Fig. 1a**), animals achieved such control by developing a running routine that could be broadly divided in three phases: 1) passive displacement toward the treadmill end, 2) steady run at the end portion of the treadmill and 3) progressive acceleration toward stop area (**Fig. 1c**). Consistent with a habit-based behavior, this routine was highly stereotyped once learning was achieved (**Fig. 1c**) and persisted for several days when the task structure was modified by shortening the treadmill length (**Supplementary Fig. 1**).

After this behavior was over-trained, we investigate how ensemble of neurons in the DLS contribute to

the execution of habits. Each recording session consisted in 50 to 100 trials. Tetrode recordings were obtained when animals displayed highly habitual running pattern (at least 8 weeks of training and running routine persisted when the treadmill length was shortened). Two-thirds of the recorded units had their spiking activity task-modulated (**Supplementary Table 1**) from which ~10 % had a firing pattern compatible with gating function and fired before and after the runs (**Fig. 3a,** first and last panels). The other 90 % of the task-modulated neurons fired at different timed during the runs (**Fig. 3a**). Strikingly, sorting in time the normalized perivent histograms of all modulated units revealed that the activity of DLS neurons is sequentially modulated during the entire task (**Fig. 3b**). This suggests that DLS ensembles are involved in the ongoing execution of this habit, rather than its gating. To confirm this possibility, we examined the relationship between, spiking activity in the DLS and the locomotor dynamics and kinematics of the habit. First, we found that about 10% of the task-modulated neurons fired in a phase-locked manner with the locomotor limb movements **(Fig. 4a)**. Second, using partial correlation and multiple regression analysis, we found that the firing rate of a large fraction of the task-modulated units (> 80%) significantly correlated with running acceleration, speed, position of the animal, time or a combination of these kinematic variables (**Fig. 4b-e)**.

The impact of the results generated by this project is fundamental for our understanding of the role of the basal ganglia in motor control during habit execution. Indeed, only a limited number of studies have so far directly related patterns of spiking activity of striatal neurons and skill execution. Those studies have generated a view of the role of the basal ganglia in skill performance as an "abstract" traffic light disengaged from movements execution itself. We found that the firing rate of a large fraction of neurons was either locked to the locomotor limb movements or correlated with the kinematics of the habit. These results are hardly compatible with an exclusive gating function of the DLS during habit execution but can be readily explained if the DLS also control the on-going execution of habits. Such function is well supported by several studies showing that inactivation of the DLS selectively impair habits but spare recently learned behaviors. Habits are assumed to arise from stimulus-response associations in the DLS and we hypothesis that the orderly sequences of kinematics-related spiking activity in the DLS constitute correlates of the sequential sensorimotor associations required to perform the habit. Spiking activity in the DLS could control habits execution directly, via basal ganglia output projections to brainstem motor regions, and/or indirectly via output projections to the thalamocortical neurons targeting frontal cortical regions. More generally, the fact that DLS continuously controls the ongoing execution of movements sequences (rather than gates them) is in agreement with the observation that, when comparing sequential movements performed by patients with PD and healthy controls, movements times lengthened progressively as the sequence progressed. To explain such impairments we propose that "healthy" ordered sequences of neuronal activity in the DLS are altered by the excessive network synchrony associated with Parkinson disease.
The results of this project are submitted for publication to Nature Neuroscience.

FIGURES

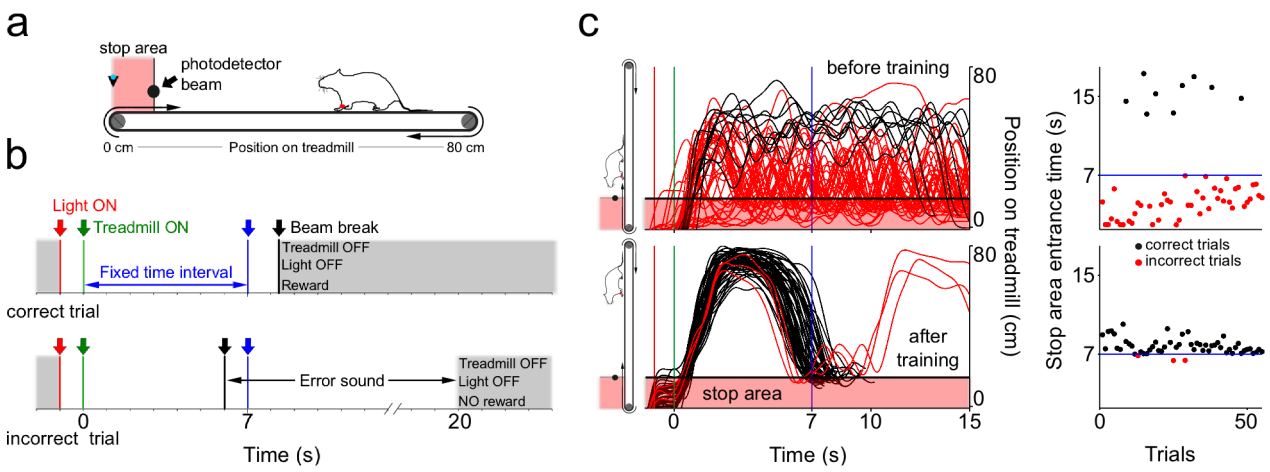

Figure 1
The left part of the figure shows the behavioral apparatus (a) and illustrates a correct and incorrect trials (b). The right part of the figure shows the behavior of one animal during two representative sessions before (c, top panels) and after (c, bottom panels) learning. Right panels show the trajectories of the animal and right panels show the stop area entrance times.

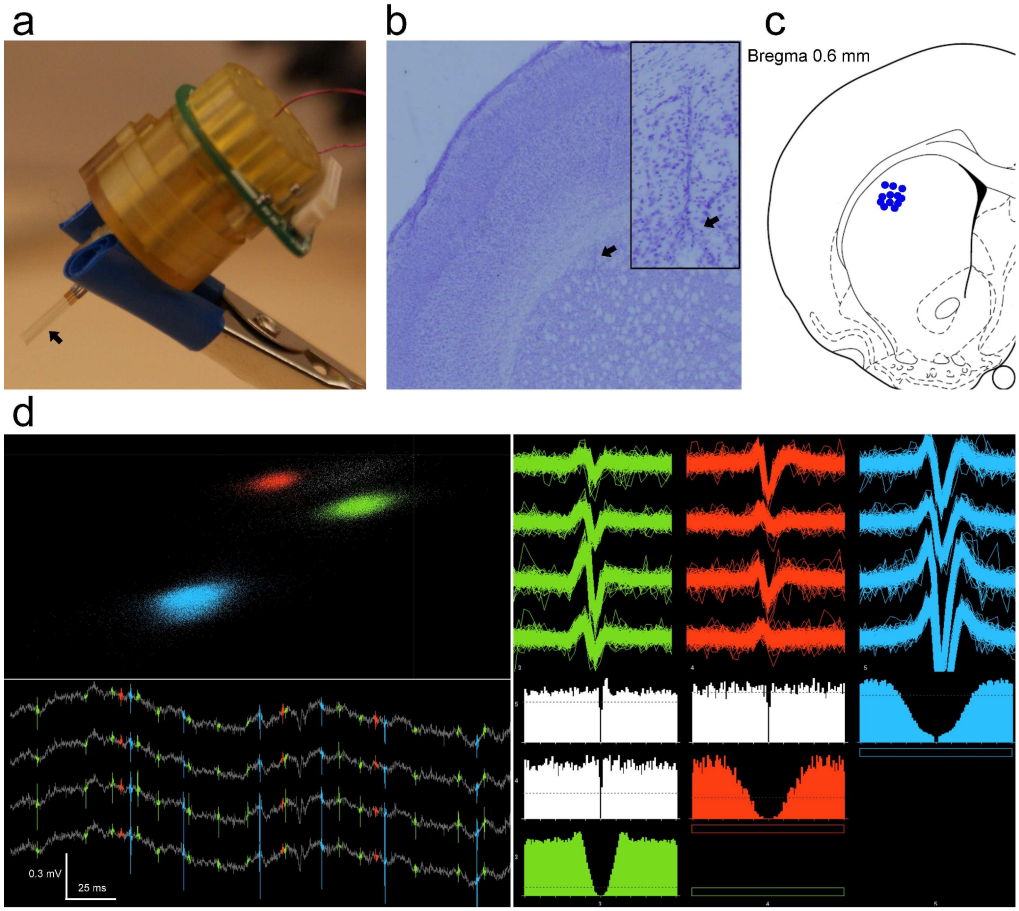

Figure 2
Electrophysiological procedures. (a) Microdrive (NLX-9) loaded with 8 tetrodes (arrow) before chronic implantation. (b) Example of tetrode localization. Arrow show tetrode track and tip (inset) on Nissl-stained coronal section of the striatum. (c) Schematic representation of the localization of the tip of all tetrodes used in this study. (d) Illustration of striatal unit recorded with tetrodes. Top left, projection of 2 principal components extracted of the waveform of spikes recorded with a tetrode. Top right, spike waveforms (100 trials superimposed) on each channel of the tetrode. Bottom left, example of the wideband (0.1 Hz to 8 kHz) striatal LFP (the 4 traces corresponding to the 4 wires of a single tetrode). Bottom right, autocorrelograms of the isolated clusters (+/- 30 ms). Spikes of the three isolated clusters are consistently colored in the 4 panels.

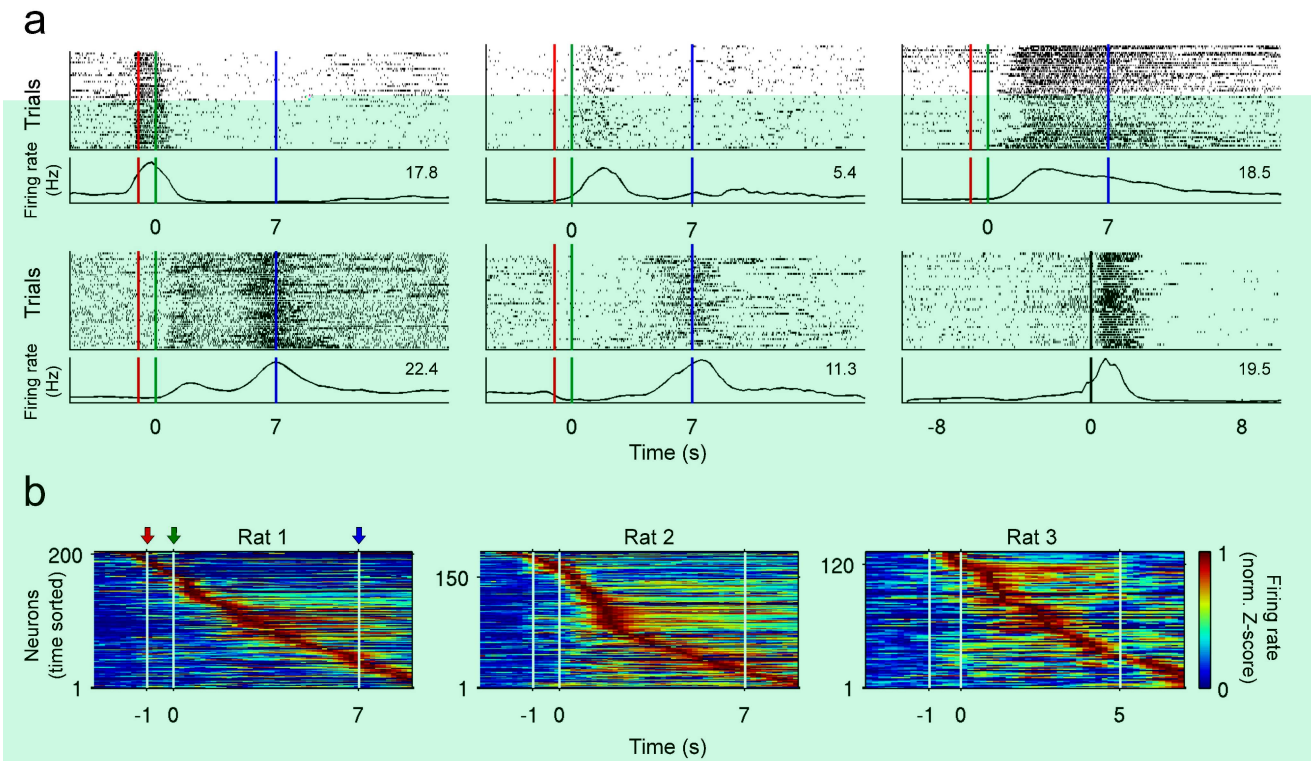

Figure 3
(a) Spike rasters of representative units firing during the task. Lower insets show average perivent histograms and peak firing rates (average, in Hz). (b) Time-sorted normalized Z-score of the spiking activity of all task-modulated units (from all recorded sessions).

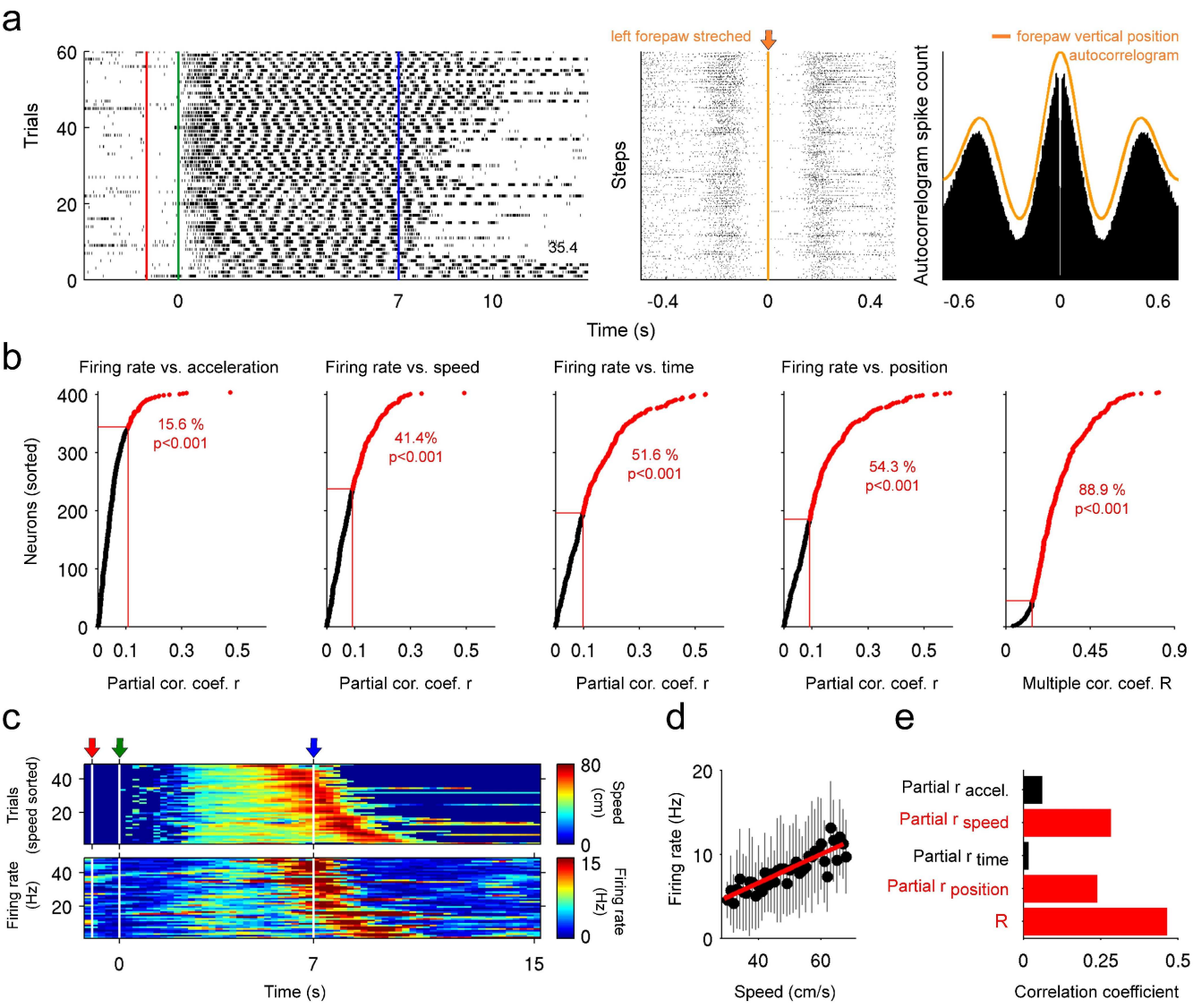

Figure 4. Spiking activity in the DLS is correlated with limb movements and the kinematics of the habit. (a) Limb-related rhythmical activity of a dorsolateral striatal unit shown through task-aligned rasters (left), limb-movement aligned spike rasters (middle) and spike auto-correlogram (right, black histogram). (b) Distributions of partial and multiple correlation coefficients (absolute values) between firing rate and running acceleration, speed, position and time for all task-modulated units. (c-e) Illustrative unit. (c) Trial-by-trial instantaneous running speed (top) and firing rate (bottom). Trials were sorted according to the time of maximum running speed. (d) Mean firing rate (black dots) and standard deviation (gray lines) versus speed. (e) Partial and multiple correlation coefficients between firing rate and kinematic parameters.