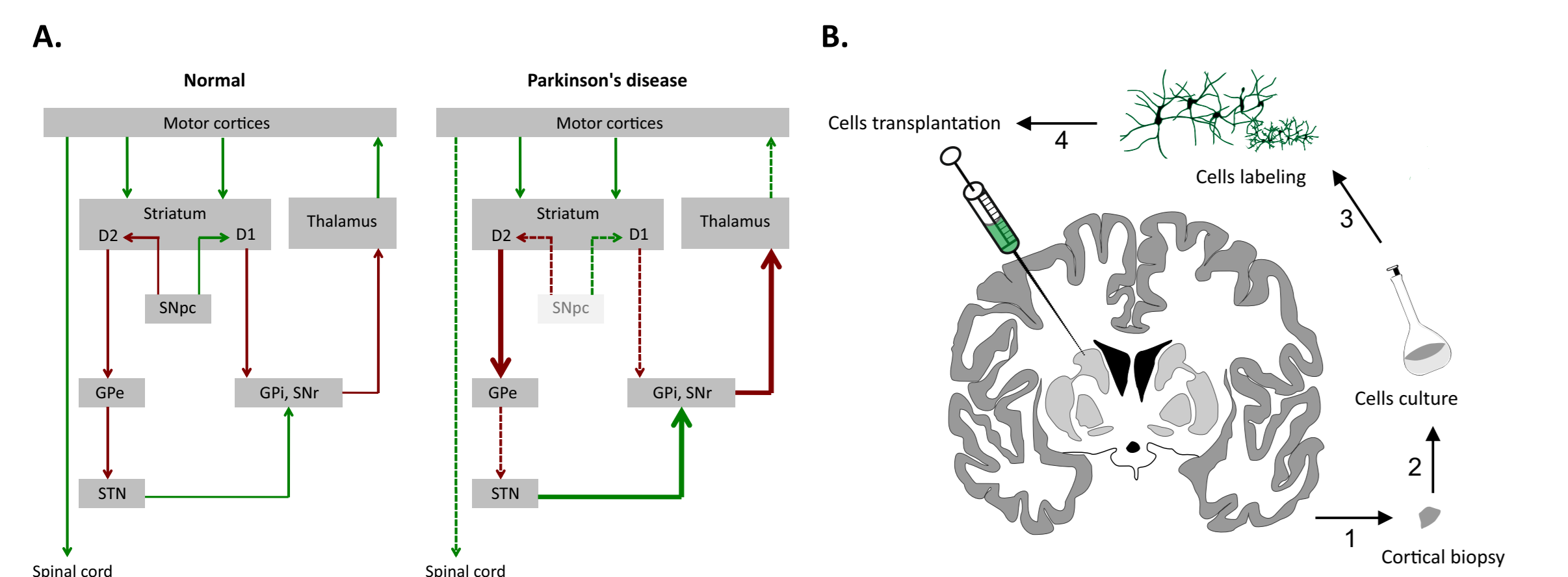


Introduction

Autologous cells transplantation overcomes several issues raised by the use of human embryonic stem cells including ethical controversies and immune limitations. Bloch J. and colleagues (2014) demonstrated the potential of the transplantation of autologous neural cells ecosystem (ANCE) in parkinsonian monkeys. The present investigation intended to assess the impact of ANCE transplantation in four cynomolgus macaque monkeys exhibiting parkinsonian symptoms, focusing on the manual dexterity and the dopaminergic system state *in vivo*.



Methods

The monkeys were extensively trained to perform fine manual dexterity tasks as well as a reach and grasp drawer task before undergoing chemical (MPTP) lesions of the SNpc. During the MPTP phase, small cortical biopsies from the prefrontal zone were performed and the gray matter material obtained put into culture according to the protocol developed by Brunet et al. (2005). Additionally, at all phases of the protocol, the integrity of the nigro-striatal system was followed-up by ¹⁸F-dopa PET scan. At the end of the experiment, histological readout was performed with immunohistochemistry technique in order to reveal the state of the dopaminergic system.

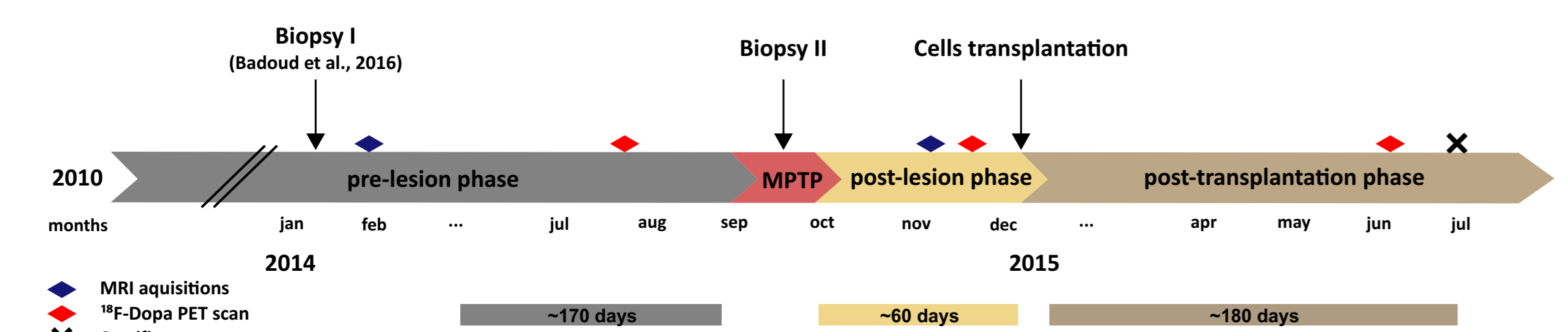


Figure 2: Experimental schedule of the project. During the pre-lesion phase a first biopsy was performed in order to assess its potential impact on the behavioral tasks and to develop the good manufacturing practice (GMP) protocol. The second biopsy, which provided the cellular material, was performed during the MPTP phase better reflecting the clinical conditions of Parkinson disease.

Fine manual dexterity

The modified-Brinkman board task

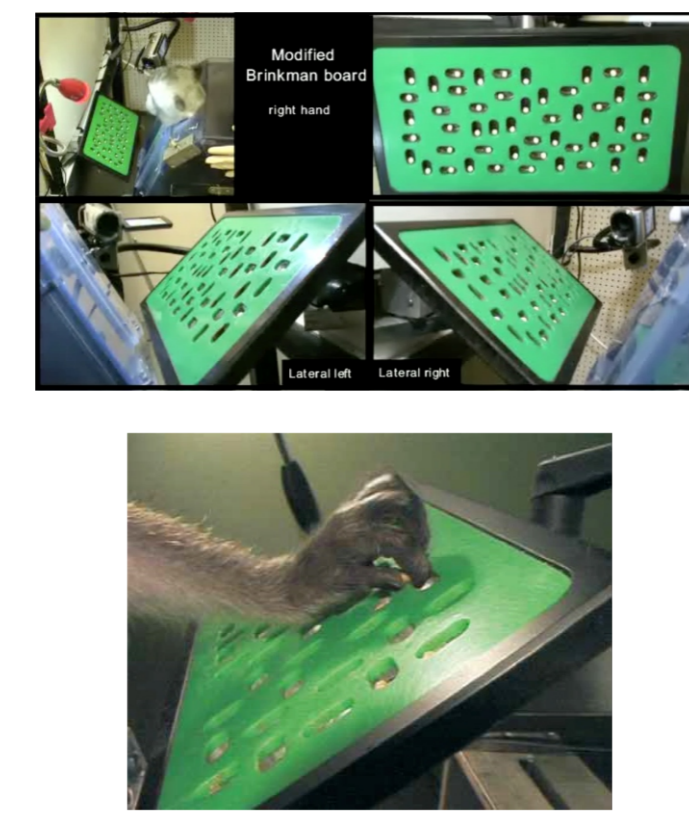
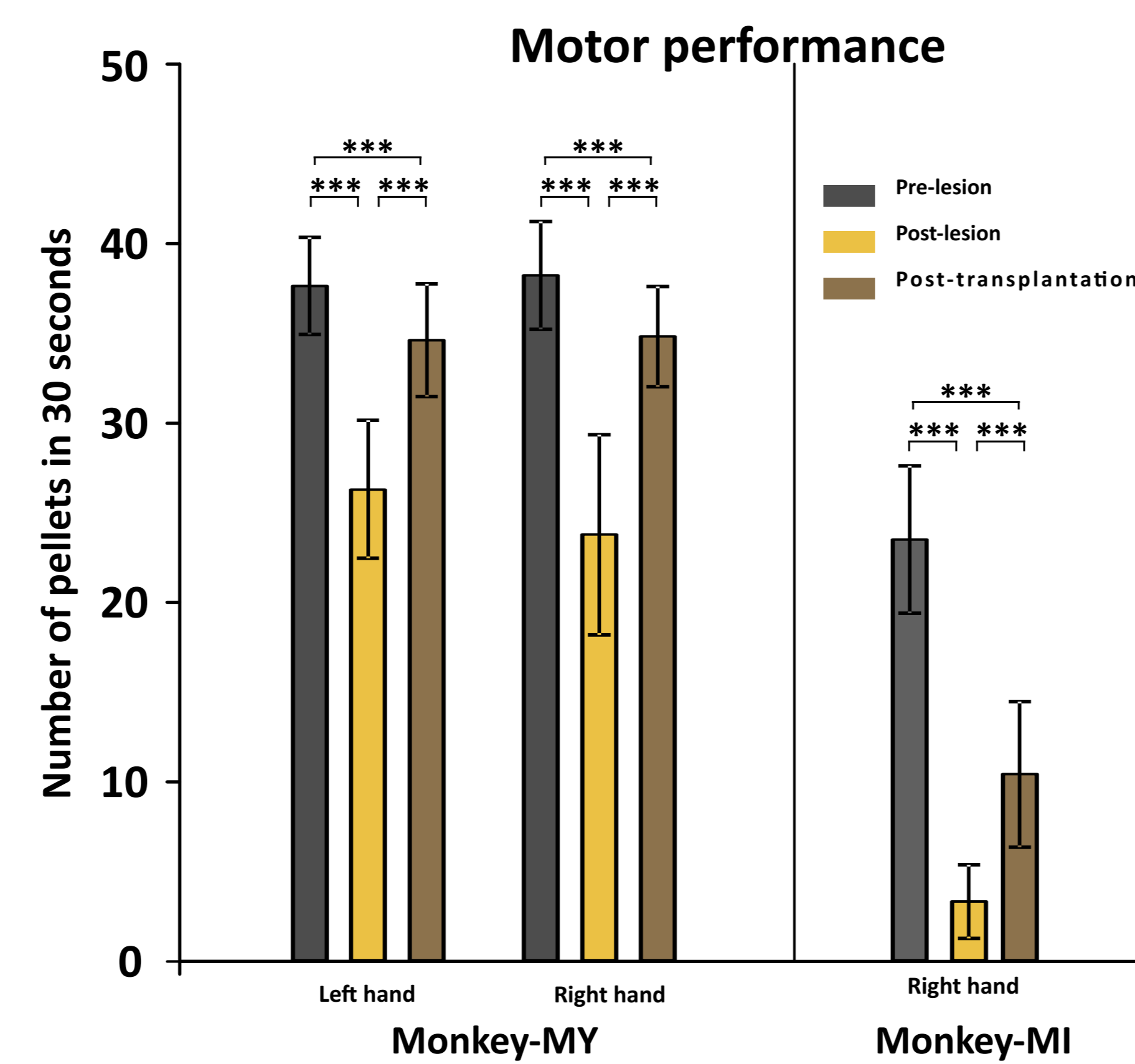


Figure 3: The «Modified-Brinkman board task», in which the monkey has to retrieve banana food pellets using the precision grip (opposition of thumb and index finger), from 25 horizontal and 25 vertical slots. Dimension of slots: 15 mm long, 8 mm wide and 6 mm deep.



After MPTP intoxication: impairments of fine manual dexterity in Monkey-MY and Monkey-MI, whereas Monkey-LL and Monkey-LY were affected to a lesser extent (data not shown).

After ANCE transplantation: improvement of the fine manual dexterity in Monkey-MY and Monkey-MI (6 months post-transplantation).

Reach and grasp movements

The drawer task

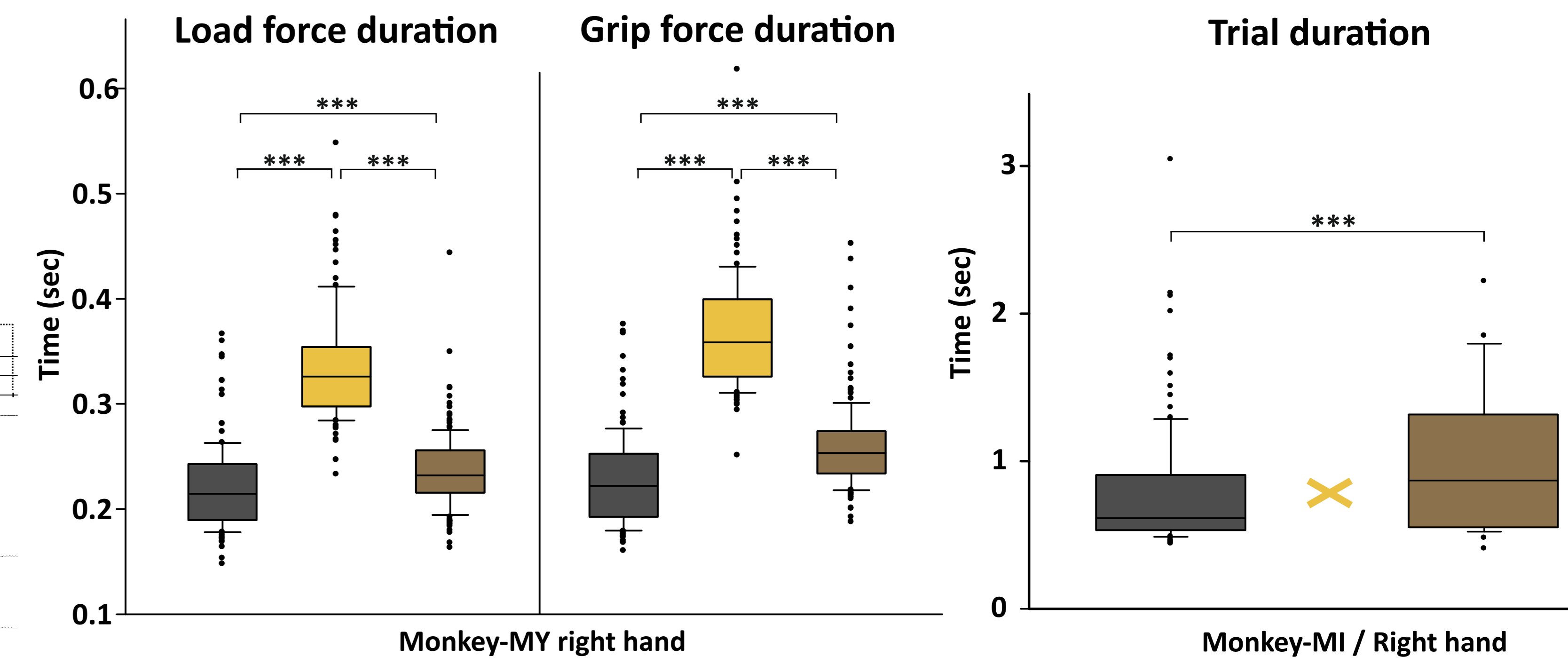
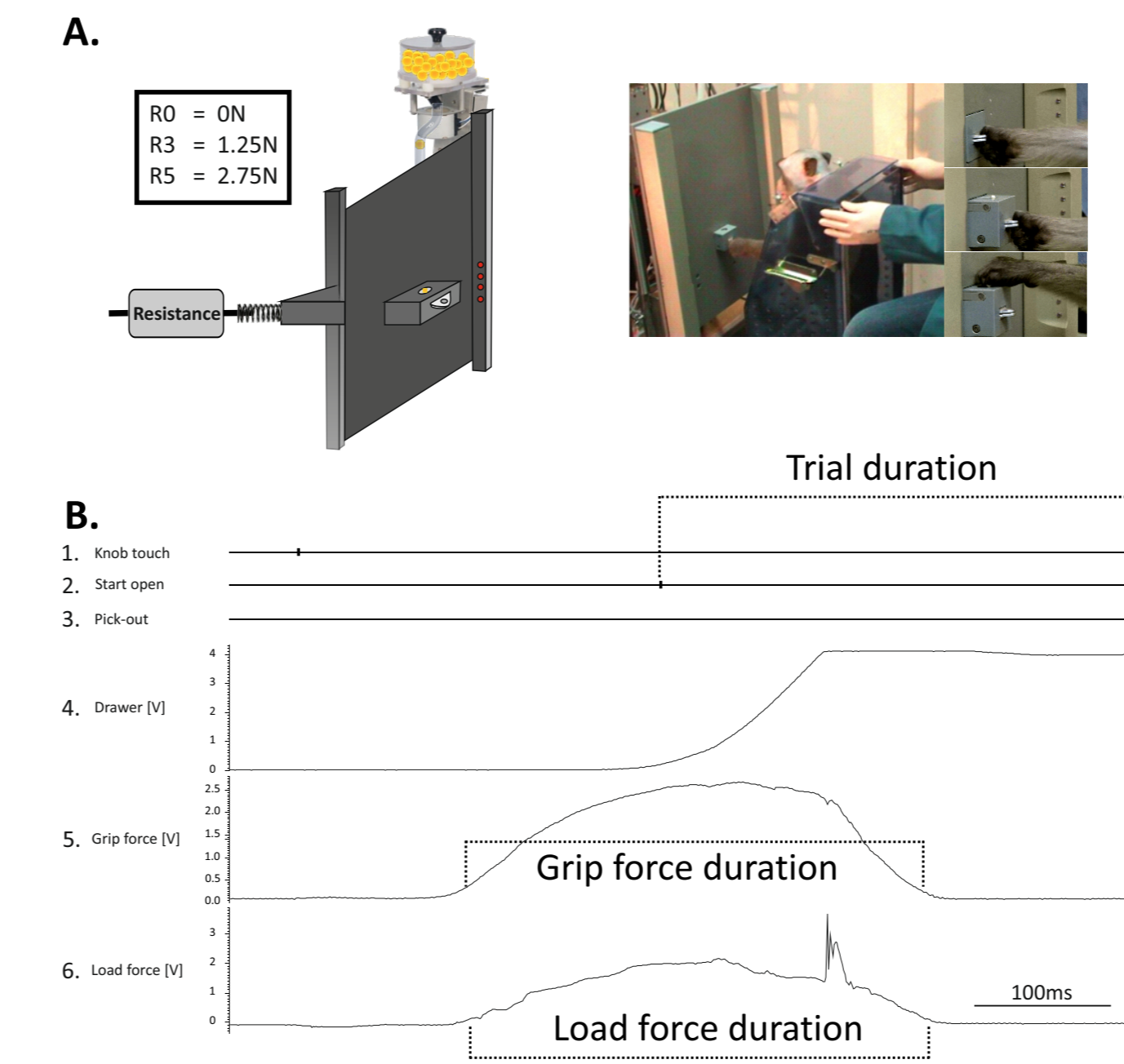


Figure 4: A. Representation of the «reach and grasp drawer task» setup with the adjustable resistances in Newtons. B. Raw data curves are shown: 1. The «tic» represents the time point when the monkey touches the knob of the drawer. 2. The «tic» represents the time point when the drawer starts opening. 3. The «tic» represents the time point when the monkey retrieve the pellet. 4. The displacement of the drawer. 5. The grip force (the force applied on the knob) and 6. The load force (the force applied to open the drawer).

After MPTP intoxication: in Monkey-MY, increase of the load and the grip force durations at all resistances for both hands (R0, R3 and left hand not shown). Monkey-MI was not able to perform the task. Monkey-LY and Monkey-LL were affected to a lesser extent (data not shown).

After ANCE transplantation: in Monkey-MY, recovery of the load force durations at all resistances for both hands (R0 and R3 not shown). Monkey-MI regained its capacity to perform the task only at R0.

Spontaneous activity

Tracking video system

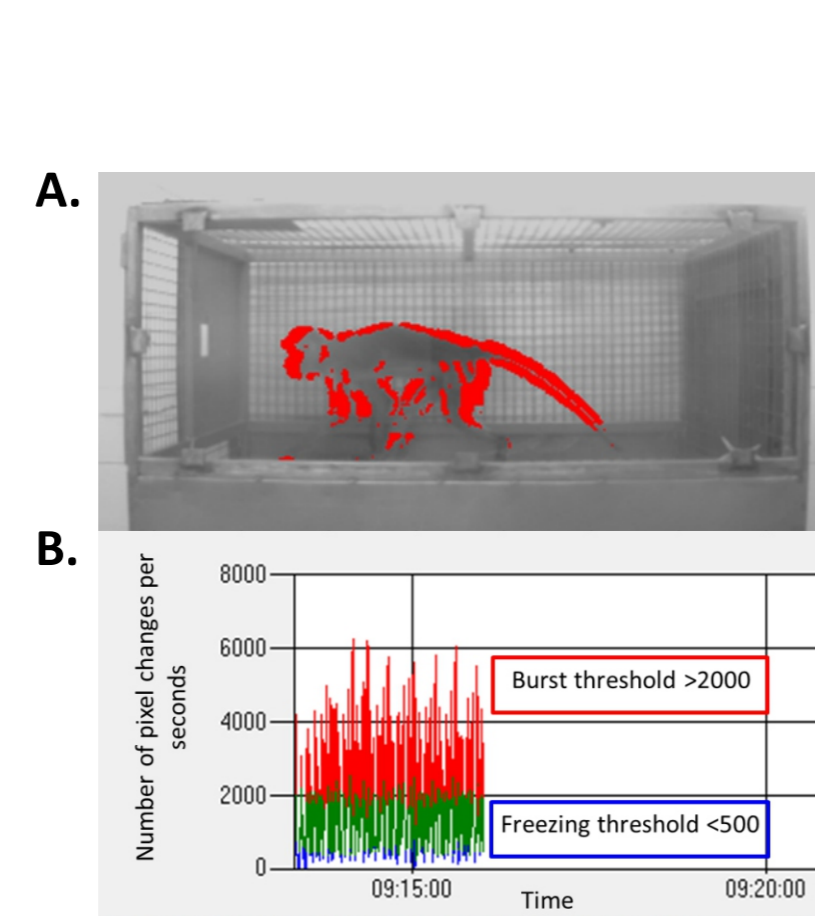
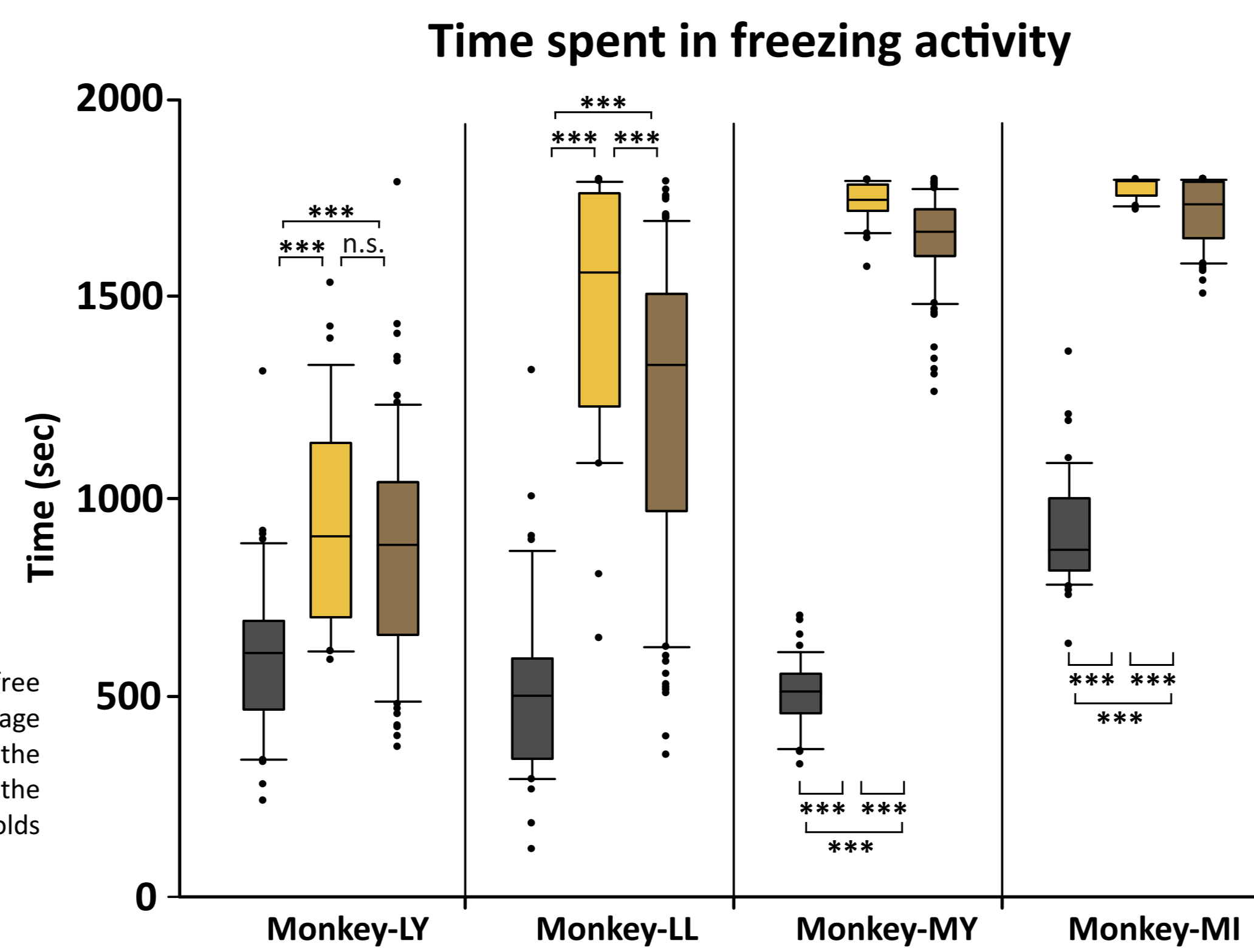


Figure 5: A. Locomotor and other activities (free behaving) were assessed using the VigiePrimate image analyzer system. The system calculated in real time the quantity of movements, obtained by change of the different 256 pixels grey level. B. Different thresholds defined freezing, middle and burst activities.

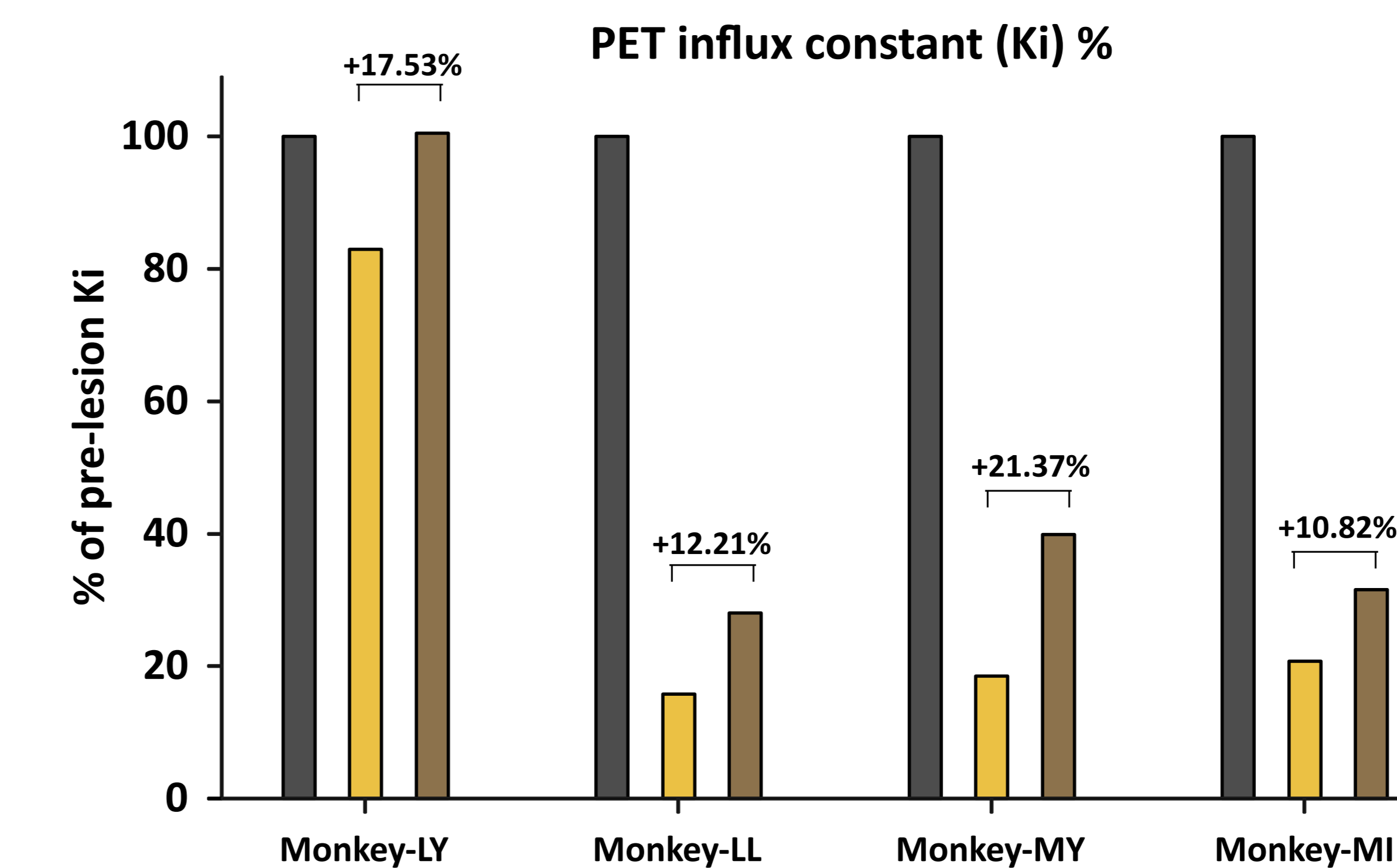
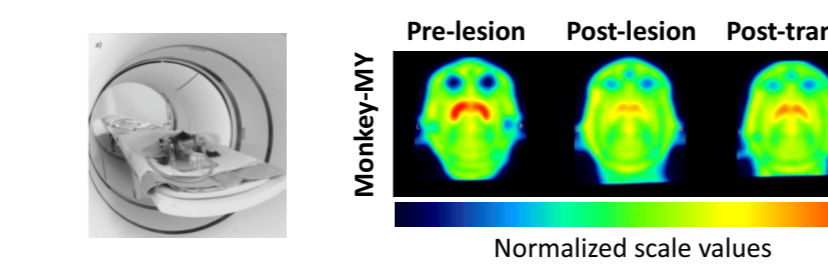


After MPTP intoxication: increase of the time spent in freezing activity in all four animals.

After ANCE transplantation: slight decrease of the time spent in freezing activity in all four animals.

Dopaminergic state

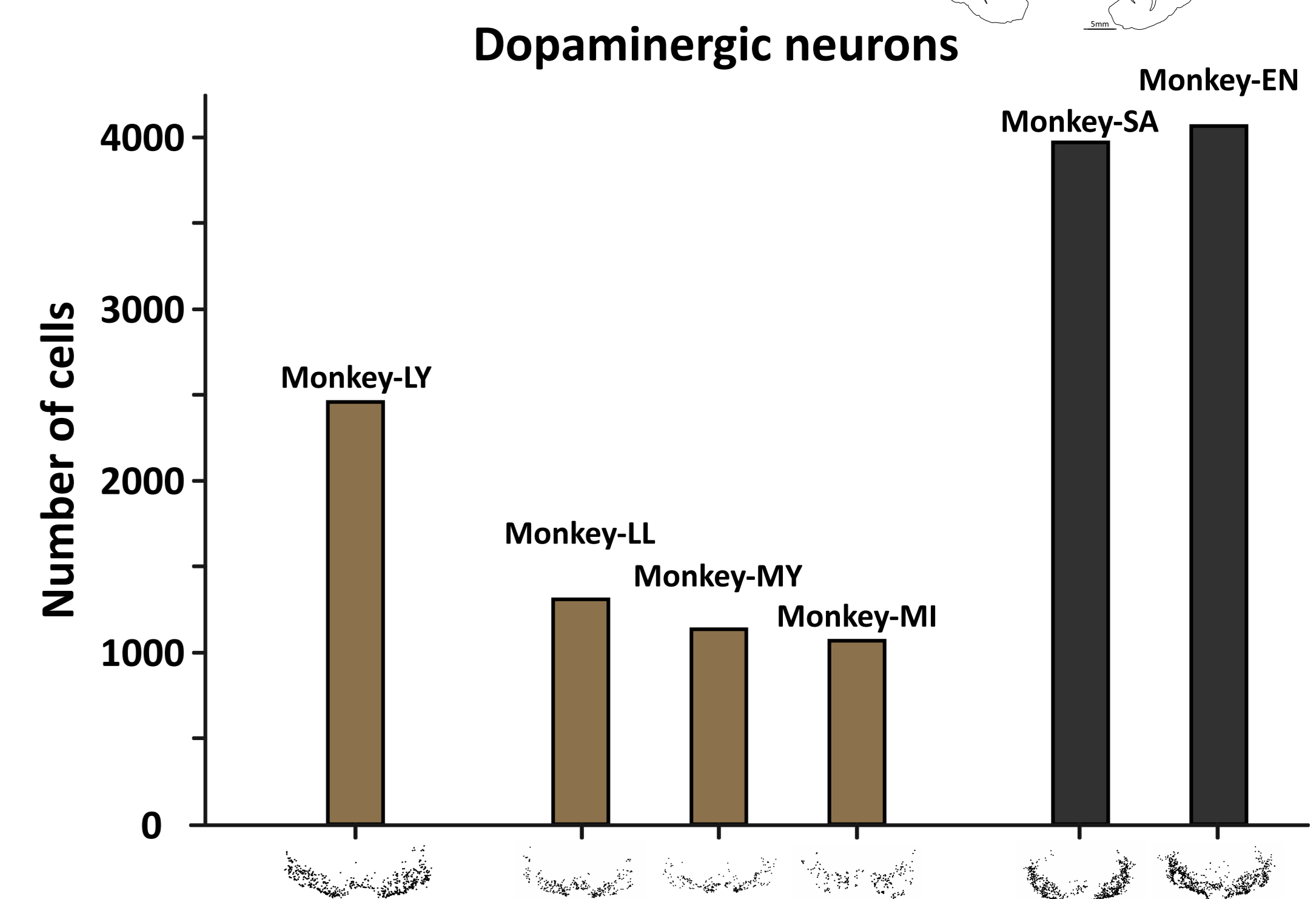
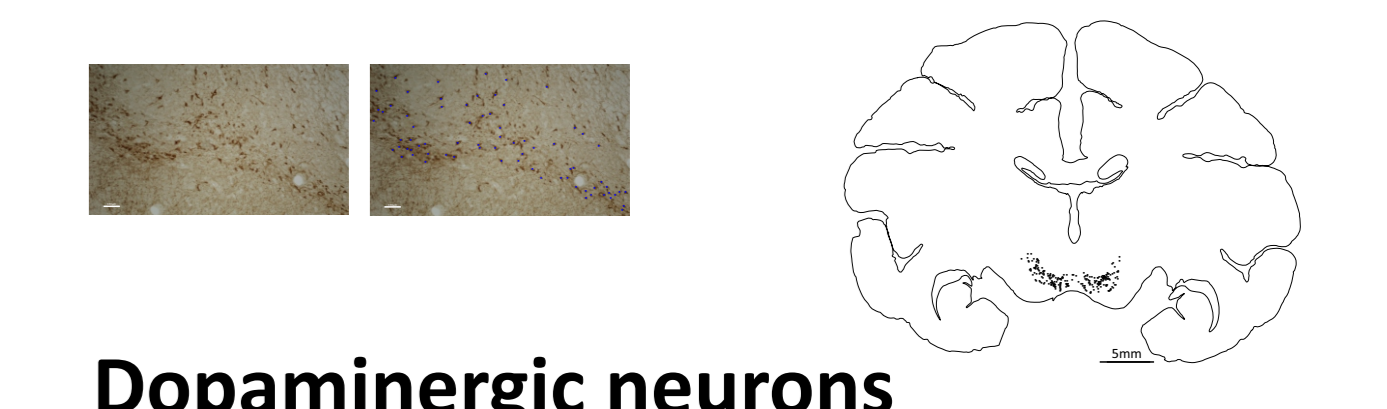
¹⁸F-Dopa PET scan



After MPTP intoxication: dramatic decrease of striatal uptake (loss>80%) in all animals except in Monkey-LY (17%).

After ANCE transplantation: all subjects showed a significant but limited increase of their striatal uptakes, ranging from 10% to 21%.

Histology



In the 4 parkinsonian monkeys: in the SNpc, the number of the dopaminergic neurons (tyrosine hydroxylase -positive neurons) was 70% lower than in the healthy monkeys (Monkey-SA and Monkey-EN) in all the four animals except in Monkey-LY (40%).

Discussion

Out of the four animals, two were severely affected by the MPTP lesions whereas the other two exhibited mild symptoms. Furthermore, the ¹⁸F-dopa striatal uptake was reduced by about 80% in three of them. Six months following ANCE transplantations, all monkeys presented significant improvement of their motor impairments. This functional recovery was accompanied by an increase of ¹⁸F-dopa striatal uptake. These results were consistent with the number of dopaminergic neurons within the SNpc. Taken together these new data open new therapeutic perspectives for the ANCE approach regarding neurodegenerative disorders like Parkinson's diseases.