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Fine Manual Dexterity Assessment After Autologous Neural Cell Ecosystem (ANCE) Transplantation in a Non-human Primate Model of Parkinson's Disease

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Abstract

Background. Autologous neural cell ecosystem (ANCE) transplantation improves motor recovery in MPTP monkeys. These motor symptoms were assessed using semi-quantitative clinical rating scales, widely used in many studies. However, limitations in terms of sensitivity, combined with relatively subjective assessment of their different items, make inter-study comparisons difficult to achieve. *Objective*. The aim of this study was to quantify the impact of MPTP intoxication in macaque monkeys on manual dexterity and assess whether ANCE can contribute to functional recovery. *Methods*. Four animals were trained to perform 2 manual dexterity tasks. After reaching a motor performance plateau, the animals were subjected to an MPTP lesion. After the occurrence of a spontaneous functional recovery plateau, all 4 animals were subjected to ANCE transplantation. *Results*. Two of 4 animals underwent a full spontaneous recovery before the ANCE transplantation, whereas the 2 other animals (symptomatic) presented moderate to severe Parkinson's disease (PD)-like symptoms affecting manual dexterity. The time to grasp small objects using the precision grip increased in these 2 animals. After ANCE transplantation, the 2 symptomatic animals underwent a significant functional recovery, reflected by a decrease in time to execute the different tasks, as compared with the post-lesion phase. *Conclusions*. Manual dexterity is affected in symptomatic MPTP monkeys. The 2 manual dexterity tasks reported here as pilot are pertinent to quantify PD symptoms and reliably assess a treatment in MPTP monkeys, such as the present ANCE transplantation, to be confirmed in a larger cohort of animals before future clinical applications.

Keywords

Macaque monkeys, Parkinson's disease, MPTP, autologous cell therapy, manual dexterity, precision grip

Introduction

Parkinson's disease (PD) is characterized by progressive appearance of the cardinal symptoms: (1) rigidity, (2) bradykinesia/akinesia, and (3) resting tremor because of the gradual degeneration of the dopaminergic neurons in the substantia nigra pars compacta. The impact of nigro-striatal denervation on motor functions has been extensively described, in both human pathology and non-human primate (NHP) 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models.[1-7] Among the most common of readouts used to assess the impact of the MPTP lesions and the potential effects of a treatment in NHP, the clinical rating scales represent the most frequently used semi-quantitative assessment.[8] However, limitations in terms of sensitivity, combined with the relatively subjective assessment of their different items, make inter-study comparisons difficult to achieve. Therefore, precise quantitative assessments of behavioral deficits provide better hints and higher reproducibility,[8] such as quantitative assessments of motor

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functions conducted in NHPs exposed to MPTP, but mostly focused on locomotion, posture, and proximal limb movements.[9-13] In contrast, the effect of MPTP intoxication on manual dexterity in NHPs was clearly investigated less often.

In humans, the pegboards were widely used by clinicians and therapists to assess manual dexterity deficits and/or to promote functional adaptations.[14,15] This test consists of filling a board containing small holes with cylindrical objects, called pegs, as fast as possible. In the context of PD, it has been demonstrated that the degree of dopaminergic depletion correlates very well with the behavioral scores derived from the pegboard task.[16,17] According to Bohnen et al,[18] this manual dexterity test could even be considered as a good biomarker of the extent of nigro-striatal denervation. Moreover, several investigations were conducted on the impact of PD on different arm movement parameters, including proximal and distal muscle control.[19-21] Fellows et al[19] reported an increase of the timing when lifting an object using the precision grip (opposition of the thumb and the index finger).

In NHP models of PD, among several of the existing rating scales, only a few aim at testing manual dexterity impairments, such as the ability to manipulate food.[22] However, as previously mentioned, the interpretation of those scales is not exhaustive, and the data are hardly reproducible. Nevertheless, the manual dexterity in macaque monkeys can be better assessed by training the animals to perform specific motor tasks, including, among others, the modified-Brinkman board and reach and grasp drawer tasks.[23] For instance, the effects of spinal or cortical lesions on different motor parameters were extensively investigated in NHPs, based on these 2 tasks. In those studies, a therapeutic agent was administered post-lesion and a functional recovery measured.[23-29] In particular, the autologous neural cell ecosystem (ANCE) transplantation approach[30] showed an increase in the manual dexterity performance in animals subjected to a cortical M1 lesion (hand representation) as compared with untreated monkeys.[27] Similarly, ANCE therapy was also found to improve motor recovery in PD monkeys.[13,31] However, the ANCE benefit on MPTP monkeys has been assessed using a clinical rating scale that allowed one to rate different items (mainly motor aspects) in a semi-quantitative manner.[13,31] Nevertheless, the animals that received the ANCE showed a decrease in parkinsonian symptoms[13,31] and an increase in striatal dopaminergic function, even though the transplanted ANCE cells did not become dopaminergic neurons.[13,31,32] The global behavior of a typical MPTP monkey is illustrated in the form of video sequences, comparing pre-lesion, post-lesion, and post-transplantation periods (http://www.unifr.ch/neuro/rouiller/research/own-projects/motor/parkinson/mptp).

In the present study, the aim was to focus on the impact of MPTP intoxication on manual dexterity in NHPs and assess whether the ANCE approach can contribute to the enhancement of functional recovery of the ability to precisely control finger movements. Four animals were trained to perform 2 fine manual dexterity tasks—namely, the modified-Brinkman board task and the reach and grasp drawer task.[23] After reaching a motor performance plateau, the animals were subjected to an MPTP lesion. After the occurrence of a spontaneous functional recovery plateau, all 4 animals were subjected to the ANCE transplantation. Therefore, manual dexterity was assessed during 3 experimental phases: pre-lesion phase, post-lesion phase, and post-transplantation phase. A further goal was to investigate whether the modified-Brinkman board and reach and grasp drawer tasks are as pertinent to assess manual dexterity in PD macaque monkeys as demonstrated to be for spinal or cortical lesions.[23]

Material and Methods

General Survey of the Experimental Protocol

This study was composed of 3 phases: the pre-lesion phase, the post-lesion phase, and the post-transplantation phase (Figure 1A). The pre-lesion phase started in spring 2010 and ended in summer 2014. It included training the animals to complete different motor tasks, with a focus on manual dexterity: the modified-Brinkman board task and the reach and grasp drawer task.[23] The post-lesion phase was aimed at assessing the behavioral impact of the MPTP lesion and the extent of subsequent spontaneous functional recovery. Finally, the post-transplantation phase, spanning a period of 6 months after the ANCE transplantation, aimed at monitoring the animals in order to assess any potential effect of the ANCE transplantation, representing an enhancement of functional recovery in addition to spontaneous recovery. In addition, the state of the dopaminergic system was assessed at each phase by ^{18}F-DOPA PET scans, as reported in Borgognon et al.[13] Two cortical biopsies were conducted in the prefrontal cortex. The first one took place during the pre-lesion phase to investigate its effect on the behavioral tasks, as reported in Badoud et al.[33] The second one was performed in the middle of the MPTP intoxication protocol and was used to obtain the cellular material required for the subsequent ANCE transplantation. As reported in Badoud et al,[33] the biopsy in the prefrontal cortex affects the ability of the animal to apply a consistent grip force during the reach and grasp drawer task but not the temporal course to open the drawer. Moreover, no effect of the biopsy itself was observed on the modified-Brinkman board task (motor performance and strategy). Therefore, the measurement of the grip force in the reach and grasp drawer task was excluded from the current study. Magnetic resonance imaging (MRI) scans were used to determine the biopsy locations and implantation sites. At the end of the experiment, the animals were euthanized humanely, and standard

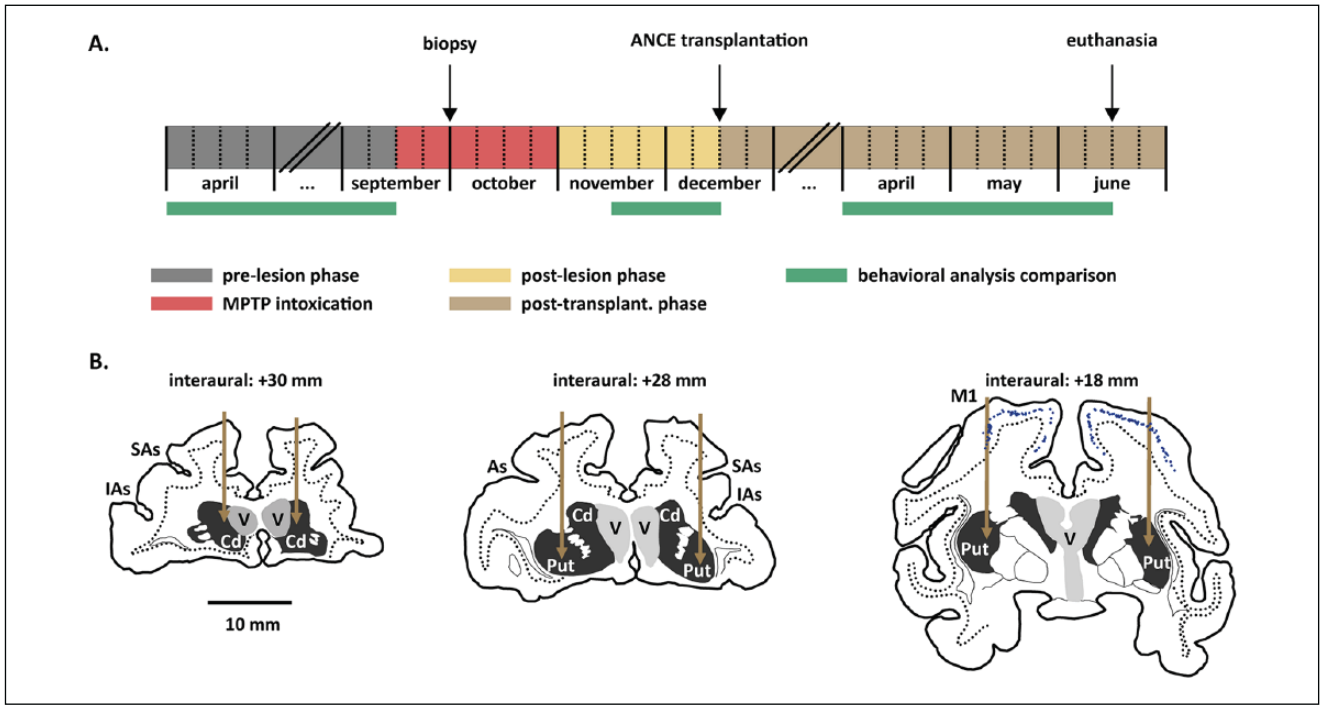

Figure 1. A. Experimental schedule of the research project. The gray zone represents the pre-lesion phase, which consisted of 6 months before the first MPTP injection. The red zone corresponds to the MPTP intoxication protocol during which the behavioral experiment could not be assessed. In the middle of the MPTP intoxication protocol, the cortical biopsy was performed. The yellow zone corresponds to the post-lesion phase. The brown zone corresponds to post-ANCE transplantation. Further details can be found in a previous report.[13] The behavioral data taken into account for the analysis for each period (green epochs) were 1 session per week for the pre-lesion phase, because the monkeys were considered to be at a plateau of motor performance, and all sessions for the post-lesion and post-ANCE transplantation phases. **B.** Reconstructions on 3 Nissl-stained histological sections in Mk-MI of the vertical needle tracts performed to reimplant the ANCE cells at 3 rostro-caudal levels, given by the interaural stereotaxic levels. The precise site of ANCE delivery corresponds to the tip of the vertical arrows. In the rightmost section, dots in the gray matter point to Betz cells in layer V, typical of the primary motor cortex (M1).

Abbreviations: ANCE, autologous neural cell ecosystem; As, arcuate sulcus (genu); Cd, caudate nucleus; IAs, inferior branch of the arcuate sulcus; Put, putamen; SAs, superior branch of the arcuate sulcus; V, ventricle.

histology was performed, as previously reported in Borgognon et al.[13] The ANCE transplantation outcomes as previously reported[13] are summarized as a reminder in Table 1. The present report focuses on the behavioral aspects of the study—namely, the impact of the MPTP lesion and ANCE transplantation on fine manual dexterity.

Animals

The experiments were conducted on the same 4 female adult macaques (*Macaca fascicularis*) as previously reported,[13] ranging from 6 to 10 years old at the beginning of the pre-lesion phase (weight between 3 and 5 kg). All 4 animals were housed in a group in the animal facility of the University of Fribourg in an enriched indoor room of 45 m^3 for a group of 2 to 5 monkeys (as required by the Swiss law on animal protection), with additional access to an outdoor space (at least 15 m^3). Animals could interact with each other within the group and were free to move (see video at http://www.unifr.ch/spccr/about/housing). The monkeys had free-access to water, and they were not food deprived. Their identities were Mk-LY, Mk-LL, Mk-MY, and Mk-MI. The overall experimental protocol was elaborated in compliance with the law on animal protection and approved by the Federal and local veterinary authorities (authorization numbers 2012_01E_FR and 2012_01-FR).

Behavioral Assessments

The assessment of the fine manual dexterity was based on 2 different tasks: the modified-Brinkman board and the reach and grasp drawer tasks.[23] These 2 motor tasks were validated and used intensively in our laboratory to assess and quantify the motor behavior, mostly manual dexterity, in NHP models of spinal cord injury[26,34-36] or motor cortex lesion.[24,25,27,29,37] For both types of lesions, the aim was to affect the corticospinal tract, crucial for the control of

Table 1. Summary as Recapitulation of the ANCE Transplantation Outcomes in All 4 Animals Derived From Borgognon et al.[13,a]

	Percentage of Pre-lesion ^{18}F-DOPA PET scan influx rate			Percentage of TH-Positive Neurons in SN Compared With Healthy Animals	Percentage of Behavioral Recovery	
	Pre-lesion	Post-lesion	Post-transplant		Schneider Rating Scale	Traveled Distance
Mk-LY	100	83	100	−39	100	16
Mk-LL	100	16	28	−67	100	38
Mk-MY	100	19	40	−72	100	45
Mk-MI	100	17	28	−74	80	70

Abbreviations: ANCE, autologous neural cell ecosystem; SN, substantia nigra; TH, tyrosine hydroxylase

[a]In the left panel, the influx constant (K_i) of ^{18}F-DOPA measured in the striatum with PET scan is expressed as percentage of the baseline pre-lesion K_i value set to 100%. Post-lesion, 3 of 4 monkeys exhibited a decrease of more than 80% of striatal ^{18}F-DOPA uptake, whereas in the fourth animal (Mk-LY), there was a decrease of only 17%; however, note that in the 4 MPTP monkeys, there is a reincrease of the striatal ^{18}F-DOPA uptake post-ANCE transplantation. In the middle panel, derived from the histology, the percentage decrease of tyrosine hydrolxylase (TH)-positive neurons in the substantia nigra (SN) pars compacta was obtained by comparing the total neuron numbers in each MPTP animal with the average neuron numbers derived from 2 healthy animals. In line with the ^{18}F-DOPA data, the same 3 MPTP monkeys showed a substantial percentage decrease of TH-positive neurons in the SN, ranging from −67% to −74%). In the fourth monkey (Mk-LY), the decrease was less prominent. In the right panel, a brief summary of the global behavioral data are provided, which was previously reported.[13] In the Schneider score, the percentage of recovery represented the proportion of the total deficit present after the MPTP lesion, which disappeared after the ANCE transplantation. In other words, 3 of 4 monkeys fully recovered based on the Schneider rating scale (100% recovery), whereas it was limited to 80% in the fourth monkey (Mk-MI). The traveled distance was quantitatively measured by using the VigiePrimate image analyzer system (View Point, Lyon, France) for each animal.[13] The percentage represents the extent of functional recovery, calculated by comparing the median values post-MPTP lesion and post-ANCE transplantation (percentage of change of the post-lesion median value).

manual dexterity, a prerogative of primates.[38-40] The 2 tasks were found to be pertinent and sensitive enough to quantify manual dexterity, especially to follow deficits post-lesion as well as functional recovery post-lesion (either spontaneous or enhanced with various treatments). In the present study, the modified-Brinkman board and the reach and grasp drawer tasks were introduced as pilot behavioral tests to assess manual dexterity in the case of PD-like deficits.

In the modified-Brinkman board task, the animal had to grasp/retrieve banana-flavored food pellets from 50 wells oriented either horizontally (25) or vertically (25) using the precision grip (opposition of the thumb and the index finger). Two parameters were analyzed in this task. The first one was the score in 30 seconds, which corresponded to the number of pellets successfully retrieved during the first 30 seconds from either horizontal wells, vertical wells, or both summed together. The motor performance in Mk-LL was assessed in a different manner. Indeed, Mk-LL adopted a mix of 2 behaviors: either grasping 1 pellet after the other, as expected, or sometimes retrieving several pellets in a row to store them into the hand palm before bringing all of them to the mouth, as illustrated in Kaeser et al[41] and Badoud et al.[33] As a consequence, for some retrievals, the time of transport to the mouth was included, whereas it was not for other individual retrievals (when storing several consecutive pellets in the hand). Moreover, in between these 2 types of successful trials, Mk-LL performed a variable number of erroneous trials in which the animal expulsed pellets out of some of the wells with the index finger, without collecting them. In such cases, Mk-LL exhibited a kind of neglect of the pellet rewards, most likely reflecting a fluctuating motivation. Because of such random variation, introducing a possible bias between vertical and horizontal wells, Mk-LL's motor performance was, thus, calculated by summing the total numbers of single pellets correctly retrieved and of multiple pellets stored in the hand and correctly retrieved during the entire task, corresponding to the total score, irrespective of the orientation of the wells. The second parameter was the contact time (CT), which was defined as the time interval between the insertion of the finger (usually the index finger) into the well and the complete retrieval of the pellet out of the well. This time interval was measured by analyzing a frame-by-frame video recording of each session. In a given individual session, the CT was measured for the first 5 horizontal wells and the first 5 vertical wells visited by the monkey. The CT parameter was assessed in Mk-LL considering only the correct trials.

In the reach and grasp drawer task, the monkey had to pull open a drawer against a resistance using one hand. Once opened, the monkey could take the food pellet hidden inside the drawer. The shape of well containing the pellet obliged the monkey to use the precision grip to retrieve the reward, after which the drawer closed automatically. Two different resistances against opening were used: (1) R0 corresponding to 0 N and (2) R5 corresponding to 2.75 N. One standard session was composed of 10 successful trials for each resistance executed with each of the 2 hands. The reach and grasp drawer task was quantified based on the parameter *trial duration*, corresponding to the time interval between the beginning of the drawer opening and the complete retrieval of the pellet out of the drawer. The monkeys

performed the 2 tasks every week day until they reached a plateau, a stable performance.

MPTP Lesion

The 4 monkeys were subjected to an acute low-dose MPTP intoxication protocol adapted from Mounayar et al.[11] A series of daily intramuscular MPTP injections (Sigma-Aldrich Co; 0.5 mg/kg, dissolved in saline solution) were performed, alternated with break periods (see the detailed protocol in Borgognon et al[13]). Based on the mild symptoms exhibited by one animal (Mk-MI), the amount of injected MPTP was increased in the last week. At the end of the protocol, 3 animals (Mk-LL, Mk-LY, Mk-MY) received a total amount of 6.25 mg/kg of MPTP and the fourth one (Mk-MI) received 7.75 mg/kg of MPTP. The safety procedures followed the guidelines of Przedborski et al.[42]

Cortical Biopsies

For each animal, 2 cortical biopsies were performed in the dorsolateral prefontal cortex (dlPFC). The first one was performed 9 months before the MPTP protocol onset and was carried out to assess the possible behavioral impact of a cortical dlPFC biopsy in itself. In addition, it aimed at refining the good manufacture practice (GMP) cell culture protocol at the Cell Production Center (Lausanne University Hospital [CHUV], Lausanne, Switzerland). The second cortical biopsy took place during the MPTP protocol to better mimic the clinical reality and was performed at the vicinity of the first biopsy in dlPFC. This biopsy provided the cellular material needed for the ANCE production that was subsequently reimplanted in the same monkey (see Borgognon et al[13] and Badoud et al[33] for further details).

Cell Transplantation

After reaching a spontaneous functional recovery, each animal received its own ANCE divided into 6 implantation sites (2 in the putamen and 1 in the caudate nucleus in each hemisphere). Each injection site was determined based on a T1-weighted MRI scan performed during the post-lesion phase and compared with coordinates derived from the atlas of the macaque brain. The surgical procedures were the same as previously described.[13] The ANCE transplantations were performed with a Hamilton microsyringe (100 µL, 22G) inserted vertically to precisely reach each site. A volume of 10 µL of culture medium was infused at each injection site, which corresponded to approximately 300 000 implanted cells in total. The injections were performed using a nano-injector (Stoelting, Wood Dale, IL) at the rate of 2 µL/min. Once the injection was completed, the needle was gently withdrawn to minimize the reflux along the needle tract, ensuring the precise location of the grafts.[13] The accurate and correct locations of ANCE deliveries were verified histologically by reconstructing the needle tracts, as illustrated in Figure 1B for Mk-MI (representative of all 4 monkeys). One rostral bilateral needle tract delivered the ANCE in the caudate nucleus, whereas 2 bilateral, more caudal needle tracts reached the putamen, as expected.

Statistical Analysis

Each monkey was its own control because the motor performances were individually compared pre-lesion versus post-lesion and/or post-transplantation for each hand, except in Mk-MI, in which only the right hand could be assessed because its left hand was injured (finger bitten by another monkey in the group housing facility). The pre-lesion plateau data encompass 1 d/wk during 6 months, whereas the post-lesion and post-transplantation data consist of all recorded daily sessions. The behavioral data were analyzed using the non-parametric Mann-Whitney U test. The threshold of statistical significance was set at P values smaller than .05 ($P \leq .05$). All graphs and statistical tests were generated using MATLAB_R2015b.

Results

Modified-Brinkman Board Task

Scores in 30 s (Mk-LY, Mk-MY, Mk-MI) and Total Score (Mk-LL). The manual dexterity as reflected by the modified-Brinkman board task is illustrated for the 4 monkeys in Figure 2, allowing a visual comparison along time of the dexterity score pre-lesion, post-MPTP lesion, and then post-ANCE transplantation. The goal to compare the post-lesion performance with a possibly ANCE-enhanced performance requires that a plateau be reached before the ANCE transplantation. In our previous studies, also based on the modified-Brinkman board task, the onset of a plateau was defined as follows: "In the recovery curve approaching saturation, the onset of the plateau was defined as the first individual data point (score) for which, among the next 3 individual data points, none exhibits a higher score (p. 1409)."[27] The red arrows in Figure 2 point to the onset of plateau, as defined by this criterion in Mk-LY, Mk-MY, and Mk-MI. No plateau could be defined for Mk-LL because of hectic behavior in this asymptomatic monkey.

The score for Mk-LY (Figure 2A) showed a moderate drop after the MPTP lesion for each hand and then progressively and spontaneously increased to reach a stable level (plateau: red arrow) during the post-lesion phase. The box plots showed no statistically significant difference between the 3 phases for each hand. These data show that the MPTP lesion in Mk-LY did not strongly affect manual dexterity; a minor deficit was transient because there was nearly complete spontaneous recovery.

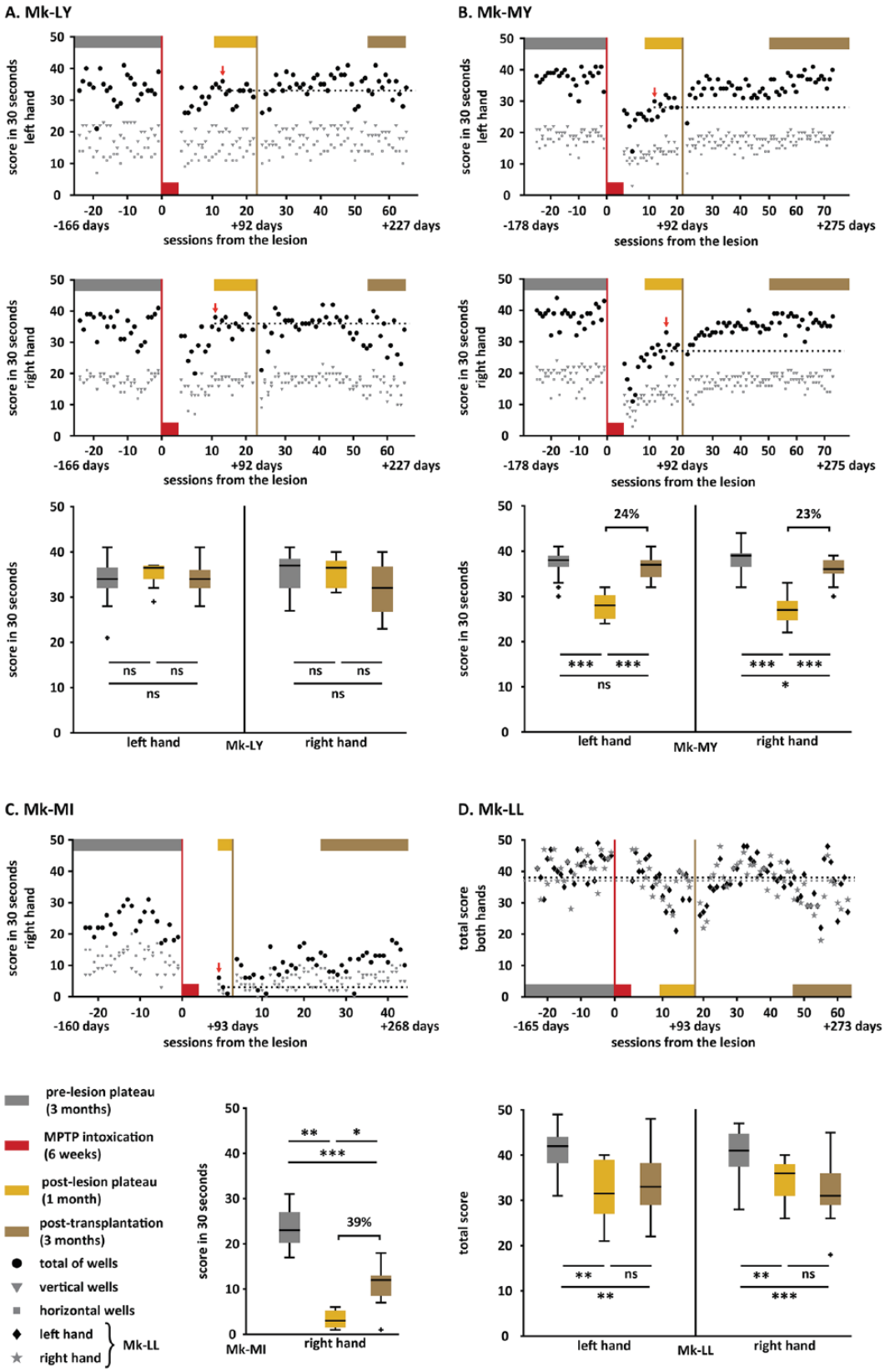

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Figure 2. Results derived from the modified-Brinkman board task, in which the score represents the number of pellets correctly retrieved during the first 30 s of the task. The *X*-axis corresponds to the behavioral sessions, with indications of the corresponding calendar days at 3 time points. Days without behavioral tests (such as weekends) were not considered along the abscissa. The *Y*-axis represents the number of pellets (horizontal, vertical, and both wells) correctly retrieved in the first 30 s. The red vertical line represents the first day of MPTP injection. The brown vertical line represents the day of ANCE transplantation. The black dots correspond to the total number of pellets (horizontal plus vertical wells), the gray triangles represent only the vertical wells, and the gray square represents the horizontal wells only (Mk-LY, Mk-MY, and Mk-MI). In Mk-LL, both hands are represented on the same graph because its motor strategy could not be assessed because the monkey did not perform the modified-Brinkman board task following the standard individual pellet grasping procedure (see Material and Methods). Therefore, in Mk-LL (D), the black diamonds represent the left hand and the gray stars represent the right hand. In the monkeys Mk-LY, Mk-MY, and Mk-MI, the onset of the plateau post-MPTP lesion is indicated by the red arrows (see text). The vertical dashed line shows the median value of the post-lesion plateau. No plateau could be defined for Mk-LL (asymptomatic and hectic score unfolding with time). Therefore, in this particular animal, the horizontal dashed lines (black for left hand and gray for the right hand) show the median value of all the 3 experimental phases. The pre-lesion plateau zone (gray rectangle), the post-lesion plateau (yellow rectangle), and the post-transplantation zone (brown rectangle) represent the data taken into account for the corresponding box and whisker plots for each hand (except in Mk-MI that had the left hand injured during the entire experiment). In monkeys Mk-LY and Mk-MY, a couple of data points before the onset of plateau were considered for the statistics to increase the number of data points (justified by the fact that they are close to the plateau data points). The Mann-Whitney *U* test was applied for the statistical test. Statistically significant differences are indicated as follows: $*P \leq .05$; $**P \leq .01$; $***P \leq .001$; "ns" refers to statistically nonsignificant ($P > .05$). The percentages above the Mk-MY and Mk-MI box plots are the extent of recovery, calculated by comparing the median values post-lesion and post-ANCE transplantation (percentage of change of the post-lesion median value). Note that we did not perform the extent of recovery in Mk-LY and Mk-LL because the animals were not behaviorally impaired after the MPTP intoxication (asymptomatic monkeys). A. Data for Mk-LY: the upper graph shows the score in 30 s for the left hand, the middle graph shows the score in 30 s for the right hand, and the lower graph shows the box-and-whisker plots for both hands. B. Data for Mk-MY, with the same convention as for Mk-LY. C. Data for Mk-MI, with the same convention as for Mk-LY. D. Data for Mk-LL, the upper graph shows the total score for both hands, and the lower shows the box-and-whisker plot for both hands.

The scores in Mk-MY (Figure 2B, Movie 1 [available online]) showed a stronger decrease after the MPTP lesion for both hands as compared with Mk-LY. There was also some spontaneous recovery, reaching a post-lesion plateau (red arrow). After the ANCE transplantation, the scores increased further and reached a stable post-transplantation plateau close to the pre-lesion level. The box plots comparing the pre-lesion and post-lesion phases showed a significant decrease in score for both hands. Furthermore, the post-transplantation scores were significantly higher for both hands than the corresponding post-lesion scores. The percentage of subsequent functional recovery after the ANCE transplantation was 24% for the left hand and 23% for the right hand. For the left hand, the post-transplantation values were not significantly different from the pre-lesion values, whereas the right hand showed a significantly lower post-transplantation score, indicating that the enhanced recovery was not quite complete.

In Mk-MI (Figure 2C, Movie 1), there was a dramatic decrease in the right hand score after the MPTP lesion. Indeed, a couple of sessions after the MPTP lesion, Mk-MI was not at all able to retrieve a single pellet. After a few weeks, Mk-MI retrieved some pellets in 3 different sessions, which were considered to reflect the post-lesion phase (red arrow). After the ANCE transplantation, Mk-MI started to retrieve more pellets and reached a post-transplantation plateau after several weeks. The box plots comparing the pre-lesion and post-lesion values showed a significant decrease. The box plots comparing the post-lesion values and the post-transplantation values showed a significant increase of the score in the latter phase. The percentage of enhanced functional recovery after the ANCE transplantation was 39%, representing a significant though incomplete recovery.

Mk-LL (Figure 2D) showed no decrease of the score immediately after the MPTP lesion. After a few sessions, the scores started to decrease. After the ANCE transplantation, the score of Mk-LL showed an increase followed by a decrease and then a quite variable manual performance. In other words, the manual behavior of this monkey was largely hectic, most likely because of a fluctuating motivation level. Nevertheless, the box plots comparing the pre-lesion values and the post-lesion values showed a significant decrease in both hands, but surprisingly delayed with respect to the MPTP administration. The box plots comparing the post-lesion values and the post-transplantation values showed no significant difference for both hands.

Contact Time. The analysis of the CT derived from the modified-Brinkman board task was conducted on the same time windows as for the score data. The CT of Mk-LY (Figure 3A) showed no significant increase after the MPTP lesion, except for the CT on the vertical wells for the left hand. After ANCE transplantation, the CT on the vertical wells for the left hand was restored to pre-lesion values. The CT on the horizontal wells remained unchanged for the left

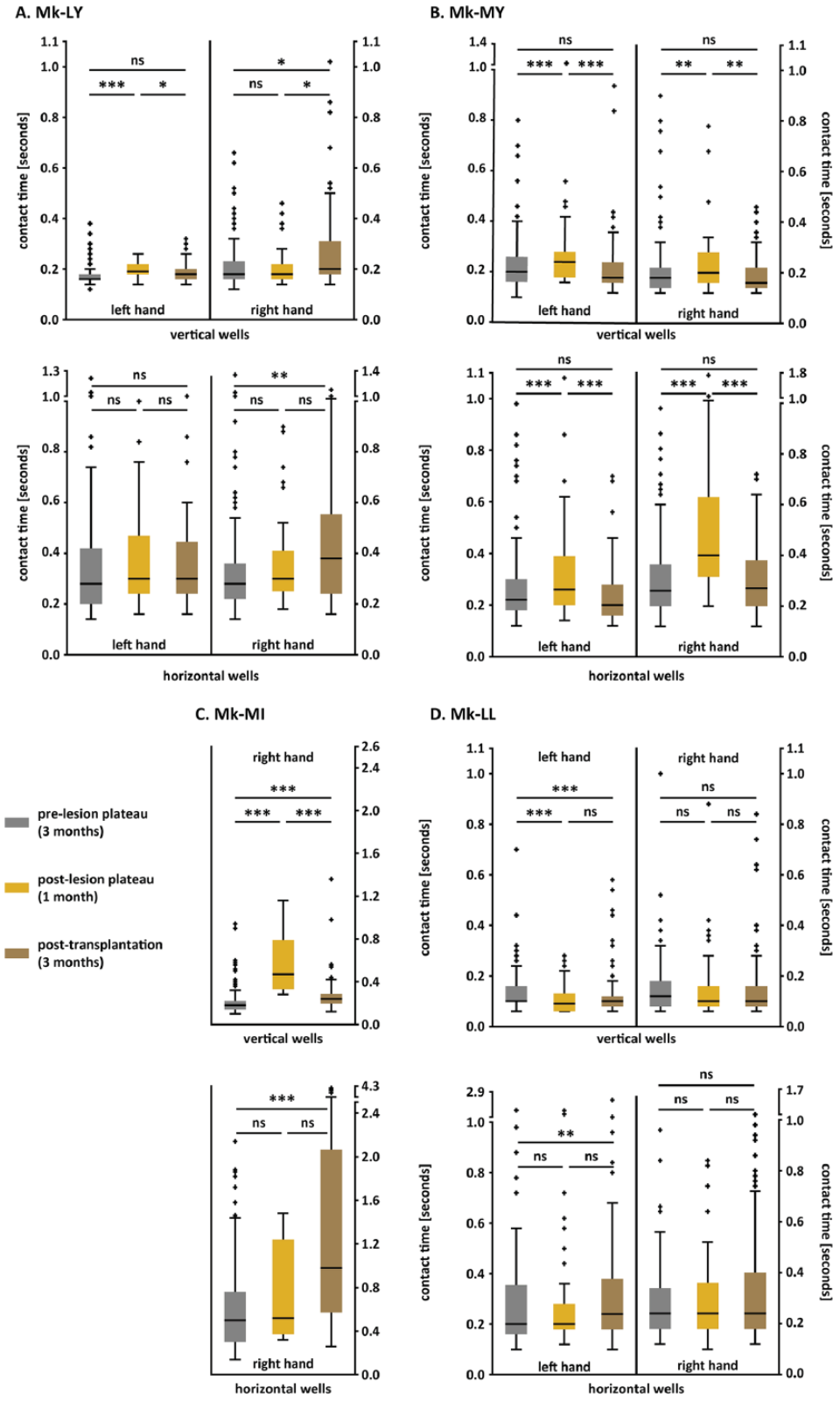

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Figure 3. Results derived from the modified-Brinkman board task, representing the contact time (CT), which was the time spent with the fingers in the well to correctly retrieve the pellet. The *Y*-axis represents the CT in seconds (note that the *Y*-axis was truncated for a better visualization of the data because of some longer CTs). The Mann-Whitney *U* test was applied for the statistical test. Statistically significant differences are indicated as follows: $*P \leq .05$; $**P \leq .01$; $***P \leq 0.001$; "ns" refers to statistically nonsignificant ($P > .05$).

hand but increased for the right hand after the ANCE transplantation. The CT on the vertical wells for the right hand also increased as compared with the pre-lesion and post-lesion values.

After the MPTP lesion, the CT in Mk-MY (Figure 3B, Movie 1) showed significant increases for both vertical and horizontal wells and for both hands. After the ANCE transplantation, all CTs showed a significant decrease as compared with post-lesion values. They returned to values comparable to the pre-lesion CT (non-statistically significant differences), in line with the ANCE enhanced recovery observed based on the score.

After the MPTP lesion, the CT in Mk-MI (Figure 3C, Movie 1) showed a significant increase for the vertical wells but not for the horizontal ones. After the ANCE transplantation, the CT on the vertical wells significantly decreased as compared with post-lesion values but stayed significantly higher than the pre-lesion values. For the horizontal wells, the CTs were significantly higher than the pre-lesion values and more variable.

The CT in Mk-LL (Figure 3D) showed no significant change for the right hand in both vertical and horizontal wells (neither after the lesion nor after the ANCE transplantation). For the left hand, a significant decrease was seen after the lesion in the vertical wells, which remained unchanged after the transplantation. For the horizontal wells, no significant change was seen after the MPTP lesion, but the values were significantly higher than the pre-lesion values after the ANCE transplantation.

Reach and Grasp Drawer Task

Trial Duration. In both monkeys Mk-LY and Mk-LL (Figures 4A and 4D), there were no systematic and coherent changes of trial durations with respect to the MPTP lesion and the ANCE transplantations, although some differences were statistically significant. As observed for the modified-Brinkman board task, the MPTP lesion only marginally affected the manual dexterity in these 2 asymptomatic monkeys.

The trial durations in Mk-MY (Figure 4B, Movie 2) showed a significant increase after the MPTP lesion for both hands and at both resistances. After the ANCE transplantation, the trial durations decreased for both hands at R5 and were even lower as compared with the pre-lesion values at R0.

Mk-MI was not able at all to open the drawer after the MPTP lesion (Figure 4C, Movie 2). After the ANCE transplantation, Mk-MI regained its capacity to perform the task, but only for the smallest resistance—namely, R0. The post-transplantation values remained higher than the pre-lesion ones.

Discussion

In the present study, the impact of ANCE transplantation in 4 MPTP intoxicated monkeys was assessed with emphasis on manual dexterity, thus representing an original report because manual dexterity has received little attention so far in PD-like monkeys. The ANCE approach, as developed by Brunet and colleagues has been shown to promote functional recovery of other motor attributes in MPTP-treated monkeys.[30-32,43] As previously reported,[13] the 4 animals in the present study showed an enhancement of the striatal dopaminergic function as well as a recovery of global motor functions assessed semi-quantitatively with the Schneider rating scale and with an automatic video image analyzer of spontaneous movements (see recapitulation in Table 1). In addition, those 4 animals were trained to perform fine manual dexterity tasks—the modified-Brinkman board and the reach and grasp drawer tasks[23]—which were the focus of the present quantitative analysis.

Pertinence of Modified-Brinkman Board and Reach and Grasp Drawer Tasks in the NHP MPTP Model

In the present study, the modified-Brinkman board and reach and grasp drawer tasks were introduced as a pilot for the first time to the MPTP macaque model. Are they pertinent and sensitive enough tests to assess deficits and functional recovery from PD-like symptoms in macaques? As illustrated in Figure 2 for the modified-Brinkman board task, this is the case in the 2 clearly symptomatic monkeys Mk-MY and Mk-MI, exhibiting deficit and recovery properties comparable to those observed after spinal cord or motor cortex lesions. Importantly, in these 2 monkeys, there was a clear deficit following MPTP administration, followed by a spontaneous recovery until reaching a plateau of incomplete recovery. As far as the reach and grasp drawer task is concerned, Mk-MY and Mk-MI, which exhibited the more severe parkinsonian symptoms, were impaired in terms of trial durations (time interval between the beginning of the drawer opening and the complete retrieval of the pellet out of the drawer). In Mk-MY, the time to execute the task was

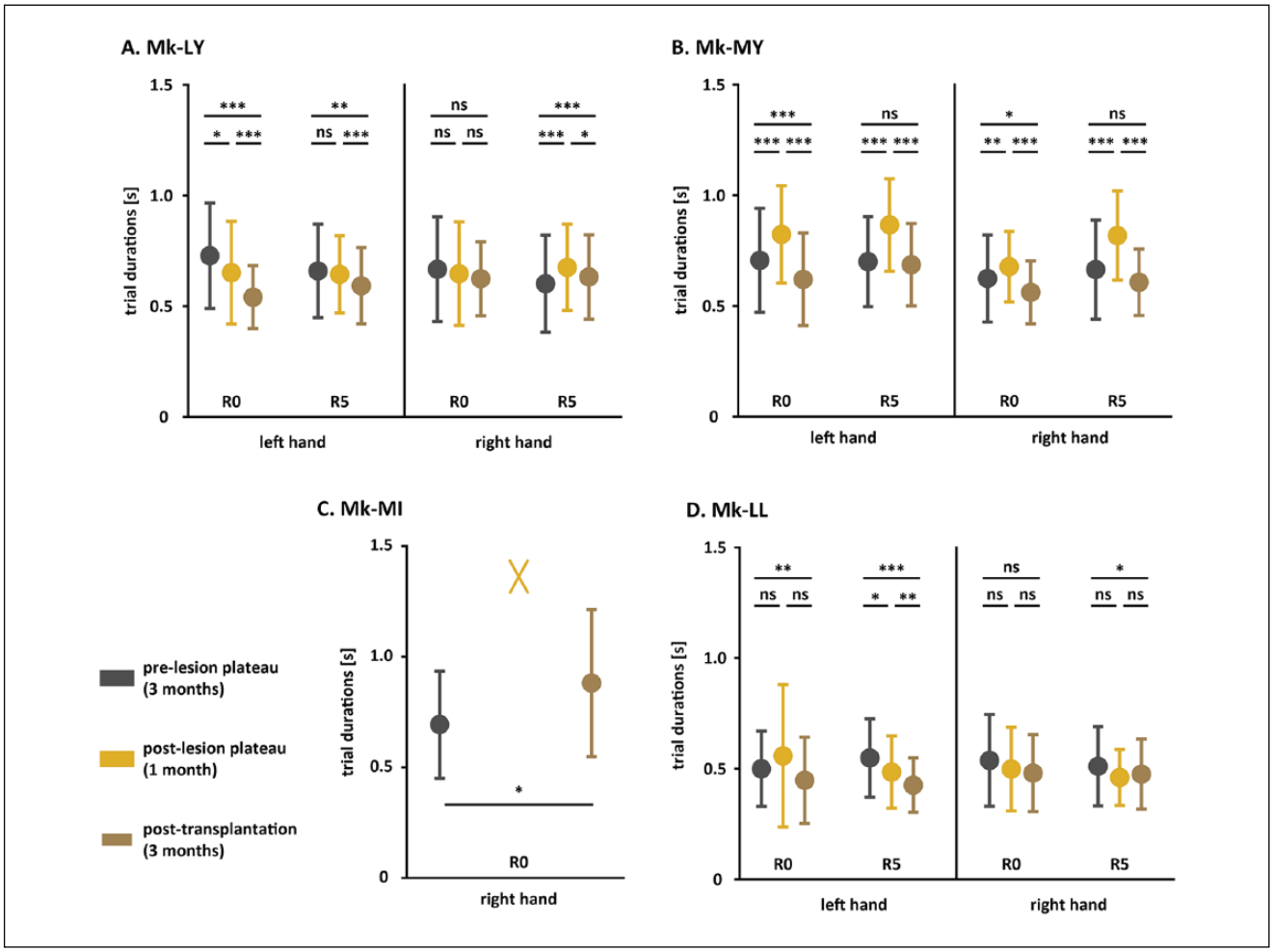

Figure 4. Results derived from the reach and grasp drawer task, representing the trial duration, which corresponds to the time interval between the beginning of the drawer opening and the complete retrieval of the pellet out of the drawer. Two different resistances were used—namely, R0 corresponding to no resistance (0 N) and R5 corresponding to 2.75 N. The *Y*-axis represents the trial duration in seconds. The error plots represent the mean (dot) and the SD (bars) for each experimental phase. Statistical analysis (Mann-Whitney *U* test) compared the trial durations between pre-lesion and post-lesion, pre-lesion and post-transplantation, and post-lesion and post-transplantation for each hand. Statistically significant differences are indicated as follows: $*P < .05$; $**P < .01$, $***P < .001$; "ns" refers to statistically nonsignificant ($P > .05$).

significantly increased in the post-MPTP lesion phase for both hands at both resistances, whereas Mk-MI was once again almost completely unable to perform the task after the MPTP lesions because of a severe akinetic state and tremors. Similar to the modified-Brinkman board task results, the 2 asymptomatic monkeys, Mk-LY and Mk-LL, were not affected in the reach and grasp drawer task after the MPTP lesion even though some significant changes were observed (see reasons below).

Overall, the data derived from the 2 symptomatic monkeys, Mk-MY and Mk-MI, suggest that the modified-Brinkman board and the reach and grasp drawer tasks are promising tools to assess manual dexterity in the case of PD, although this remains to be confirmed on a larger number of symptomatic monkeys (see reasons below).

Post-ANCE Transplantation in Mk-MY and Mk-MI

Mk-MY and Mk-MI, after reaching a plateau post–MPTP lesion (Figure 2), exhibited a significant post-transplantation enhancement of functional recovery for the modified-Brinkman board task. The occurrence of such a post-lesion plateau was crucial in order to test the potential effect of the ANCE treatment. In this respect, the behavioral outcome is comparable to what has been reported after motor cortex lesions[27]: a first plateau of spontaneous recovery, followed by a second plateau time linked to the ANCE treatment. Could the enhancement of functional recovery be attributed to some training effect? It is unlikely because, as reported earlier[44] from a large cohort of macaques over many years,

a training effect in the modified-Brinkman board task occurred only during a few weeks immediately after the first exposure to the task, but not later in the midterm and long term.

As far as the reach and grasp drawer task is concerned, Mk-MY showed significant improvement (reduction) of its trial duration during the post-transplantation phase. Mk-MI, unable to perform the task after the MPTP injections, regained this capacity a few weeks after ANCE transplantation. This recovery was nevertheless incomplete, with significantly slower performances as compared with the pre-lesion state.

Limitations

One of the major limitations of this study is related to the small number of animals. Taken together with the intrinsic behavioral variability of the NHPs[45] and the well-known inter-individual variations in terms of MPTP sensitivity, only 2 symptomatic monkeys (Mk-MY and Mk-MI) were ultimately suitable to assess the benefit of the ANCE treatment. In the 2 asymptomatic monkeys (MK-LY and Mk-LL), the modified-Brinkman board task appears less pertinent. In Mk-LY, the deficit post–MPTP administration was modest, whereas there was none in Mk-LL. As shown previously,[13] this was also the case for other motor attributes, indicating that the limitation is not the task in itself, but that these 2 monkeys were asymptomatic, a phenomenon well known in cohorts of MPTP monkeys (intoxication resistance), affecting a significant proportion of them, especially when, as is the case here, one adopts a careful and progressive MPTP administration to avoid massive deficits, which may call for anticipated euthanasia (on ethical grounds). Spontaneous recovery mechanisms after MPTP intoxication remain uncertain. Among hypotheses, MPTP resistance in Mk-LY and Mk-LL could be explained as follows: (1) metabolic differences and/or clearance of MPP+[46-48]; (2) a dysfunctional downregulation of the tyrosine hydroxylase enzyme during the MPTP protocol, such that after the MPTP lesion protocol, dopamine neurons may be reactivated[49]; (3) the associative territory of the striatum (less affected by MPTP) could compensate by sprouting some fibers to the sensorimotor territory of the striatum (areas more affected by MPTP)[49] and increasing the dopamine release from residual dopaminergic systems[50]; and (4) a role of the serotonin neurotransmission dynamics that can compensate the motor deficits.[11,12,50] Moreover, Mk-LY and Mk-LL were 4 years younger than Mk-MY and Mk-MI. It has been shown that younger-onset PD patients seem to have more efficient compensatory mechanisms as compared with older-onset patients. Indeed, the disease progresses slower and seems to endure more damage of the nigrostriatal system before the appearance of the first motor symptoms.[51]

The second limitation of the present investigation is associated with the absence of control subjects in the same conditions. However, as mentioned above, control subjects were involved in a recent largely comparable ANCE experiment conducted on MPTP monkeys.[31] Yet, and for the same reasons as mentioned above, a cohort limited to only 4 monkeys cannot generate a relevant group for statistical comparisons. The much larger number of animals that would be required to achieve such a comparison would not be feasible in terms of infrastructure and would not be ethically accepted because of restrictions on the number of NHPs that can be used in a protocol in Switzerland. This problem was already tackled in several articles from our laboratory.[26,27] Nevertheless, Bloch et al[31] published a similar study in which they implanted ANCE produced according to the exact same protocol as in the present study in a similar NHP MPTP model.[31] They were able to show a significant motor improvement in 4 of 5 treated monkeys, whereas the control subjects (no cells or killed cells) remained parkinsonian. For this reason, the present investigation did not aim at providing an additional proof of efficacy of ANCE transplantation itself but, rather, tried to demonstrate additional evidence related to manual dexterity, a motor attribute that was not investigated by Bloch et al.

Translational Validity of the NHP MPTP Model

The modified-Brinkman board task has been used and validated in several studies that, among others, investigated the impact of therapeutic strategies after spinal and cortical lesions in NHPs.[24-27] By its construction, the task is comparable to the pegboard task used in the clinic. Both force the participants to perform fine hand movements representing independent use of different fingers. In these 2 cases, the subject has to generate a transport movement, mostly involving proximal muscles, and a grasp movement that requires fine control of the distal muscles.[52,53] Our results are relatively in line with some studies conducted on MPTP monkeys,[9,54-56] where they showed an increased time to execute reach and grasp movements. Moreover, our results are in agreement with some studies conducted on human subjects showing that the reach and grasp movements are generally slower in PD patients compared with controls, suggesting that this could be a result of global bradykinesia and rigidity.[16-18,57-59] Moreover, one current manifestation of PD is a decrease in hand dexterity. If the patient's ability to control their proximal muscles (arms) remains relatively spared, their capacity to perform fine movements with their fingers deteriorates more and more with disease progression.[60-64] These observations are in line with the increased CT observed in Mk-MY and Mk-MI. It has been hypothesized that this motor manifestation could be linked to the fact that the pallidal outputs are projecting on the ventrolateral thalamus that itself selectively innervates the hand

representation of M1.[65,66] As a further development of behavioral tests for PD in monkeys, based on previous reports in human PD patients,[67] one may consider the option of examining bimanual versions of the modified-Brinkman board task[45] or of the reach and grasp drawer task.[68-70]

In contrast to the modified-Brinkman board task, the execution of the reach and grasp drawer task required the animal to systematically perform the same trajectory movement in order to reach the target (drawer's knob), generate a certain amount of force to open the drawer and access the reward, and allow fine quantification of a specific motor action.[23] This task also demands the involvement of more varied muscle groups than the modified-Brinkman board task. On one hand, the score of the modified-Brinkman board task encompasses the transport movement from the board to the mouth and involves the biceps and supinator muscles, whereas on the other hand, the movements to execute the drawer task are linear and involve the triceps and pronator muscles more (during the recorded phase). The increases in trial durations are in line with PD patients showing a prolonged time to lift the charge after grasping a knob in a vertical linear task, certainly associated with bradykinesia.[19,20] Taken together, those 2 tasks could be commonly used for assessing quantitatively Parkinson-like symptoms in a NHP MPTP model, which would reinforce data interpretation and reproducibility compared with clinical rating scales.[8]

Place of ANCE Transplantation

In general, the post-ANCE transplantation results exhibited improvement in comparison to the post–MPTP lesion phase. Yet in the absence of controls as a result of the limited number of animals included in this study, it is not possible to definitively conclude that there was a therapeutic effect of the ANCE transplantation. Nevertheless, there are some indications that the compensatory mechanisms described in the literature cannot be considered to be the unique contributors responsible for this functional recovery after an MPTP lesion. Because the generation of stable motor symptoms is mandatory for the assessment of the efficacy of a new treatment, several laboratories have tackled this important question.[11,71-73] Taylor et al[72] reported that the most severely affected monkeys exhibited stable symptoms that lasted for months. Soderstrom et al[73] estimated that stable motor symptoms were present with a striatal dopaminergic depletion of at least 80%.

In this study, it is evident that Mk-LL and Mk-LY, over and above resistance to MPTP, underwent functional recovery, possibly as a result of some compensatory mechanisms.[74] However, Mk-MY and Mk-MI were both more severely affected by the MPTP intoxication, with a decrease of at least 80% of the ^{18}F-DOPA striatal uptake.[13] In particular, Mk-MI was so severely affected that it was totally unable to grasp any food with one or the other hand, requiring external assistance to eat and drink for a few weeks. This supports the view that the recovery of Mk-MI and Mk-MY is unlikely to be a result of spontaneous compensatory mechanisms only. Indeed, the results published by Brunet et al[32] with the same protocol of cell development presume that the ANCE may also produce neurotrophic factors (GDNF/BDNF). Consequently, based on the observations presented above, we can reasonably suggest that ANCE transplantations most likely played a role in the functional recovery exhibited by the 2 symptomatic animals (Mk-MY and Mk-MI).

Conclusion

The present study is original because of its emphasis on manual dexterity, which is clearly affected in the symptomatic MPTP monkeys. The 2 tasks reported here are also pertinent to quantify PD symptoms and contribute to a reliable assessment of a treatment in MPTP monkeys, in line with their pertinence reported for other motor pathologies (spinal or cortical lesions). Finally, the protocols of culture of the ANCE used for the implantation were conducted in strict accordance with GMP in a Swissmedic-accredited facility. The GMP implementation is a crucial and necessary step to move toward future clinical applications.

Authors' Note

S Badoud, JB, J-FB, and EMR designed the study; S Badoud, EMR, S Borgognon, and JC performed the MPTP intoxication protocol, including its daily survey; S Borgognon, JC, VM, PC, and S Badoud trained the monkeys and collected the behavioral data; S Borgognon, JC, VM, PC, LC, MF, and S Badoud analyzed the behavioral data; J-FB supervised the cell cultures; JB, S Badoud, JC, S Borgognon, and EMR performed the cellular transplantations; VM produced the video documents; S Borgognon, JC, S Badoud, and EMR drafted the manuscript. All authors revised the final version of the manuscript.

This study has led to 1 PhD thesis and 2 master's thesis manuscripts, which can be accessed from the laboratory website (www.unifr.ch/neuro/rouiller/).

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