Corticobulbar projections change according to motor pathology in macaque monkeys

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INTRODUCTION

The corticobulbar projection, together with the corticospinal tract (CST), act in parallel with projections from the brainstem (such as the reticulospinal tract) to ensure direct or indirect control of movement on motoneurons in the spinal cord. In monkeys little is known about the projections coming from the motor cortex on the brainstem as well as on their influence. Previous studies suggested a role of the reticulospinal tract in the control of reaching movement and in the recovery after a lesion of the CST, spinal cord or cerebral cortex.

The aim of the present study was to anatomically analyze the corticobulbar projections coming from different motor cortical areas (premotor cortex (PM), primary motor cortex (M1) and supplementary motor cortex area (SMA)) on the reticular formation of the brainstem, possibly influencing the reticulospinal neurons. We analysed the projections in **intact** monkeys (n=7, tracer injections in either PM, M1 or SMA) and in monkeys subjected to different pathologies: **M1 cortical lesion** (PM injection (n=4)); **spinal cord injury** (M1 injection, (n=5)) or **Parkinson's disease** (PD; tracer injection in either PM (n=2) or M1 (n=2)).

METHODS

The anterograde tracer biotinylated dextran amine (BDA) was injected unilaterally in either PM, SMA or M1 in twenty macaque monkeys (*Macaca fascicularis*). The corticobulbar projections labeled anterogradelly by BDA were then analyzed in 12 consecutive histological sections (50 µm thick), 250 micrometers apart. Axons and terminals, including boutons *en passant*, were then plotted using the software Neurolucida.

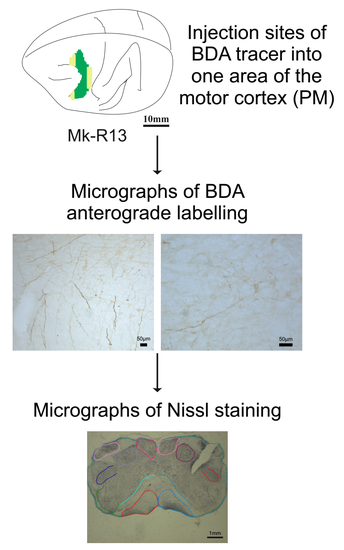
An adjacent series of 12 sections was stained with Creysil violet revealing Nissl bodies. On these sections we delineated the brainstem nuclei.

The Neurolucida software is connected to a light microscope (Olympus BX40). We used the objective 4x to trace the contours of the sections and the Pyramidal tract, the 10x to trace the axons and finally the 20x to plot the boutons *en passant* and *terminaux*. For the series stained with Nissl we used the 1.25x objective to delineate the nuclei and to acquire pictures.

Both series of sections (BDA and Nissl staining) were overlapped in order to match the zone of terminals and the nuclei delineated with Nissl staining.

In the group of the **intact monkeys** (n=7), 3 were injected in PM, 3 in M1 and one in SMA; monkeys subjected to **cortical lesion** of M1 hand area (n=4) were injected in PM; animals subjected to **MPTP lesion mimicking PD** (n=4) were injected in either PM (n=2) or M1 (n=2); finally monkeys subjected to **unilateral cervical hemisection** at C7/C8 (n=5; SCI) were injected in M1.

Statistics were calculated on the basis of the number of boutons in each nucleus and were derived from the Paired t-test /Wilcoxon; * $p \leq 0.05$; ** $p \leq 0.01$, *** $p \leq 0.001$ (bilateral comparison).



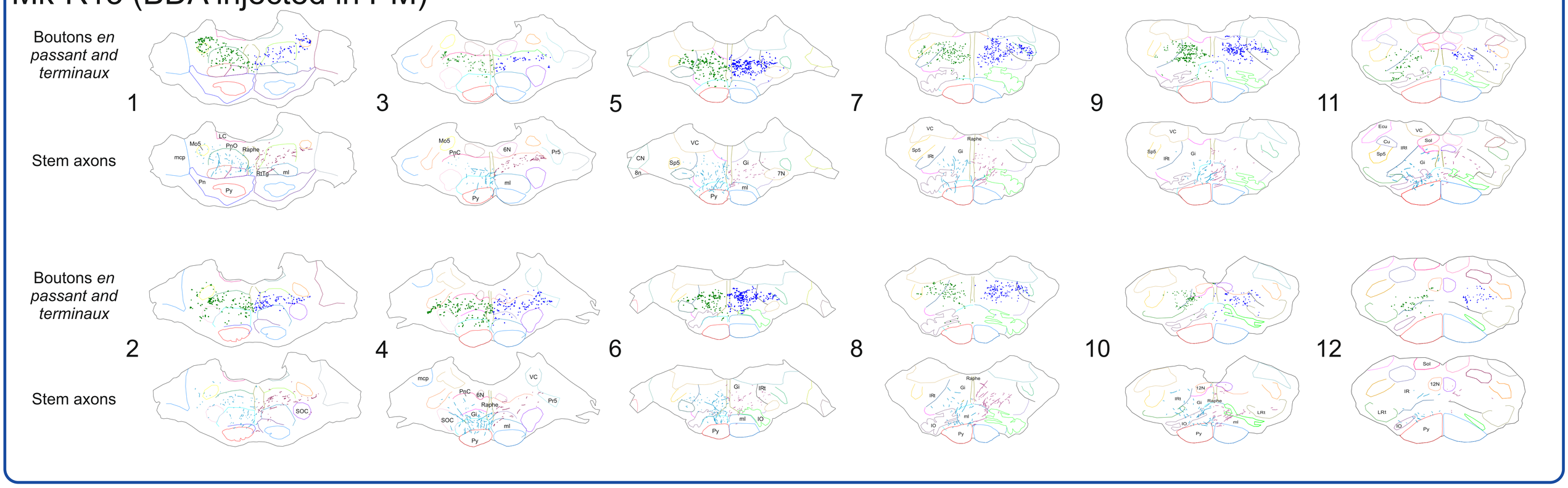
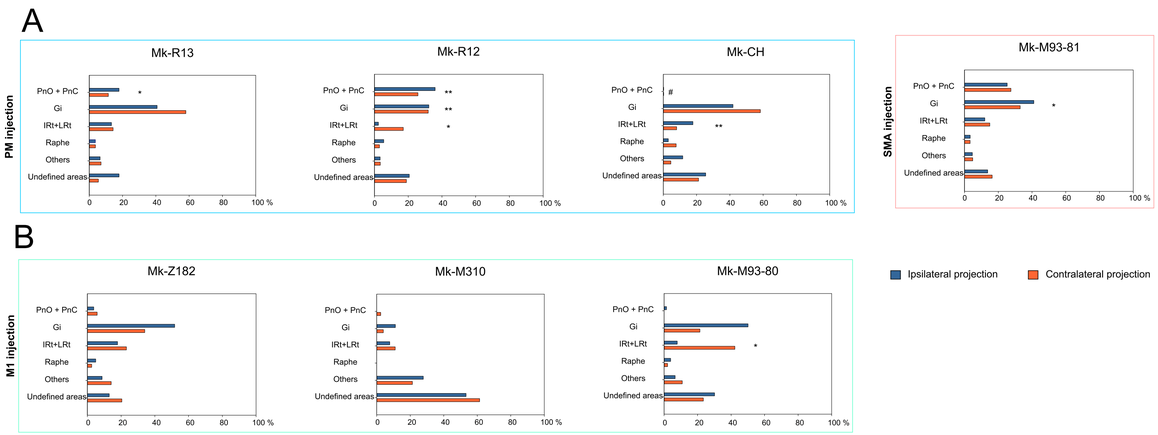
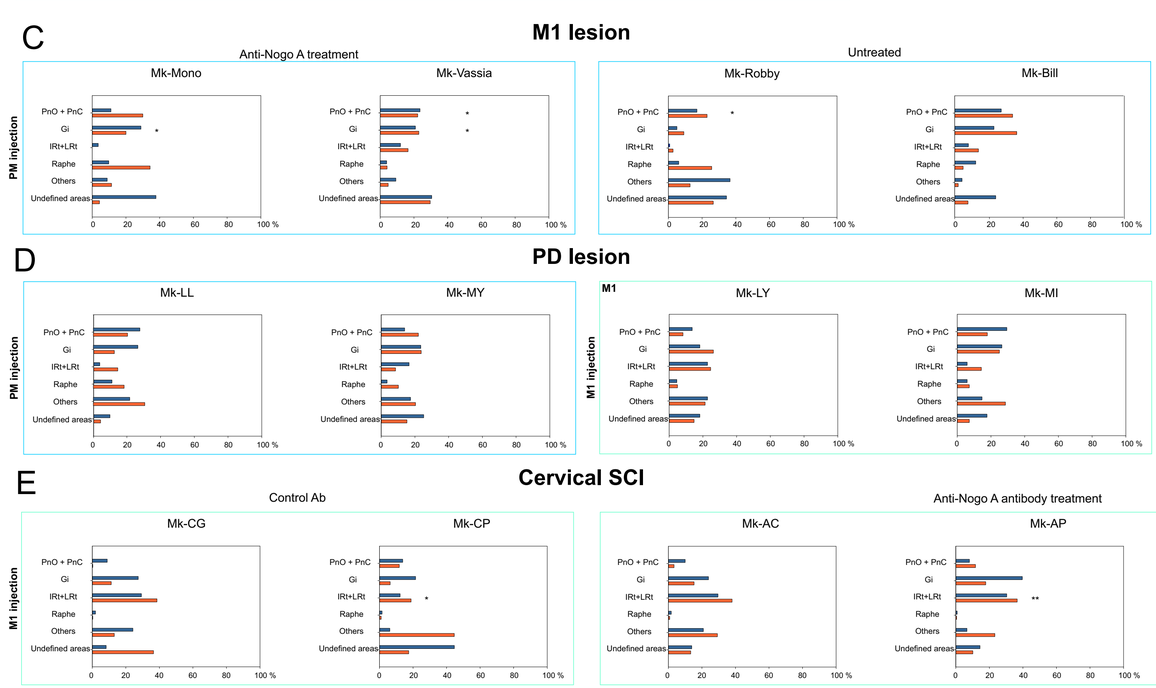


Figure 1: brainstem drawings of coronal sections of Mk-R13 arranged from rostral (section 1) to caudal (section 12). All nuclei are delineated with a different color (see list of abbreviations). Axons located ipsilateral to the BDA injection are marked in blue whereas those located contralaterally are marked in bordeaux. Boutons *en passant* and *terminaux* ipsilateral to the BDA injection are marked as green circles whereas the contralateral terminals are marked as blue squares.





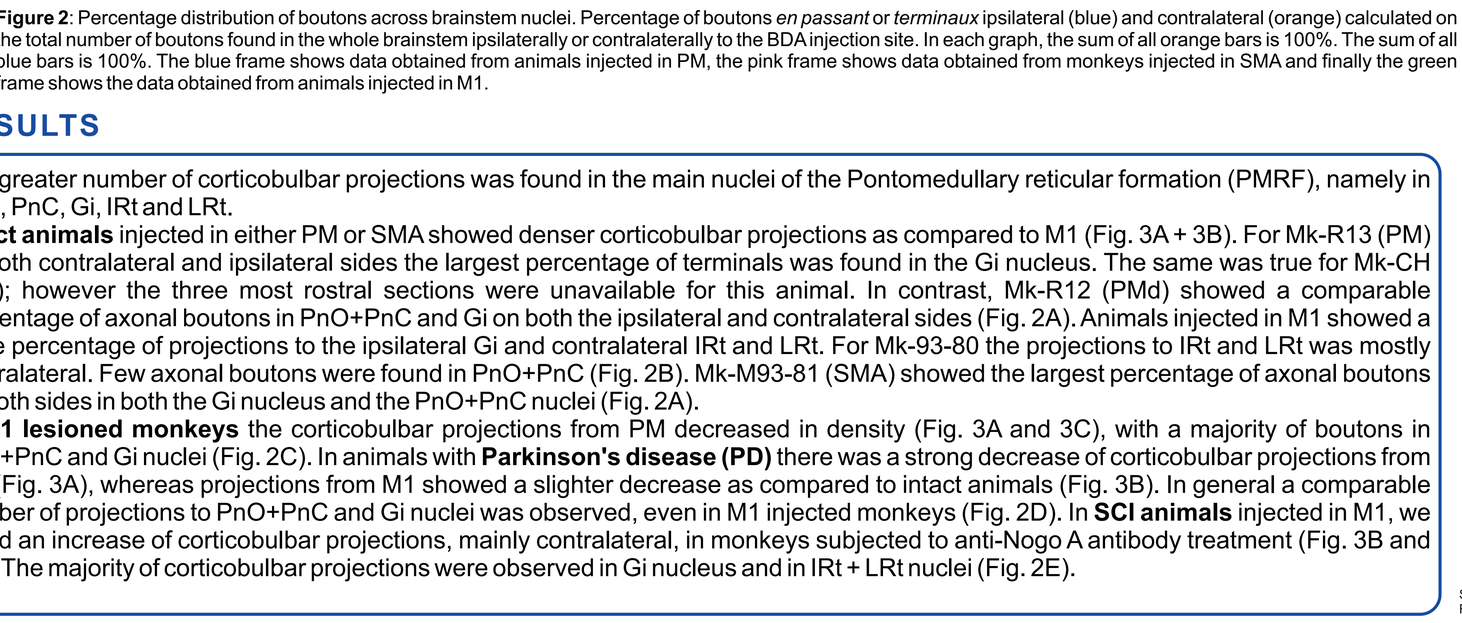


Figure 2: Percentage distribution of boutons across brainstem nuclei. Percentage of boutons *en passant* or *terminaux* ipsilateral (blue) and contralateral (orange) calculated on the total number of boutons found in the whole brainstem ipsilaterally or contralaterally to the BDA injection site. In each graph, the sum of all orange bars is 100%. The sum of all blue bars is 100%. The blue frame shows data obtained from animals injected in PM, the pink frame shows data obtained from monkeys injected in SMA and finally the green frame shows the data obtained from animals injected in M1.

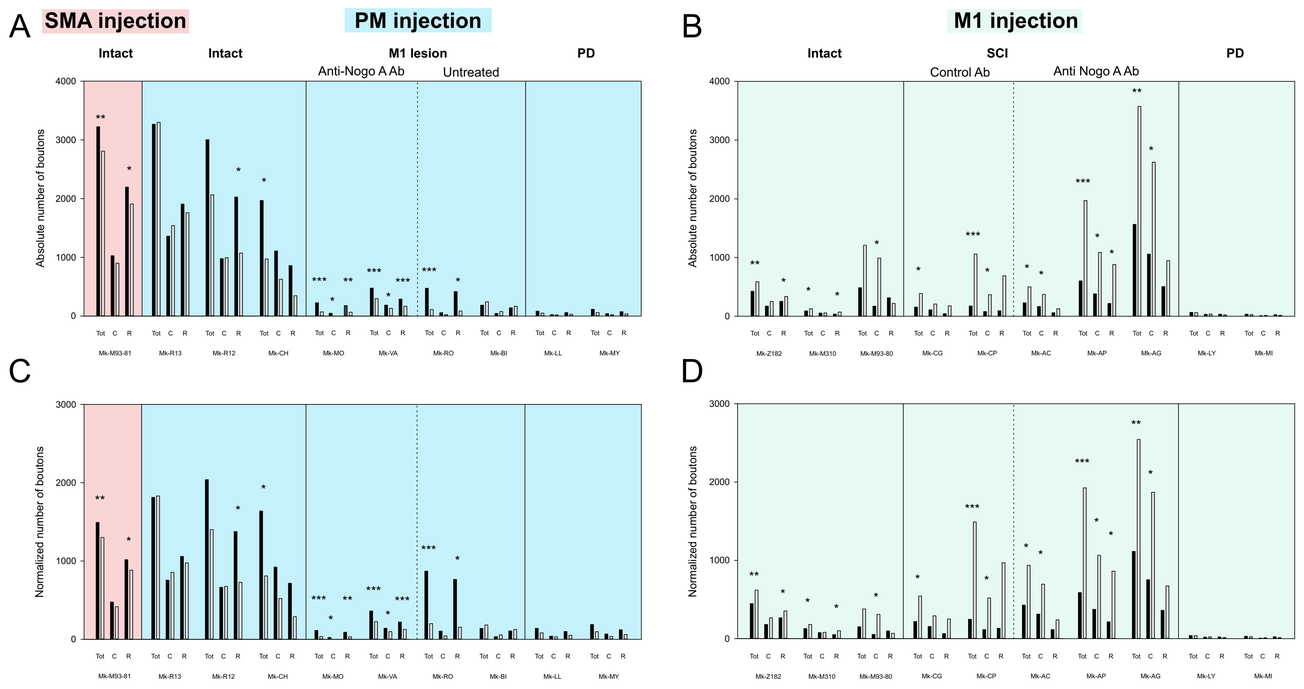


Figure 3: Histograms showing the total numbers of boutons *en passant* and *terminaux* in the whole brainstem (Tot), in its caudal half (C; from section 7 to section 12) and in its rostral half (R; from section 1 to section 6). Black bars are for ipsilateral projections and white bars for contralateral ones.

Panel A) Histogram representing the row data (absolute numbers of boutons) for projections arising either from SMA (pink background) or from PM (blue background) in **intact animals**, in monkeys subjected to **M1 hand lesion** and in **PD animals**. Panel B) Histogram showing the row data for animals injected in M1 (green background) in **intact animals**, in monkeys subjected to **cervical SCI** and in **PD** animals. Panel C) The same data as in A but normalized. Panel D) The same data as in B but normalized. Normalization was performed with reference to the number of BDA labelled corticospinal axons observed above the Decussatio pyramidum.

Abbreviations

6N	Abducens nucleus	ml	Medial leminiscus
7N	Facial nucleus	Mo5	Trigeminal motor nucleus
5n	Trigeminal nerve	PMRF	Ponto Medullary Reticular Formation
7n	Facial nerve	Pn	Pontine nuclei
8n	Vestibulocochlear nerve	PnC	Pontine reticular nucleus caudalis
9n	Glossopharyngeal nerve	PnO	Pontine reticular nucleus oralis
12N	Hypoglossal nucleus	Pr	Prepositus nucleus
CN	Cochlear nucleus	Pr5	Principal sensory trigeminal nucleus
Cu	Cuneate nucleus	Py	Pyramidal tract
Ecu	External cuneate nucleus	Raphe	Raphe nuclei
Gi	Gigantocellular reticular nucleus	RtTg	Reticulo tegmental nucleus of Pons
IO	Inferior olive	Sol	Solitary nucleus
IRt	Intermediate reticular nucleus	SOC	Superior olivary complex
LC	Locus coeruleus	Sp5	Spinal sensory trigeminal nucleus
LRt	Lateral reticular nucleus	VC	Vestibular complex
mcp	Middle cerebellar peduncle		

RESULTS

The greater number of corticobulbar projections was found in the main nuclei of the Pontomedullary reticular formation (PMRF), namely in PnO, PnC, Gi, IRt and LRt.

Intact animals injected in either PM or SMA showed denser corticobulbar projections as compared to M1 (Fig. 3A + 3B). For Mk-R13 (PM) on both contralateral and ipsilateral sides the largest percentage of terminals was found in the Gi nucleus. The same was true for Mk-CH (PM); however the three most rostral sections were unavailable for this animal. In contrast, Mk-R12 (PMd) showed a comparable percentage of axonal boutons in PnO+PnC and Gi on both the ipsilateral and contralateral sides (Fig. 2A). Animals injected in M1 showed a large percentage of projections to the ipsilateral Gi and contralateral IRt and LRt. For Mk-93-80 the projections to IRt and LRt was mostly contralateral. Few axonal boutons were found in PnO+PnC (Fig. 2B). Mk-M93-81 (SMA) showed the largest percentage of axonal boutons on both sides in both the Gi nucleus and the PnO+PnC nuclei (Fig. 2A).

In **M1 lesioned monkeys** the corticobulbar projections from PM decreased in density (Fig. 3A and 3C), with a majority of boutons in PnO+PnC and Gi nuclei (Fig. 2C). In animals with **Parkinson's disease (PD)** there was a strong decrease of corticobulbar projections from PM (Fig. 3A), whereas projections from M1 showed a slighter decrease as compared to intact animals (Fig. 3B). In general a comparable number of projections to PnO+PnC and Gi nuclei was observed, even in M1 injected monkeys (Fig. 2D). In **SCI animals** injected in M1, we found an increase of corticobulbar projections, mainly contralateral, in monkeys subjected to anti-Nogo A antibody treatment (Fig. 3B and 3D). The majority of corticobulbar projections were observed in Gi nucleus and in IRt + LRt nuclei (Fig. 2E).

CONCLUSIONS

Intact monkeys: The corticobulbar projections originating from PM and SMA are denser then from M1 (Fig. 3C and D). Moreover, the corticobulbar projection from PM and SMA tend to be more prominent on the ipsilateral PMRF than contralaterally; this is the other way around for the corticobulbar projection from M1.

M1 lesion and PD: For both pathologies, there was a decrease of corticobulbar projections (as compared to intact monkeys), from PM after M1 lesion and from both PM and M1 in case of PD (Fig. 3C and D).

Spinal cord injury (SCI): As compared to intact monkeys, no change in the two control SCI monkeys; in contrast, 2 out of 3 anti-Nogo A antibody treated monkeys showed an increase of the corticobulbar projections from M1, with contralateral predominance as well (Fig. 3D).

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