Corticobulbar projections from distinct motor cortical areas in intact macaque monkeys and following lesion of the primary motor cortex (M1)

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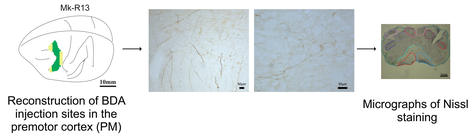
INTRODUCTION

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 and compare the corticobulbar projections on the reticular formation of the brainstem, possibly influencing the reticulospinal neurons. Corticobulbar projections were analysed in intact monkeys, originating from the premotor cortex (PM), the supplementary motor area (SMA) and the primary motor cortex (M1) as well as in M1 lesioned monkeys from PM.

METHODS

The tracer biotinylated dextran amine (BDA) was injected unilaterally in either PM, SMA or M1 of seven intact macaque monkeys and in PM of four M1 lesioned macaque monkeys (*Macaca fascicularis*). The corticobulbar projections anterogradelly labeled by BDA were then analyzed in 12 consecutive histological sections (50 µm thick), 250 µm apart. Stem axons and terminals, including boutons *en passant*, were then plotted using the software Neurolucida.
An adjacent series of 12 sections was stained with Cresyl Violet revealing Nissl bodies, on which the brainstem nuclei were delineated.
One animal (Mk-M90-60) was injected with cholera toxin subunit b (CB) in the spinal cord at C5-C8 levels to label reticulospinal neurons in the PMRF. 15 consecutive histological sections were analyzed for CB staining (50 µm thick, 300 µm apart) , and the corresponding sections were stained on an adjacent series for Cresyl Violet.
The Neurolucida software is connected to a light microscope (Olympus Bx40). We used the magnification 40x to trace the contours of the sections and the Pyramidal tract, the 100x to trace the axons, or neurons in case of Mk-M90-60. Finally the magnification 200x was used to plot the boutons *en passant* and *terminaux*. For the series stained for Nissl we used 12.5x magnification to delineate the nuclei and to acquire pictures.
Both series of sections (BDA, or CB, and Nissl staining) were overlapped in order to match the zones of BDA or CB staining and the nuclei delineated with Nissl staining.
The intact monkeys Mk-R13, Mk-R12, Mk-CH were injected in PM; Mk-Z182, Mk-M310, Mk-M93-80 were injected in M1 and finally Mk-M93-81 was injected in SMA. Notice that only Mk-M93-80 and Mk-M93-81 were subjected to intracortical microstimulation (ICMS) mapping and thus the injection was precisely located in the hand area (Rouiller et al., 1996). The M1 lesioned monkeys Mk-MO, MK-VA, Mk-RO and Mk-BI were all injected in PM; the M1 cortical lesion was performed with ibotenic acid injections. In Mk-M90-60 the injection was localized unilaterally between C5 and C8 levels of the spinal cord.
Statistics were calculated on the basis of the number of boutons in each nucleus and were derived from the Paired t-test /Wilcoxon, represented with asterisks: * $p \leq 0.05$; ** $p \leq 0.01$, *** $p \leq 0.001$.



Reconstruction of BDA injection sites in the premotor cortex (PM)

Micrographs of Nissl staining

Abbreviations

6N	Abducens nucleus	ml	Medial leminiscus (sometimes including the Trapezial body)
7N	Facial nucleus		
5n	Trigeminal nerve	Mo5	Trigeminal motor nucleus
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		
Ecu	External cuneate nucleus	Py	Pyramidal tract
Gi	Gigantocellular reticular nucleus	Raphe	Raphe nuclei
IO	Inferior olive	RtTg	Reticulo tegmental nucleus of Pons
IRt	Intermediate reticular nucleus		
ll	Lateral lemniscus	Sol	Solitary nucleus
LC	Locus coeruleus	SOC	Superior olivary complex
LRt	Lateral reticular nucleus	Sp5	Spinal sensory trigeminal nucleus
mcp	Middle cerebellar peduncle	VC	Vestibular complex

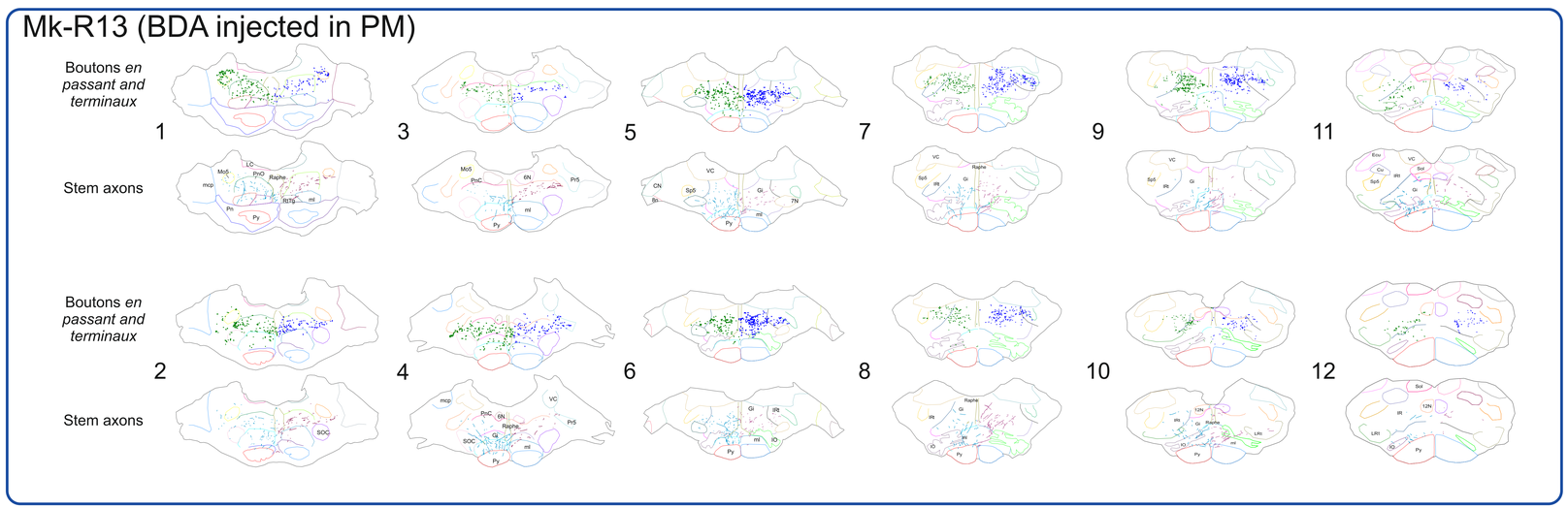


Figure 1: Brainstem drawings of coronal sections of Mk-R13 arranged from rostral (section 1) to caudal (section 12). All nuclei are delineated with a different color (see list of abbreviations). Axons located ipsilateral to the BDA injection are marked in blue whereas those located contralaterally are 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.

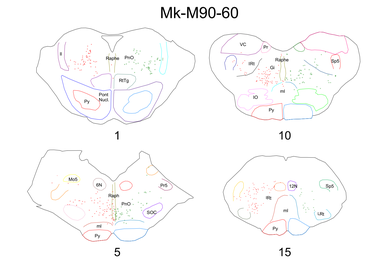


Figure 2: Brainstem drawings representing the distribution of reticulospinal neurons on the ipsilateral (red stars) and the contralteral sides (green triangles) with respect to the retrograde tracer CB injected in C5-C8 of the spinal cord.

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. These nuclei corresponds to the area were the reticulospinal neurons are located in the PMRF (Figure 2).

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 similar percentage of axonal boutons in PnO+PnC and Gi for both the ipsilateral and contralateral sides to the injection. Animals injected in M1 showed a large percentage of projections in the ipsilateral Gi and contralateral IRt and LRt. For Mk-93-80 the projection in IRt and LRt was mostly contralateral. Few axonal boutons were found in PnO+PnC. Mk-M93-81 (SMA) showed the largest percentage of axonal boutons on both sides in both the Gi nucleus and the PnO+PnC nuclei (Figure3). For the M1 lesioned monkeys, most boutons were in PnO+PnC and Gi nuclei (Figure 5).

Overall, the monkeys injected in non-primary motor cortical areas (PM, SMA) showed a statistically significant stronger corticobulbar projections on the ipsilateral side than on the contralateral one (except Mk-R13 (Figure 4A). This was the reverse in the monkeys subjected to BDA injections in M1: predominance of corticobulbar contralateral projections (Figure 4A). In M1 lesioned monkeys. corticobulbar projections from PM showed, as in intact animals, a statistically significant higher projection on the ipsilateral side than contralateral side; however the density of corticobulbar projection was strongly decreased.

A main result of the present study was that the corticobulbar projection in intact animals was denser when originating from PM or SMA, as compared to M1 (Figure 4B). In M1 lesioned monkey, corticobulbar projections from PM decreased in density (Figure 4B). Moreover, the density of normalized boutons tended to decrease as the lesion volume increased (Figure 6).

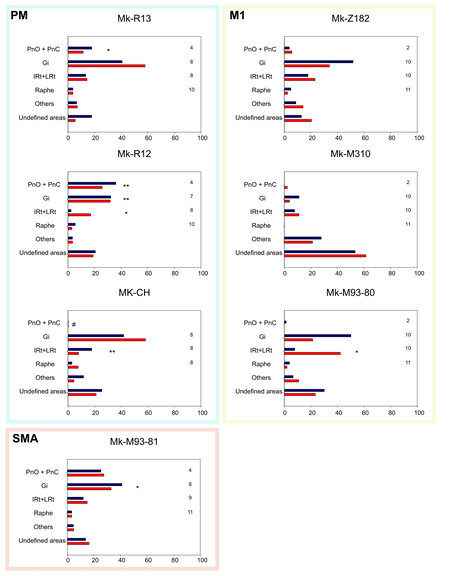


Figure 3: Percentage distribution of axonal boutons across brainstem nuclei. Percentage of boutons *en passant* or *terminaux* ipsilateral (blue) and contralateral (red) calculated on the total number of boutons found in the whole brainstem ipsilaterally or contralaterally to the BDA injection site. In each graph, the sum of all red bars is 100%. The sum of all blue bars is 100%.
The blue frame shows data obtained from animals injected in PM. In Mk-R13 and Mk-CH the BDA injection was located in both PMd and PMv, whereas in Mk-R12 the injection was restricted to PMd. The yellow frame shows data obtained from animals injected in M1. In Mk-Z182 and Mk-M310 the injection was larger than that performed in Mk-M93-80, which was restricted to the hand area. The pink frame shows data obtained from a single animal injected in SMA, the injection was localized in the hand area.
Numbers on the right of the histogram bars represent the number of sections including each nucleus or group of nuclei.

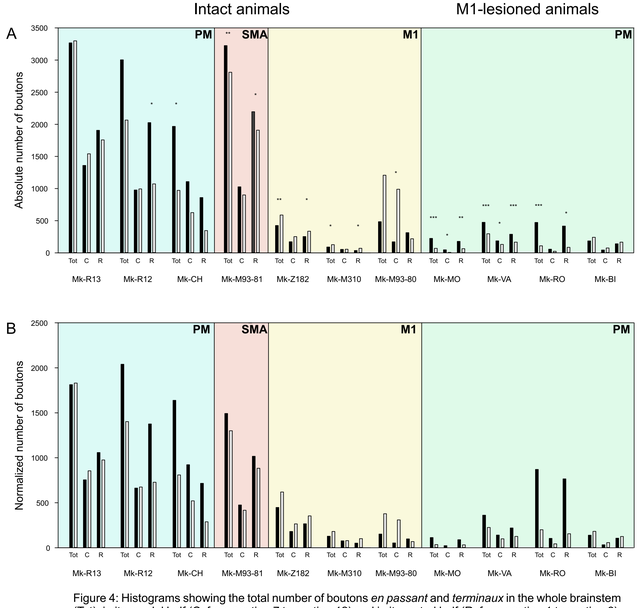


Figure 4: Histograms showing the total number of boutons *en passant* and *terminaux* in the whole brainstem (Tot), in its caudal half (C; from section 7 to section 12) and in its rostral half (R; from section 1 to section 6). Black bars are for ipsilateral projections and white bars for contralateral ones. A) Histogram representing the row data (absolute numbers of boutons), B) Histogram showing the same data as in A but normalized according to the number of BDA labelled corticospinal axons observed above the *Decussatio pyramidum*. Intact animals were injected in PM (blue background), in SMA (pink background) and in M1 (yellow background). Lesioned animals were all injected in PM (green background).

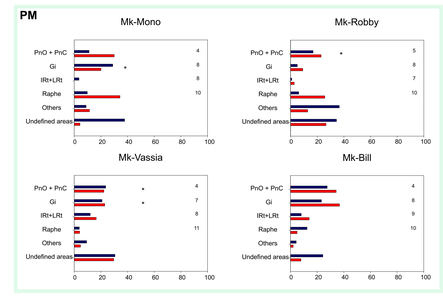


Figure 5: Percentage distribution of axonal boutons across brainstem nuclei. Percentage of boutons *en passant* or *terminaux* ipsilateral (blue) and contralateral (red) calculated on the total number of terminals found in the whole brainstem ipsilaterally or contralaterally to the injection site. In each graph, the sum of all red bars is 100%. The sum of all blue bars is 100%.
The green frame shows animals injected in PM and previously lesioned in the hand area of M1. Mk-MO, Mk-VA and Mk-BI injection of BDA was located in both PMd/PMv, whereas in Mk-RO the injection was restricted to PMd. Mk-MO and Mk-VA were treated with Anti-NOGO-A antibody during the post-lesion phase (4 weeks).
Numbers on the right of the histogram bars represent the number of sections including each nucleus or group of nuclei.

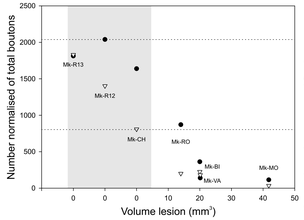


Figure 6: Graph representing the number of normalized boutons (according to the number of BDA labelled corticospinal axons observed above the *Decussatio pyramidum*) as a function of the volume of the M1 lesion. All the represented animals were injected in PMd/PMv, except Mk-R12 and Mk-RO whose injection was restricted to PMd.
The grey background includes the intact animals (lesion volume = 0). The black circles are for ipsilateral boutons , whereas the white triangles are for contralateral boutons.

CONCLUSION

A tendency for the corticobulbar projection to preferentially terminate ipsilaterally in the PMRF was found in monkeys injected in PM and SMA (both intact and lesioned). This was the reverse in the monkeys injected in M1 (tendency to preferentially terminate contralaterally). Moreover, the corticobulbar projection was less dense when originating from M1 as compared to PM or SMA, as well as after a lesion of M1 hand area for the PM projection.
Reticulospinal neurons were found to be more numerous on the ipsilateral side of the PMRF with respect to the injected side in the spinal cord.

In the future the same analysis will be performed in monkeys subjected to spinal cord injury or Parkinsonian (MPTP treated). Moreover, the corticotecal projection will be compared to the corticobulbar projection.

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