Corticobulbar projections from the motor cortex in macaque monkeys

Fregosi M., Hamadjida A., Rouiller E.M.

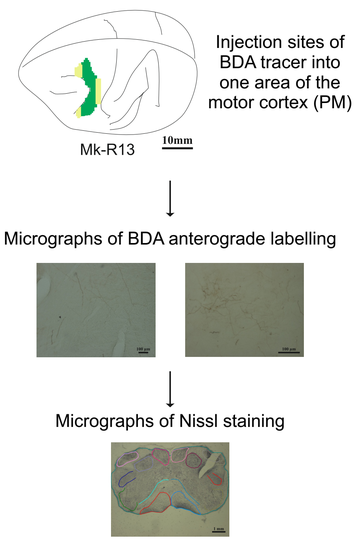
University of Fribourg, Departement of Medecine, Neuroscience,
Fribourg Center of Cognition

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 distinct motor cortical areas: the premotor cortex (PM), the supplementary motor area (SMA) and the primary motor cortex (M1) on the reticular formation of the brainstem, possibly influencing the reticulospinal neurons.

METHODS

The tracer biotinylated dextran amine (BDA) was injected unilaterally in either PM, SMA or M1 of seven intact 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.
Mk-R13, Mk-R12 and Mk-CH were injected in PM; Mk-Z182, Mk-M310 and 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) and thus the injection was precisely located in the hand area (Rouiller et al., 1996).



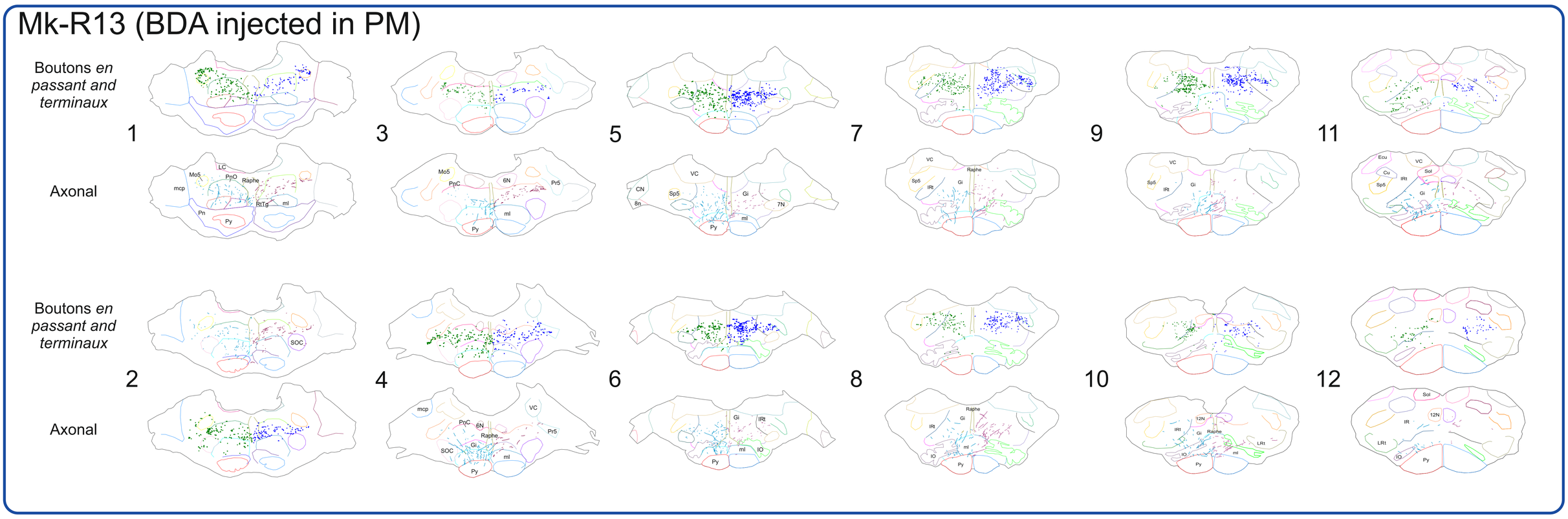
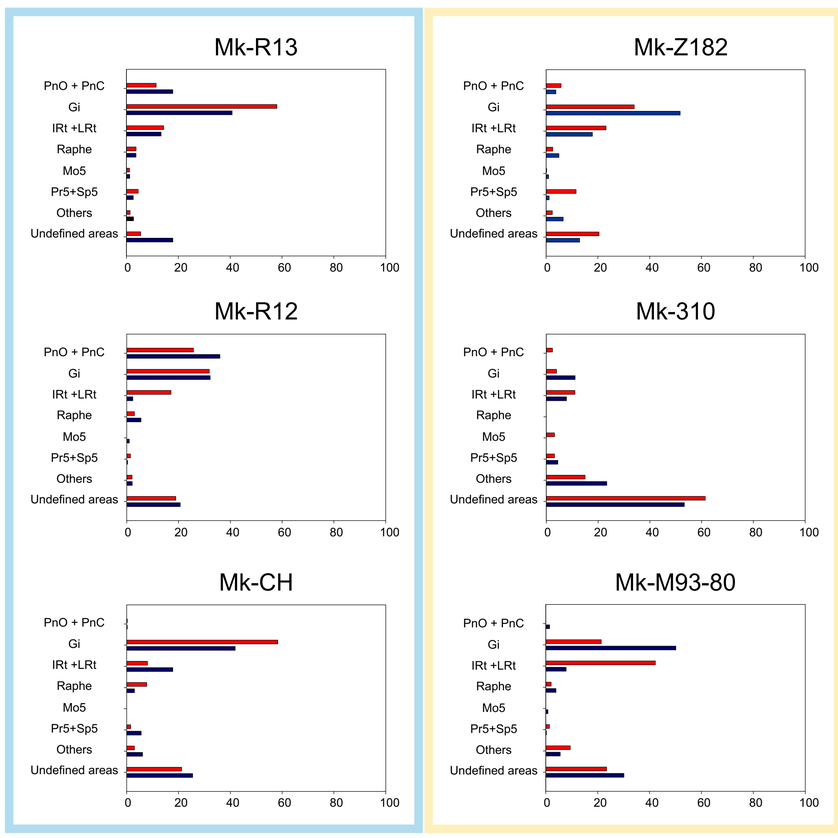


Figure 1: brainstem drawings of coronal sections of Mk-R13 arranged from rostral (section 1) to caudal (section 12). All nuclei are delineated with a different color (see list of abbreviations). Axons located ipsilateral to the BDA injection are marked in blue whereas those located contralaterally are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



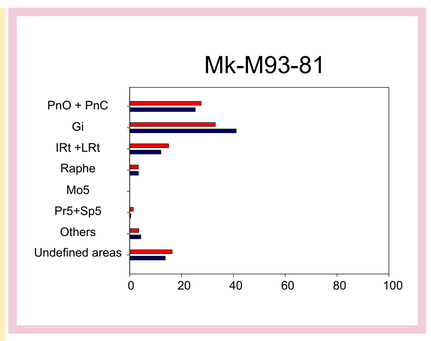


Figure 2: Distribution of axonal boutons across brainstem nuclei. Percentage of boutons *en passant* or *terminaux* ipsilateral (blue) and contralateral (red) calculated on the total number of terminals found in the whole brainstem ipsilaterally or contralaterally to the injection site.
The blue square 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 square 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 square shows data obtained from the only animal injected in SMA, the injection was localized in the hand area.

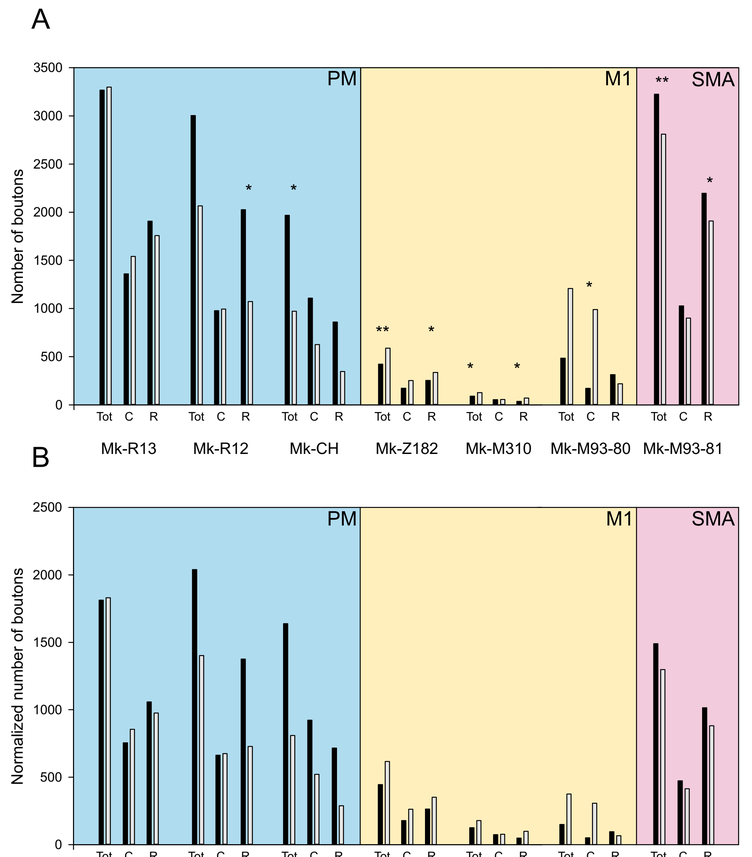


Figure 3: Histograms showing the total number of boutons *en passant* and *terminaux* in the whole brainstem (Tot), in the caudal half (C; from section 7 to section 12) and in the rostral half (R; from section 1 to section 6). Black bars are for ipsilateral projections and white bars for contralateral ones. A) Histogram representing the row data, 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*. The blue square shows animals injected in PM, the yellow square shows those injected in M1 and finally the pink square shows the one injected in SMA. Statistically significant comparisons (ipsi- versus contra-) derived from the Paired t-test /Wilcoxon test are represented with asteriscs: * $p \leq 0.05$; ** $p \leq 0.01$, *** $p \leq 0.001$.

Abbreviations

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

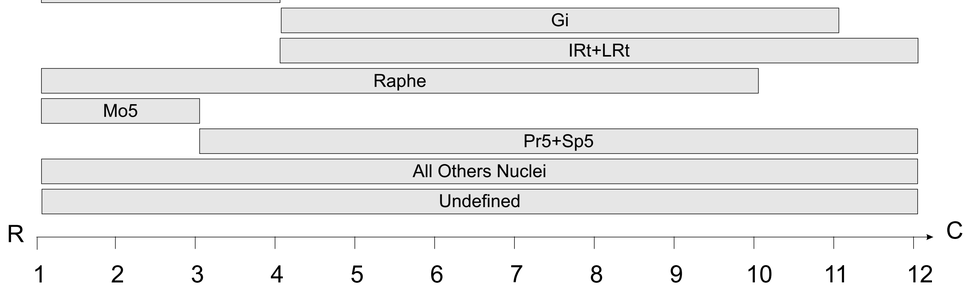


Figure 4: Schema representing the location of brainstem nuclei or group of nuclei according to their rostral (R) to caudal (C) extent.

CONCLUSION

A tendency to preferentially terminate ipsilaterally in the PMRF was found in monkeys injected in PM and SMA. On the contrary, the monkeys injected in M1 showed a tendency to preferentially terminate contralaterally in the PMRF. Moreover, the corticobulbar projection was less dense when originating from the primary motor cortex area as compared to PM or SMA.

In the future the same analysis will be performed on monkeys subjected to cortical lesion, to test if after a lesion of the primary motor cortex a reorganization of the corticobulbar projections coming from PM occurs, in line with the notion that PM contributes to the functional recovery from M1 lesion (Liu and Rouiller, 1999).

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. The rostro-caudal localization of the brainstem nuclei is shown in Figure 4.
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 section were unavailable for this animal. In contrast, Mk-R12 (PM) showed a similar percentage of connections in PnO+PnC and Gi for both the ipsilateral and contralateral sides to the injection. Animals injected in M1 showed a larger percentage of projections in the contralateral Gi and ipsilateral IRt and LRt. For Mk-93-80 the projection in IRt and LRt was mostly ipsilateral. Few projections were found in PnO+PnC. Mk-M93-81 showed the largest percentage of terminals on both sides in both the Gi nucleus and the PnO +PnC nuclei (Figure 2).

Overall, the monkeys injected in non-primary motor cortex areas (PM, SMA) showed a statistically significant stronger corticobulbar projections on the ipsilateral side than on the contralateral one (except Mk-R13 (Figure 3). This was the reverse in the monkeys subjected to M1 injections: predominance of corticobulbar contralateral projections (Figure 3).
A main result of the present study was that the corticobulbar projection was denser when originating from PM or SMA, as compared to M1 (Figure 3, Panel B).

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