Corticobulbar projections from the premotor cortex in the macaque monkey

Fregosi M. , Hamadjida A., Rouiller E.M.
University of Fribourg, Departement of Medecine, Neuroscience

UNIVERSITÉ DE FRIBOURG
UNIVERSITÄT FREIBURG

SPCCR

Swiss Society for Neuroscience

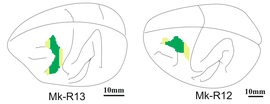
INTRODUCTION

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 action on its nuclei. Some studies suggest 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 a stroke.
The aim of the present study is to analyze the projections coming from the premotor cortex (PM) on the reticular formation of the brainstem, influencing the reticulospinal neurons.
In the future the same analysis wll be performed on lesioned monkeys to assess whether, after a lesion of the primary motor cortex (M1), a reorganization of the projections in the brainstem occurs and its relations with the spontaneous recovery or with Anti-Nogo-A antibody treatment.

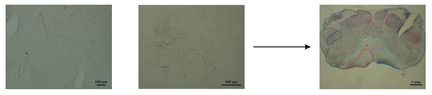
METHODS

The tracer biotinylated dextran amine (BDA) was injected unilaterally in the premotor cortex of two intact macaque monkeys (*Macaca fasciucularis*). The corticobulbar projections labeled anterogradely by BDA were then analyzed in 12 consecutive histological sections, 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 for 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 nuclei, the 10x to trace the axons and finally the 20x to plot the terminals and the boutons. For the series stained with Nissl we used the 1.25x objective to delineate the nuclei and to acquire pictures.
Once we have both series of sections (BDA and Nissl staining), we overlapped them in order to visualize if there is a correspondence between the zone of terminals and the nuclei delineated with Nissl staining. In Mk-R13 the BDA injection was located in both PMd and PMv whereas in MK-R12 the BDA injection was restricted to PMd.

Injection sites of BDA tracer on the left hemisphere in PM



Microphotographs of BDA anterograde labeling



Microphotographs of Nissl staining

Abbreviations

6N	Abducens nucleus	Mo5	Trigeminal motor nucleus
7N	Facial nucleus	Pn	Pontine nuclei
7n	Auditory nerve	PnC	Pontine reticular nucleus caudalis
8n	Vestibulocochlear 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
Gr	Gracilis nucleus	Sal	Salivatory nucleus
IO	Inferior olive	SOC	Superior olivary complex
Irt	Intermediate reticular nucleus	Sp5	Spinal sensory trigeminal nucleus
LC	Locus coeruleus	VC	Vestibular complex
Lrt	Lateral reticular nucleus		
mcp	Middle cerebellar peduncle		
ml	Medial leminiscus (sometimes including the Trapezial body)		

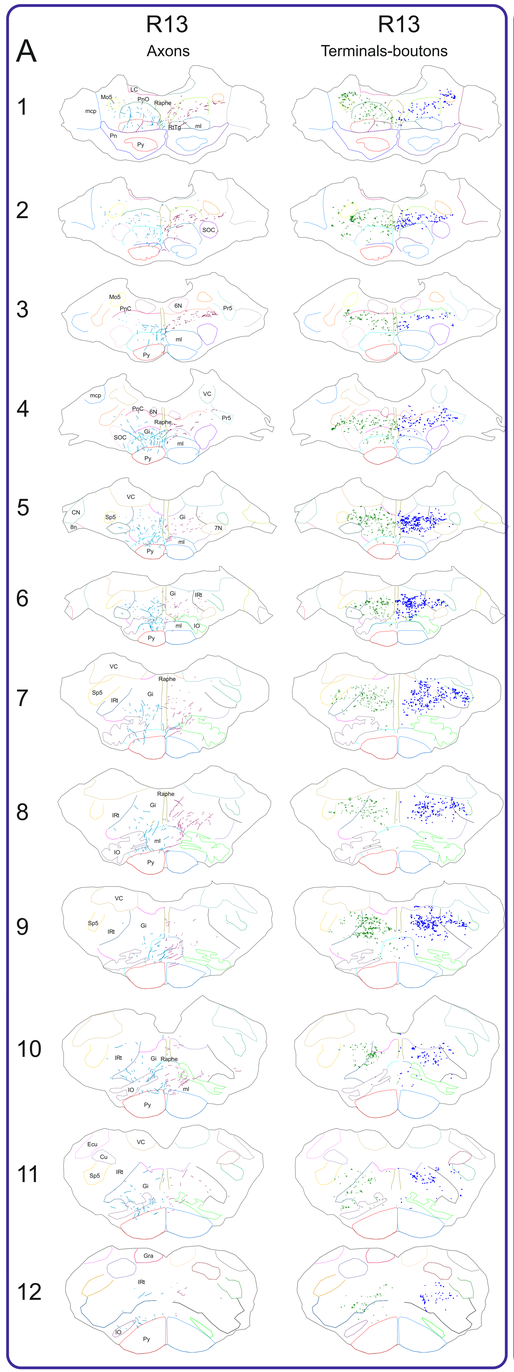


Figure 1: brainstem drawings on coronal sections of Mk-R13 (A) and Mk-R12 (B) arranged from rostral (R) to caudal (C) going from sections 1 to 12. All nuclei are delineated with a different color (see list of abbreviations). Axons located ipsilateral to the BDA injection in PM are marked in blue whereas those located contralaterally are marked in bordeaux. Terminals, or boutons, ipsilateral to the BDA injection are marked as green circles whereas the contralateral terminals are marked as blue squares.

Rostral

Caudal

	Mk-R13		Mk-R12	
	Ipsi	Contra	Ipsi	Contra
PnO + PnC	583 (61%)	378 (39%)	1080 (67%)	532 (33%)
Gi	1321 (41%)	1924 (59%)	968 (60%)	658 (40%)
IRt+LRt	459 (49%)	469 (51%)	72 (17%)	353 (83%)
Raphe	120 (50%)	122 (50%)	165 (73%)	61 (27%)
Mo5	41 (52%)	38 (48%)	29 (100%)	0
Pr5 + Sp5	86 (37%)	149 (63%)	9 (24%)	29 (76%)
Others	78 (63%)	46 (37%)	61 (59%)	42 (41%)
Undefined	579 (77%)	176 (23%)	620 (61%)	390 (39%)
Tot	3267	3299	3004	2066

Table 1: table showing the total number of ipsilateral and contralateral terminals or boutons with respect to the PM injection site for a nucleus only or a group of nuclei. In brakets, the percentage of ipsilateral versus contralateral number of terminals .

Section number	Mk-R13			Mk-R12		
	Boutons' number		Δipsi-contra	Boutons' number		Δipsi-contra
	Ipsi	Contra		Ipsi	Contra	
12	99	88	11	43	78	-35
11	142	138	4	138	146	-8
10	196	164	32	140	104	36
9	452	425	27	288	295	-7
8	202	273	-71	147	201	-54
7	269	453	-184	221	170	51
6	248	527	-279	64	92	-28
5	359	570	-211	138	136	2
4	390	216	174	292	145	147
3	236	107	129	382	173	209
2	363	173	190	512	238	274
1	311	165	146	639	288	351
	3267	3299		3004	2066	

Table 2: table showing the number of ipsilateral and contralateral terminals-boutons in every section. The difference between the ipsilateral side and the contralateral side is given in the Δipsi-contra column. When the number is greater on the ipsilateral side it is colored in orange whereas the reverse is in violet.

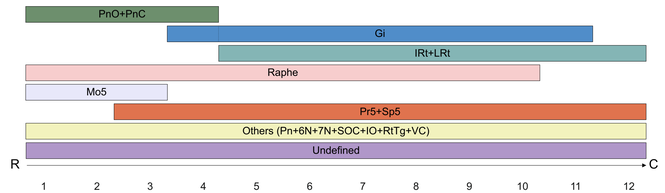


Figure 2: schema representing the location of a nucleus or a group of nuclei in the brainstem according to their rostral (R) to caudal (C) extent.

	R13		R12	
PnO + PnC	18	12	36	26
Gi	40	58	32	32
IRt+LRt	14	14	2	17
Raphe	4	4	6	3
Mo5	1	1	1	0
Pr5 + Sp5	3	5	0	1
Others	2	1	2	2
Undefined ares	18	5	21	19
Tot	100	100	100	100

Table 3: percentage of terminals, or boutons, ipsilateral or contralateral calculated on the total number of terminals found in the whole brainstem ispilaterally or contralaterally to the PM injection site (the total number is the one given in Table 2).

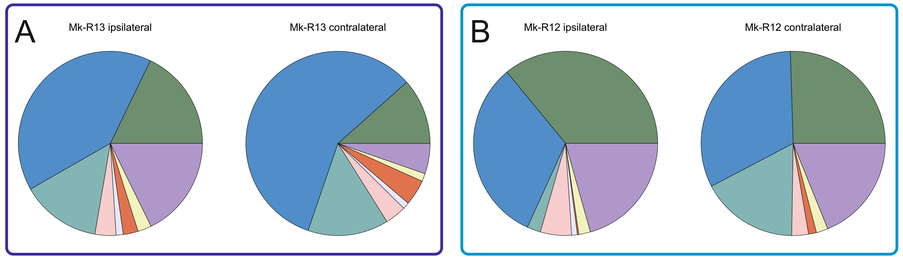


Figure 3: pie graphs (same data as in Table 3) for Mk-R13 (A) and Mk-R12 (B) showing the percentage of terminals-boutons ipsilateral, or contralateral, with respect to the total number of terminals. The colors correspond to those colors used in Figure 2 to define the areas of one nucleus or a group of nuclei.

RESULTS

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

Mk-R13 did not show a systematic difference of laterality on the PMRF for both the rostral and caudal halves. On the contrary Mk-R12 showed a statistically significant difference (paired t-test, p=0.05) for the rostral half (from section 1 to 6) with more projections on the ipsilateral side. No difference was found for the caudal half (Table 2).

For Mk-R13 on both contralateral and ipsilateral sides with resspect to the PM injection sites the largest percentage of terminals was found in the Gi nucleus. In contrast, Mk-R12 showed a similar percentage of connections in PnO+PnC and Gi for both the ipsilateral and contralateral sides to the injection (Figure 3). This interindividual difference may be releated to injection site characteristics.

CONCLUSION

A tendency to preferentially terminate ipsilaterally in the rostral half of the PMRF was found for Mk-R12 whereas Mk-R13 did not show such trend. Whether the difference in the location of BDA injection is involved in this difference remains a question for further analyses on additional intact animals.

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 projections coming from PM occurs, in line with the notion that PM contributes to the functional recovery from M1 lesion (Liu and Rouiller, 1999). Moreover, we aim at analysing the relation of the rearrangement with the spontaneous functional recovery of the animal or with the Anti-Nogo-A antibody treatment to which some of the animals have been subjected.

Supported by: Swiss National Science Fundation (SNF), Grants to E.M.R. No.31-61857.00, 310000-110005, 31003A-132465; The National Center of Competence in Research (NCCR) on «Neural Plasticity and Repair»; Novartis Foundation; The Swiss Primate Competence Center for Research (SPCCR): http://www.unifr.ch/spccr/home/