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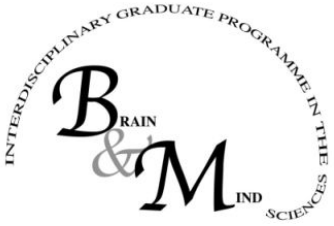

Neurophysiological Study of the Perception of Biological Motion

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Doctoral Thesis

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ΕΥΧΑΡΙΣΤΙΕΣ

Η παρούσα διδακτορική διατριβή εκπονήθηκε στο πλαίσιο του διατμηματικού μεταπτυχιακού προγράμματος σπουδών *Εγκέφαλος & Νους,* κατά τα έτη 2006-2010, στο εργαστήριο *Λειτουργικής Απεικόνισης Εγκεφάλου* του τομέα *Βασικών Ιατρικών Επιστημών* στο Πανεπιστήμιο Κρήτης.

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LIST OF ABBREVIATIONS

2-D	Two-dimensional	Δισδιάστατος (2-Δ)
2-DG	2-deoxy-D-[^{14}C]glucose	[^{14}C]-2-δεοξυγλυκόζη
2-DG-6-P	[^{14}C] 2-DG-6-phosphate	[^{14}C]-2-δεοξυγλυκόζη-6-φωσφορική
3-D	Three-dimensional	Τρισδιάστατος (3-Δ)
BB	Biceps brachii (Biceps) muscle	Δικέφαλος μυς
C1-C8	Cervical spinal segments, 1-8	Αυχενικά νωτιαία μυελοτόμια 1-8
Cm	Motion-control Monkeys	Πίθηκοι που παρακολουθούσαν το κινούμενο άνω άκρο του πειραματιστή να εκτελεί κινήσεις προσέγγισης
CMA-r,-d,-v	Cingulate motor area-rostral,-dorsal,-ventral	Προσαγώγιος κινητική περιοχή-πρόσθια, ραχιαία, κοιλιακή
CNS	Central nervous system	Κεντρικό νευρικό σύστημα (ΚΝΣ)
Cs	Central sulcus	Κεντρική αύλακα
E	Grasping-execution monkeys	Πίθηκοι που εκτελούσαν κινήσεις σύλληψης 3-Δ αντικειμένου με την άκρα χείρα (Ε)
E*DC*	Extensor digitorum communis	Κοινός εκτείνων μυς των δακτύλων (της άκρας χείρας)
EMG	Electromyogram	Ηλεκτρομυογράφημα
F2	Frontal area 2	Μετωπιαία περιοχή 2
F5	Frontal area 5	Μετωπιαία περιοχή 5
F7	Frontal area 7	Μετωπιαία περιοχή 7
FDI	First dorsal interosseus muscle	Πρώτος ραχιαίος μυς μεσόσταιος (της άκρας χείρας)
FDS	Flexor digitorum superficialis	Επιπολής καμπτήρας μυς των δακτύλων (της άκρας χείρας)
fMRI	Functional magnetic resonance Imaging	Λειτουργική απεικόνιση μαγνητικού συντονισμού
G-6-P	Glucose-6-phosphate	Γλυκόζη-6-φωσφορική
GABA	γ-aminobutyric acid	γ-αμινοβουτυρικό οξύ
GM	Grey matter	Φαιή ουσία
H-Reflex	Hoffmann reflex	Αντανακλαστικό Hoffmann
HRP	Horseradish peroxidase	Υπεροξειδάση του ραπανακιού
IN	Interneuron (spinal/cortical)	Ενδονευρώνες (νωτιαίοι/φλοιώδεις)
LCGU	Local cerebral glucose utilization	Τοπική κατανάλωση γλυκόζης (ΤΚΓ)
MEG	Magnetoencephalography	Μαγνητοεγκεφαλογραφία
MEP	Motor evoked potential	Κινητικό προκλητό δυναμικό
MI/FI	Primary motor cortex/ frontal area I	Πρωτοταγής κινητικός φλοιός/μετωπιαία περιοχή I
MN	Motoneuron	Κινητικός νευρώνας νωτιαίου μυελού
O	Grasping-observation monkeys	Πίθηκοι που παρακολουθούσαν την άκρα χείρα του πειραματιστή να εκτελεί κινήσεις σύλληψης 3-Δ

		αντικειμένου (Π)
OP	Opponens pollicis	Αντιθετικός του αντίχειρα (μυς)
PET	Positron emission tomography	Τομογραφία εκπομπής ποζιτρονίων
PF/7b	Cytoarchitectonic subdivision of area 7 in the inferior parietal lobule	Κυτταροαρχιτεκτονική υποδιαίρεση της περιοχής 7 στο κάτω βρεγματικό λοβίο
PM-d,-v	Premotor cortex-dorsal, -ventral	Προκινητικός φλοιός-ραχιαίος, -κοιλιακός
SI	Primary somatosensory cortex	Πρωτοταγής σωματαισθητικός φλοιός
SII	Secondary somatosensory cortex	Δευτεροταγής σωματαισθητικός φλοιός
STS	Superior temporal sulcus	Άνω κροταφική αύλακα
T1-T2	Thoracic spinal segments, 1-2	Θωρακικά νωτιαία μυελοτόμια, 1-2.
TMS	Transcranial magnetic stimulation	Διακρανιακός μαγνητικός ερεθισμός
WM	White matter	Λευκή ουσία

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ΠΕΡΙΛΗΨΗ

Πρόσφατες μελέτες νευροαπεικόνισης στον πίθηκο *Rhesus* (*Macaca mullata*), με την ποσοτική αυτοραδιογραφική μέθοδο της ραδιενεργού δεοξυγλυκόζης (2-δεοξυ-D-[^{14}C] γλυκόζης; 2-DG) (Raos et al., *Neuroimage* 23: 193-201, 2004; Raos et al., *J Neurosci* 27: 12675-83, 2007; Evangeliou et al., *Cereb Cortex* 19: 624-639, 2009; Kilintari et al., *Cereb Cortex* 21: 949-63) κατέδειξαν ότι σχεδόν οι ίδιες σωματαισθητικές, κινητικές και συνειρμικές μετωπιαιο-βρεγματο-ινιακές περιοχές του φλοιού των εγκεφαλικών ημισφαιρίων ενεργοποιούνται τόσο κατά την παρατήρηση όσο και κατά την εκτέλεση κινήσεων σύλληψης με την άκρα χείρα. Τα δεδομένα αυτά υποδεικνύουν ότι για να κατανοήσουμε μια πράξη που εκτελείται από κάποιο άλλο υποκείμενο προσομοιάζουμε αυτή την πράξη, επαναλαμβάνοντας την νοερά. Μελέτες *διακρανιακού μαγνητικού ερεθισμού* (TMS) έδειξαν ότι η διεγερσιμότητα της φλοιονωτιαίας οδού του παρατηρητή τροποποιείται κατά την παρατήρηση κινήσεων που εκτελούνται από άλλους. Τα βιβλιογραφικά δεδομένα για τη συμμετοχή του νωτιαίου μυελού στο φαινόμενο αυτό είναι αντικρουόμενα (Pattuzzo et al., *Neuropsychologia 41: 1272-8, 2003*; Li et al., *J Neurosc* 24: 9674-80, 2004).

Στόχος της παρούσας μελέτης ήταν να ελέγξουμε, αν και σε ποιό βαθμό η παρατήρηση κινήσεων επηρεάζει τη λειτουργική δραστηριότητα στο νωτιαίο μυελό. Για το σκοπό αυτό αναλύσαμε την περιοχή αντιπροσώπευσης του άνω άκρου (αυχενικό όγκωμα) του νωτιαίου μυελού πιθήκων *Rhesus* (*Macaca mullata*), που εκτελούσαν (εκτέλεση κινήσεων σύλληψης- Ε) ή παρατηρούσαν (παρατήρηση κινήσεων σύλληψης- Ο) κινήσεις σύλληψης.

Οι τρεις πίθηκοι της συνθήκης Ε είχαν εκπαιδευτεί να προσεγγίζουν και να συλλαμβάνουν ένα τρισδιάστατο αντικείμενο με το αριστερό τους χέρι, ενώ το δεξί

ήταν ακινητοποιημένο. Ο ένας πίθηκος εκτελούσε τις κινήσεις σύλληψης υπό οπτική καθοδήγηση, ενώ οι άλλοι δύο στο σκοτάδι. Οι πίθηκοι είχαν εκπαιδευτεί να διατηρούν το βλέμμα τους σταθερό προς τη θέση του αντικειμένου μέσα σε ένα κυκλικό παράθυρο διαμέτρου 8°. Οι τρεις πίθηκοι της συνθήκης Ο είχαν εκπαιδευτεί να διατηρούν το βλέμμα τους εντός του κυκλικού παραθύρου των 8°, με τα δυο τους χέρια ακινητοποιημένα, ενώ παρατηρούσαν τον πειραματιστή να συλλαμβάνει το τρισδιάστατο αντικείμενο. Ο πειραματιστής στεκόταν στη δεξιά πλευρά του πειραματόζωου. Οι υπόλοιπες παράμετροι της δοκιμασίας Ο ήταν όμοιες με αυτές της Ε. Επιπρόσθετα, δύο πίθηκοι είχαν εκπαιδευτεί να διατηρούν το βλέμμα τους εντός του κυκλικού παραθύρου των 8° με τα δυο τους χέρια ακινητοποιημένα, ενώ παρατηρούσαν τόσο το αντικείμενο όσο και το άνω άκρο του πειραματιστή να κινείται μπροστά τους με τον καρπό και τα δάχτυλα σε έκταση, χωρίς να υπάρχει ταυτόχρονη θέαση αντικειμένου και άνω άκρου. Ο πειραματιστής στεκόταν στη δεξιά πλευρά της θέσης του πειραματόζωου. Οι πίθηκοι αυτοί αποτέλεσαν την ομάδα ελέγχου παρατήρησης της βιολογικής κίνησης (Cm).

Την περάτωση του πειράματος 2-DG, ακολουθούσε εκτομή και ψύξη του νωτιαίου μυελού (από τα πρώτα αυχενικά έως τα πρώτα θωρακικά μυελοτόμια). Στη συνέχεια λαμβάνονταν σειριακές οριζόντιες τομές πάχους 20 μm, οι οποίες εκτίθονταν σε αυτοραδιογραφικά φιλμ. Η πυκνομετρική ανάλυση των αυτοραδιογραφημάτων πραγματοποιήθηκε μέσω ενός υπολογιστικά υποστηριζόμενου συστήματος ανάλυσης εικόνας (MCID, Imaging Research, Ontario, Canada). Σε κάθε τομή μετρήθηκε η τοπική κατανάλωση γλυκόζης (ΤΚΓ) κατά μήκος της προσθιοπίσθιας έκτασης της φαιής ουσίας (ξεχωριστά για την αριστερή και δεξιά πλευρά), με χωρική διακρισιμότητα 50 μm, ώστε να δημιουργηθεί μία σειρά δεδομένων. Οι σειρές δεδομένων ανά 5 διαδοχικές τομές στοιχίζονταν στο πρόσθιο

τμήμα της κάθε τομής και ο μέσος όρος τους αποτελούσε μία γραμμή στο δισδιάστατο χάρτη της μεταβολικής δραστηριότητας. Οι επιμέρους δισδιάστατοι μεταβολικοί χάρτες του νωτιαίου μυελού μετασχηματίστηκαν γεωμετρικά έτσι ώστε τα όριά τους να συμπίπτουν με τα όρια ενός πρότυπου χάρτη.

Τα αποτελέσματα έδειξαν ότι η περιοχή του αυχενικού ογκώματος, στην οποία αντιπροσωπεύεται το άνω άκρο, παρουσιάζει, κατά την παρατήρηση κινήσεων σύλληψης, αμφοτερόπλευρη μείωση της κατανάλωσης γλυκόζης σε σχέση με τον μάρτυρα της βιολογικής κίνησης. Αντίθετα, κατά την εκτέλεση κινήσεων σύλληψης η ίδια περιοχή παρουσιάζει αύξηση της κατανάλωσης γλυκόζης, ομόπλευρα του κινούμενου άκρου, όπως ήταν αναμενόμενο. Το εύρημα αυτό υποδεικνύει ότι ο νωτιαίος μυελός είναι μία από τις δομές που συμμετέχουν στην αναστολή της κινητικής εντολής στον παρατηρητή, κατά τη θέαση κίνησης άλλου υποκειμένου.

Προτείνουμε ότι η ενεργοποίηση που παρατηρήθηκε ομόπλευρα του κινούμενου άνω άκρου κατά την εκτέλεση κινήσεων σύλληψης διαμεσολαβείται από διεγερτικές φλοιονωτιαίες προβολές από τον πρωτοταγή κινητικό φλοιό. Η αμφίπλευρη μεταβολική μείωση που παρατηρήθηκε κατά την παρατήρηση κινήσεων σύλληψης πιθανόν αντικατοπτρίζει το καθαρό αποτέλεσμα κατερχόμενων διεγερτικών προβολών από τον πρωτοταγή κινητικό φλοιό και ανασταλτικών προβολών από τον προκινητικό φλοιό οι οποίες είτε άμεσα, είτε μέσω του εγκεφαλικού στελέχους επηρεάζουν τη δραστηριότητα των ενδονευρώνων του νωτιαίου μυελού.

Συμπερασματικά, η μείωση της μεταβολικής δραστηριότητας στην περιοχή του αυχενικού ογκώματος του νωτιαίου μυελού, κατά την παρατήρηση κινήσεων σύλληψης, εξηγεί την απουσία μυϊκής δραστηριότητας και κίνησης του άνω άκρου

του παρατηρητή παρά την ενεργοποίηση της περιοχής εκπροσώπησης του άνω άκρου στον πρωτοταγή κινητικό φλοιό.

ABSTRACT

Recent brain imaging studies, using the quantitative 2-deoxy-D-[^{14}C]glucose (2-DG) imaging technique in the *Rhesus* monkey (*Macaca mullata*), have demonstrated that the forelimb representation of the primary motor (MI) and somatosensory cortices (Raos et al., *Neuroimage* 23: 193-201, 2004), as well as several premotor, parietal and occipital areas (Raos et al., *J Neurosci* 27: 12675-83, 2007 Evangeliou et al., *Cereb Cortex* 19: 624-639, 2009; Kilintari et al., *Cereb Cortex* 21 : 949-63), are activated by both action-execution and action-observation, indicating that the spectator mentally simulates the observed action. Moreover, several studies have demonstrated repeatedly that corticospinal excitability is modulated during action observation, providing evidence of an activation of the observer's motor system. However, evidence for the involvement of the spinal cord in action observation is controversial (Pattuzzo et al., *Neuropsychologia 41: 1272-8, 2003*; Li et al., *J Neurosc* 24: 9674-80, 2004).

In the present study we have explored whether and how action-observation affects the spinal cord. For this purpose, we analyzed the forelimb-representation of the spinal cord (cervical enlargement) from eight monkeys. Subjects trained to either execute reaching-to-grasp movements (grasping execution- E) or observe the experimenter performing the same movements (grasping observation- O).

Three E monkeys were trained to reach for and grasp a three-dimensional (3-D) object, with the left forelimb, whereas the right one was restricted. One of the monkeys performed the task under visual guidance and the other two in complete darkness. Three O monkeys were trained to maintain their gaze within the 8°-diameter circular window, with both hands restricted, whereas observing the experimenter

grasping the ring with the hook grip. The experimenter was standing on the right side of the monkey and performed the reaching-to-grasp movements with the right hand. The rest of the parameters of the O task were similar to the those of the E task. Two motion control (Cm) monkeys were used as control for the O monkeys. Cm monkeys were trained to maintain their gaze within the 8°-diameter circular window, while both hands were restricted, and to observe the moving hand of the experimenter, with the wrist and fingers being extended, without hand preshaping or interaction with the 3-D object. Specifically, the Cm monkeys were exposed to consecutive (and not simultaneous) view of the object and the moving arm. The experimenter was standing at the right side of the monkey.

At the end of each 2-DG experiment the spinal cord was dissected (from the first cervical to the first thoracic segments) and twenty μm thick horizontal sections were obtained, and exposed on autoradiographic films. The autoradiograms were densitometrically analyzed in a computerized image analysis system (MCID, Imaging Research, Ontario, Canada). In each section we sampled the local glucose utilization (LGU) along the rostrocaudal extent of the gray matter (separately for the left and the right side) with a resolution of 50 μm, to produce a data array for the section under analysis. The data arrays of 5 consecutive sections were aligned and averaged to produce a line in the two dimensional map (2-D) of the metabolic activity. To account for inter-individual differences the individual 2-D maps were then transformed to match a common template map.

The results showed that the metabolic activity in the cervical enlargement of the spinal cord is suppressed bilaterally in monkeys observing reaching-to-grasp movements, whereas it is activated ipsilaterally to the grasping hand in monkeys executing the same movements.

We propose that the elevated activity ipsilateral to the grasping hand, in the 'executing' monkeys, may be mediated through *MI*-corticospinal excitatory signals. The bilateral metabolic depression observed in the 'observing' monkeys may represent the sum of excitatory *MI*-corticospinal and inhibitory premotor cortico-spinal or premotor cortico-brainstem-spinal inputs to spinal local interneurons. All in all, the depression of overall activity in the cervical enlargement of the spinal cord of the O monkeys may explain the suppression of overt movements during action observation, despite the activation of the observer's motor system.

INTRODUCTION

Efficient social interpersonal communication and interaction requires understanding of the intentions of others. This cognitive faculty provides the means for the assignment of meaning to the behavior of conspecifics (Leslie, 2002; 2003). However, the assumption that interacting social subjects can recognize the state of mind of others, eludes direct empirical support (Premack and Woodruff, 1978). This neuro-philosophical concept has been described as the *problem of other minds*, alternatively, the *problem of intersubjectivity,* and attempts to answer the matter in hand, putting it forward as: "If we are aware of our own experiences, mental states and intentions, through direct access or introspection, how can we be informed of the analogue for our counterparts, which are apparently endowed to share their own mental states with other individuals" (McGinn, 1984)? In the area of philosophy of mind, any theoretical attempt to answer this conceptual issue corresponds to a *theory of mind* (Premack and Woodruff, 1978). By definition, a *theory of mind* should explain how we predict, attribute and explain others' mental states, such as, thoughts, desires, emotions, intentions and goals, which precede any behavioral manifestation (Pargetter, 1984; Avramides, 2001).

So far, two antagonistic main theories have been generated: a) the *theory* theory and b) the *simulation* theory. On one hand, *theory* theory postulates that our ability to explain, predict and interpret intentional behaviors is subserved by tacit knowledge of an internally represented theory of commonsense or *folk psychology* (Fodor, 1987; Fodor and British Psychological Society., 1987). *Folk psychology* incorporates the language-based set (propositional thinking) of concepts such as *beliefs*, *desires*, *intentions*, *expectations*, *preferences*, *hopes*, *fears*, and so forth (Sellars, 1956; Churchland, 1994). According to *theory* theory, in order to understand

what others do, we implement inferential (language-based) processes, through a set of causal laws, linking together human behavior and internal mental states (Barsalou, 1999; Bennett and Hacker, 2003; Sommerville and Decety, 2006).

On the other hand, *simulation* theory holds that we represent the mental states and processes of others by mentally simulating them, or else, by generating similar states and processes in ourselves. This precedes any inferential language-grounded mental process. Under this perspective, knowledge is embodied and fragmented in individual constituents, such as sensory/kinaesthetic, motor and introspective ones (Barsalou et al., 2003a; Barsalou et al., 2003b). In other words, *simulation* theory proposes that we understand others by simulating, by some sort of mental/motor imagery (i.e., mental rehearsal of sensory-motor programs), that we are endowed to see, hear or act through the mind's eye, ear or body-limbs (Nigel, 2010). Thus, behaving/acting and recognizing/perceiving are the "two sides of the same coin", suggesting a mutual neuronal substratum, which indicates an action-perception coupling (Jeannerod, 1995).

The conceptual underpinnings, regarding interrelation among action and perception can be traced back in some influential ideas. For instance, Carpenter (1881) had suggested that imagined movements have a qualitatively similar, but quantitatively less powerful effect on motor output, than deliberate movements. Bastian (1897) had supported the concept of *kinesthetic images*, stating that images are formed from sensory traces left by a prior movement, stored in the motor cortex, and revived when the same movement is under performance. Gibson (1979) had theorized a functional linkage between perception and action, as a prerequisite for an adaptive behavior. Specifically, Gibson's *ecological* psychology argues against a distinct division between perception and action. Furthermore, Greenwald (1970) and

James (1890) had supported the role of intentional motor-representations in the control of actual movements. Finally, Allport (1987) had suggested that the distributed set of percepts and action plans are content-specific mental-representations (for a review, see Hommel et al., (2001).

Nonetheless, William James's *Ideomotor Principle of Voluntary Action* (James, 1890) had a greater impact in the field of cognitive neurosciences. Specifically, the contemporary descriptive term, *shared representations* of actions, denotes the very similar neural substrate in explicit and implicit (observation/imagery) motor performance. James had theorized that perception of action is based on co-activation of visual and motor circuits, within the central nervous system (CNS). Characteristically, he had speculated:

> *"We may lay it down for certain that every mental representation of a movement awakens to some degree the actual movement which is its object; and awakens it in a maximum degree whenever it is not kept from so doing by an antagonistic representation, present simultaneously to the mind"*
> *William James (1980). Principles of Psychology, p. 526, Vol. II.*

At this point we should mention that action-*simulation* supports our recognition of observed actions performed by other subjects as well as our imagination of covert actions (mental motor-imagery) which do not result in overt behavior (Jeannerod, 2001; Goldman, 2006).

Neurophysiology of *Action-Simulation:* non Human Primates

Mirror neurons, originally recorded in area *F5* of the monkey ventral premotor cortex (*PMv*), discharge both when the monkey executes a particular action and when it observes another individual (monkey or human) executing a similar action (e.g.,

grasping a piece of food or a geometric 3-D solid object). *Mirror* neurons, in order to be triggered by visual stimuli, require an interaction between a biological effector (e.g., hand or mouth) and an object (di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti and Craighero, 2004).

Mirror neurons have been also described in other cortical brain areas. In the rostral part of the inferior parietal lobule (area *PF* or *7b*) (Fogassi et al., 2005; Fogassi and Luppino, 2005; Rozzi et al., 2008) neurons have been recorded to respond mainly to sensory stimuli and some do have motor and/or visual properties, with a percentage of them displaying properties of *mirror* neurons (Fogassi and Luppino, 2005). Furthermore, a specific type of cells in the superior temporal sulcus (area *STS*) found to be triggered by the visual information of the *biological motion* (cell specificity/tuning in several types of *biological motion*) (Perrett et al., 1989; Jellema et al., 2000; Puce and Perrett, 2003), as well as during the observation of goal-directed hand movements (Puce and Perrett, 2003). However, the absence of motor properties does not allow them to be characterized as *mirror neurons* (Rizzolatti and Craighero, 2004).

Subsequent single neuron studies revealed the existence of neurons with "mirror properties" also in the cortical areas of the frontal lobe. For instance, Cisek and Kalaska (2004) found a population of neurons in the dorsal premotor cortex (*PMd),* exhibiting directional tuning in all three conditions: observation, instructed delay period (before any actual observed movement) and actual-execution of the same well-learned task. The execution task included forelimb reaching movements to a sequence of visible targets in a touch screen. Authors concluded that this activity might be a single-neuron correlate of mental rehearsal of the observed or anticipated action.

Tkach et al. (2007) recorded neurons from *MI* and *PMd* in the monkey brain, while the monkey was executing or observing its own action. The results showed that *MI* and *PMd* single-unit neuronal activity was similar, for passive observation of the familiar task and for its execution. These findings suggest that observation of a well-known behavioral task elicits motor commands in *MI* and *PMd*, similar to those elicited by its overt counterpart.

Recent imaging studies employing the ^{14}C-deoxyglucose method (2-DG) demonstrated that the areas activated in the brain of an actor performing a grasping movement and those of an observer watching the performance of the very same movement by another subject are virtually the same. This common neural network includes the forelimb representations of the primary motor and somatosensory cortices, several premotor and cingulate areas, extensive regions of the posterior, lateral, medial parietal, and intraparietal cortex, as well as occipital cortical areas (Raos et al., 2004; 2007; Evangeliou et al., 2009; Kilintari et al., 2010). These findings contradict the widely held notion that the two brain areas with *mirror* neurons, (areas *F5* and *PF),* are necessary and sufficient for the recognition of observed actions. Instead, these findings support the idea that we decode others' actions by activating our own action system. Moreover, the premotor activations were stronger for action observation, in contrast to the primary somatosensory–motor ones, which were stronger for action execution. Activations induced by observation were bilateral, whereas those induced by execution were contralateral to the moving forelimb. It has been suggested that these differences in intensity and lateralization of activations between the executive and the perceptual networks help attribute the action to the correct agent, i.e., to the "self", during action-execution, and to the "other", during action-observation. Accordingly, the "sense of agency" could be

articulated within the core components of the circuitry supporting both action-execution and action observation (Raos et al., 2004; 2007; Evangeliou et al., 2009; Savaki, 2010).

Neurophysiology of *Action-Simulation*: Humans

Brain imaging studies (fMRI, PET and MEG) in human subjects have also revealed that action-*simulation*, either in the case of action-observation or in the case of motor-imagery, activates an extensive brain network similar to that activated for action-generation. Observation of hand movements activated portions of the premotor cortex (Grafton et al., 1996; Rizzolatti et al., 1996; Decety et al., 1997; Iacoboni et al., 1999), and the parietal cortex (Grafton et al., 1996; Buccino et al., 2001). Moreover, action-observation in humans activated many other cortical brain regions: primary motor cortex (Hari et al., 1998), dorsal premotor cortex (Buccino et al., 2001; Gazzola and Keysers, 2009), somatosensory cortices (*SI*, *SII*) (Avikainen et al., 2002; Gazzola and Keysers, 2009; Keysers et al., 2010), superior parietal lobule, supplementary and cingulate motor areas (Gazzola and Keysers, 2009). In a recent meta-analysis Caspers et al. (2010), examining the results of 87 studies, concluded that action-observation activated consistently in both hemispheres an extensive network of fronto-parietal areas including dorsal and ventral premotor cortex, supplementary motor area, SI, superior parietal cortex, rostral inferior parietal lobule, intraparietal cortex, posterior middle temporal gyrus at the transition to visual area *V5* and fusiform face area/fusiform body area.

Similarly to action-observation, motor-imagery activated the neuronal substrate subserving execution. For instance, areas found to be implicated in motor-imagery include: *SI* (Decety et al., 1994; Grafton et al., 1996; Porro et al., 1996; Roth et al., 1996; Lotze et al., 1999), premotor cortex (Stephan et al., 1995; Grafton et al.,

1996; Gerardin et al., 2000; Naito et al., 2002), supplementary motor area, as well as, medial cingulate frontal regions (Gerardin et al., 2000; Naito et al., 2002).

Covert-Actions and Excitability of the Motor System

A non-invasive method for the investigation of the involvement of motor cortex in action-*simulation* is the transcranial magnetic stimulation (TMS) (Barker et al., 1985; Barker, 1999). TMS is produced by passing a very brief high-current pulse through an insulated coil of wire held over the scalp. The electric pulse induces a rapidly changing magnetic field with lines of flux running perpendicular to the coil. Since the skull has little impedance to the passage of the magnetic field, electric currents are induced in the brain that flow at right angles to the magnetic field. If current amplitude, duration, and direction are appropriate, they will depolarize cortical neurons and generate action potentials. Thus, the term "magnetic cortex stimulation" is somewhat misleading, since the magnetic field simply serves as a "vehicle" for carrying an electric stimulus across the scalp and skull into the cortex (Walsh and Cowey, 2000; Rafal, 2001; Siebner and Rothwell, 2003).

TMS has been extensively used to estimate the corticospinal excitability, in relation to both overt and covert actions (Walsh and Cowey, 2000; Siebner and Rothwell, 2003). TMS effects are quantified through electromyographic (EMG) responses and dynamic measurements of motor evoked potentials (MEPs).

Action-Simulation and Corticospinal Excitability

The first evidence that corticospinal excitability is modulated during mental-simulation of an action (e.g. mere action-observation), has been provided by Fadiga and colleagues (1995). Subjects had to observe either a grasping-execution task or a moving arm tracing geometrical figures in the air, both tasks performed by the

experimenter. In control conditions, subjects had to pay attention to either a 3-D object presentation or to a randomly appearing dimming light in a screen. TMS was delivered over the left motor cortex, just before the end of each stimulus, whereas MEPs recorded from four hand muscles extensor digitorum communis (EDC), first dorsal superficialis (FDS), first dorsal interosseus (FDI) and opponens pollicis (OP*)*. They demonstrated that MEPs recorded during grasping-observation were facilitated in a muscle-specific manner, same way as in the actual performance of the same action. This facilitation was not detected in control conditions.

The aforementioned findings have been replicated and extended by subsequent studies, providing evidence that motor excitability is significantly modified when the subject observes an action performed by another individual. Brighina and colleagues (2000) explored whether the observation of meaningless voluntary hand movements, performed by the experimenter, could activate the motor cortex. The results demonstrated that meaningless actions induced facilitatory MEPs in the observer's motor cortex. Yahagi and Kasai (1998) tested whether motor-imagery of flexion, abduction or extension of the right or left index-finger affects corticospinal excitability in a lateralized pattern. They demonstrated that MEP amplitudes significantly increased in both the left and the right FDI muscles during motor-imagery of flexion and abduction but not of extension. TMS of the left motor cortex induced more pronounced MEP amplitudes in the right FDI hand muscle, than in the left. They concluded that motor-imagery affects corticospinal excitability with specific enhancement of cortical responsiveness, as well as that the left hemisphere dominates in motor programming for right-handers. Similar results were provided by Fadiga et al. (1999) through a different motor-imagery task of proximal (arm) and distal (hand) actions. However, Clark et al. (2004) failed to identify any lateralization

influence on MEP responses during action-simulation. Additionally, Aziz-Zadeh and co-workers (2002) reported that during action-observation of either left or right hand movement, each hemisphere was more strongly activated when viewing actions conducted by the contralateral hand.

Strafella and Paus (2000) used a paired-pulse TMS protocol to examine changes in cortical excitability during action-observation. They stimulated the left *MI* during rest, observation of handwriting and observation of arm movements. MEPs were recorded from the FDI and biceps brachii (BB) muscles, and they observed corticospinal facilitation, due to cortical rather than to spinal interneuronal excitation. Gangitano et al. (2001) studied the effects of different phases of an observed movement on the modulation of corticospinal excitability. The main finding was that the observer's MEP amplitude was becoming larger with increasing finger aperture and smaller during the closure phase of the hand. This indicates that response facilitation is differentially tuned during the different phases of the observed action. Schutz-Bosbach et al. (2006) demonstrated that observed hand actions which were not attributed to oneself had an increased corticospinal facilitatory effect, whereas self-attributed observed actions tended to suppress the corticospinal excitability. However, Patuzzo et al. (2003) did not detect any difference in corticospinal excitability during attribution of action to the correct agent.

The effects of action-observation and motor-imagery on corticospinal excitability have been investigated. Clark et al. (2004) tested whether action-observation or mental rehearsal of symbolic gestures could induce differential modulation of corticospinal excitability. They found that both experimental conditions led to similar facilitatory MEP amplitudes.

The TMS induced MEPs do not provide information about the level of the corticospinal pathway at which the effects arise (Petersen et al., 2003). Controversy exists in the literature regarding the relative contributions of supraspinal and spinal changes to the observed increase in the MEP amplitudes. Next, we will review studies which tested the excitability changes during action-simulation, using H-reflex and/or F-wave techniques.

Spinal Modulation and Action-Simulation

The Hoffmann reflex (H-reflex) is an electrically induced reflex analogous to the mechanically induced stretch-reflex (Abbruzzese et al., 1996). Electric stimulation of the median or radial nerve of the hand elicits the H-reflex, which reflects the efficacy of synaptic transmission (monosynaptic activity), as the stimulus travels in afferent (Ia sensory) fibers through the α-spinal motoneurons of the corresponding muscle to the efferent (motor) fibers. Accordingly, action potentials generated by α-spinal MNs travel along efferent fibers, until they reach the neuromuscular junction and produce a twitch response in the EMG. This is the resulting H-wave of the H-reflex, as opposed to F-wave, which is caused by antidromic activation of the spinal MNs through strong electrical stimulation of the peripheral nerves.

Rossini and colleagues (1999) explored whether motor-imagery affects corticospinal excitability, as well as, whether it has the potential to change the facilitatory effects in distinct hand muscles. They employed a paradigm of motor mental rehearsal which activated discrete muscles. TMS and H-reflex excitability was tested. They found that motor-imagery of different forms of finger abduction induced somatotopically similar, but less intense, amplitudes of facilitatory MEPs, as compared to those induced by actual execution. The F-wave modulation indicated inrtaspinal excitability during motor-imagery. However, they suggested that

intraspinal modulation should be viewed under skepticism, due to the correlated temporal patterns indicating that cortico-cortical facilitatory influences are stronger than spino-spinal. Li and colleagues (2004) explored whether the motor-imagery effects on modulation of the corticospinal excitability could also be recorded at the segmental level. They found that motor imagery exerts significant effects on spinal segmental circuitry.

Baldissera and colleagues (2001) tested the H-reflex during an action-observation task. They demonstrated that mere observation of hand actions modulates the excitability of the observer's spinal circuitry, in a phase-specific manner, according to the kinematics of the observed actions. However, in comparison with cortico-cortical facilitatory MEPs, H-reflex excitability changes displayed a reverse pattern. Specifically, during action-observation, motor cortical facilitatory MEPs strictly mimic the observed movements (as in the case of actual performance), but H-reflex facilitatory amplitudes showed an opposite discharge order. Specifically, H-reflex evoked facilitatory effects during the motion-phase of hand opening (finger extension), whereas in the motion-phase of hand closing (finger flexion) the effects were depressed. They attributed this result to a spinal effect opposite to the corresponding cortical one, which "prevents the overt replica of the observed action". However, in subsequent studies, Baldissera and colleagues (Borroni et al., 2005; Montagna et al., 2005; Borroni and Baldissera, 2008; Borroni et al., 2008) undermined the abovementioned finding, because they found that the temporal dynamics between H-reflex excitability and TMS facilitatory MEPs were matched, during action-observation and motor-imagery. Therefore the reflex excitability changes were attributed to the motor cortex, instead of the spinal cord.

Abbruzzese et al. (1996) employed TMS over the left motor cortex, while the subjects imagined, on their own pace, movements performed with their left hand. The imagined movements were either a repetitive movement of thumb to index opposition or a predetermined sequence of thumb opposition to each finger. The recorded MEPs were significantly increased during imagery of sequential thumb-finger oppositions, as compared with the repetitive index-finger opposition task and control conditions. Nevertheless, H-reflex modulation found to be unaffected, suggesting that subthreshold modulation of spinal neurons did not take place in the motor-imagery task. However, authors recognized the possibility of involvement of the spinal interneurons in motor-imagery, which may escape the detectable elicitation of the H-reflex. Patuzzo et al. (2003) did not detect any statistically significant H-/F-wave responses in observed or imagined hand actions. Similarly, the absence of reflex modulation during motor-imagery has been reported by many other authors (Abbruzzese et al., 1996; Kasai et al., 1997; Yahagi and Kasai, 1998; Hashimoto and Rothwell, 1999).

In the following section, the cytoarchitectonic and neuroanatomical aspects of the spinal cord are overviewed, focusing on the forelimb/hand neural representation (cervical enlargement).

Organization of the Spinal Cord

The spinal cord is a long, cylindrical structure, invested by meanings. It lies in the vertebral canal and is composed of gray (GM) and white (WM) matter. Phylogenetically, it is the oldest neural structure (Kappers et al., 1936), constituting 2% of the CNS weight. Functionally, the spinal intrinsic and extrinsic circuitries integrate sensory and motor processes.

Segments

Spinal cord is segmented to regions, each receiving a pair of dorsal and ventral root filaments. The segments in primates are 8 cervical, 12 thoracic, 5 lumbar, 5 sacral and 1 coccygeal. Along the spinal cord there are two enlargements, the cervical enlargement extending between the 5th cervical (C5) and the 1st thoracic (T1) segments and the lumbar extending between the 2nd (L2) and the 6th lumbar (L6) segments. The cervical associated nerve roots innervate the upper extremities (forelimbs), whereas the lumbar nerve roots innervate the lower extremities (hindlimbs).

White Matter

The white matter WM is composed of longitudinal running axons and glial cells. The horns of the grey matter (GM, see below) divide both sides of the WM into three columns (funiculi): the dorsal, the lateral and the ventral. Each column includes a variety of bundles of ascending or descending axons. The dorsal columns of WM are composed by the central processes of dorsal root ganglion cells (large myelinated axons). They lie between the two dorsal horns of the GM and contain exclusively ascending axons that carry somatic sensory information (i.e., skin sensation and proprioception from the limbs and trunk) to the brain stem. The lateral columns include both ascending and descending axons, which originate from the brain stem and the neocortex, innervating spinal interneurons (INs) and motoneurons (MNs). In primates, the corticospinal fibers are found in the dorsal part of the lateral column. The ventral columns also include ascending and descending axons. The lateral and ventral columns contain a variety of descending (corticospinal, vestibulospinal, rubrospinal, tectospinal and reticulospinal) and ascending (spinothalamic,

spinocerebellar and spinotectal) fiber groups. The descending motor axons control axial/proximal and distal muscles. The ascending somatosensory axons in the lateral and ventral columns constitute parallel pathways, conveying sensory information to higher levels of the CNS.

Gray Matter

The spinal GM is morphologically heterogeneous. It consists of neuronal cell bodies, dendrites, axons, and glial cells. Macroscopically, the GM can be divided into dorsal and ventral horns. The sensory relay-neurons, which receive information from the periphery, are concentrated in the dorsal horn. The motoneurons (MNs) are located in the ventral horn. The central region, which connects the dorsal and ventral horns, is called the intermediate zone of the GM and contains various types of interneurons (INs). Microscopically, the GM is organized in successive layers of cells. A cross section of the spinal cord, at the cervical level, shows that the GM is arranged in the form of a butterfly or the capital letter H (depending on the level). The cross bar of the H is termed commissural GM and encloses the central canal, which contains the cerebrospinal fluid (Carpenter, 1991; Brodal, 2010).

From dorsal to ventral horns, successive layers of cells comprise the 10 laminae of Rexed. The laminae were first described by Rexed in the cat (1952; 1954). Since then, this arrangement has been used as a reference of cytoarchitectonic boundaries in many species. As regards the rhesus monkey, Apkarian and Hodge (1989a; 1989b; 1989c) have identified the spinothalamic cells based on the cytoarchitectonic organization provided by Rexed, and by the use of spinal tract lesions and horseradish peroxidase (HRP) labeling. Till now, a detailed laminar identification has been completed in the mouse (Sidman et al., 1971), the rat

(Molander et al., 1984; Molander et al., 1989) and the human species (Schoenen, 1982b, 1982a).

The 10 laminae of Rexed are demarcated on the basis of their distinct dendritic architecture, chemoarchitecture, patterns of connections, and function. The first nine laminae are arranged from dorsal to ventral, while the tenth comprise the circle of cells surrounding the central canal. Laminae I-IV belong to the dorsal horn, which is mainly related to sensory relays. Laminae V-VIII, located among dorsal and ventral horns, constitute the intermediate zone, wherein spinal INs of various types are located. The intermediate zone is responsible for motor integration receiving input (i) from spinal afferents such as groups Ia and Ib and (ii) from the long descending motor tract systems, and sending output to spinal MNs. Along with INs, MNs are exclusively found in the most ventral part of the ventral horn, the lamina IX (Kuypers and Brinkman, 1970).

On the basis of chemoarchitecture, spinal MNs are cholinergic, but other excitatory substances, such as *dopamine* have also been identified locally (Heise and Kayaloglou, 2009). As regards the spinal INs, two predominant inhibitory neurotransmitters have been identified in all laminae, GABA (γ-aminobutyric acid) and glycine. On the other hand, the excitatory interneuronal effects are mediated mainly via dopaminergic and glutaminergic synaptic neurotransmission. In general, diverse neurotransmitters (acetylcholine, monoamines, amino-acids) and neuroactive peptides, scattered amongst spinal laminae, contribute cooperatively to the excitatory and inhibitory effects of spinal INs upon MNs (Carpenter, 1991; Heise and Kayaloglou, 2009).

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Spinal Motoneurons and Interneurons: Organization & Classification

Several types of spinal neurons are clustered into discrete cellular groups, which are connected to form functional systems (Amaral, 2000). Spinal MNs, which carry neural commands towards muscles and glands, are organized in discrete longitudinal ventral horn groups, the motor nuclei or the spinal MN pools. Spinal MNs are directly responsible for the generation of force by the muscle they innervate. By definition, each motor nucleus innervates a single skeletal muscle, although a degree of overlap is present. The motor nuclei extend throughout the length of the spinal cord, over one to four spinal segments (Sprague, 1948; Romanes, 1964). Under a simplified view, three columnar groups of motor nuclei may be recognized: the medial, the central and the lateral, each of which is further subdivided into dorsal and ventral ones. The muscles of the limbs are innervated by the lateral columns. Within the cervical enlargement, lateral column axons innervate the forelimb muscles; with the hindlimbs represented in the lumbrosacral enlargement (Reed, 1940; Loeb, 2000; Floeter, 2003).

Each spinal MN receives thousands of synapses from different sources: spinal INs, afferents from peripheral sensory organs (dorsal root ganglia) and descending axons from the brain stem and the cerebral cortex (ventral root ganglia). However, most of the synapses on MNs originate from the spinal INs (Floeter, 2003). MNs transform patterns of interneuronal activity into commands for skeletal muscle contraction and relaxation.

There are three types of MNs, the α (alpha), β (beta), and γ (gamma) ones (Leksell, 1945). The majority of somatic MNs are α-MNs, which are large multipolar cells, amongst the largest in diameter in the nervous system, more enlarged at the

cervical and lumbar spinal segments than at the thoracic. The αMNs innervate fibers of striated muscles, called extrafusal, responsible for the generation of posture and movement forces via tension by contraction. A smaller in proportion and diameter class of MNs are the γ somatic ones, which innervate the muscle spindles of the intrafusal muscle fibers (Boyd, 1962). The α- and γ-MNs are intermingled in the motor nuclei innervating the same muscles (Eccles, 1967; Willis et al., 1969; Bryan et al., 1972). The β-MNs are fewer and innervate, predominantly, distal muscles of the limbs by sending axon branches, to both intrafusal and extrafusal muscles (McHanwell, 2009). In contrast to α-MNs, the axons of β- and γ-MNs have slower conduction velocity and higher electrical threshold (Eccles et al., 1960; Brodal, 2010).In lamina IX, spinal INs and visceral MNs are distributed among the somatic MNs.

INs comprise the vast majority of spinal neurons. Within the intermediate zone they function as signal integrators. They receive many convergent and divergent inputs from multiple sources, both descending (supraspinal-motor) and ascending (sensory). On the output side, most interneuronal axons are branching and connecting with other spinal INs (local) and MNs. Overall, spinal INs, exert excitatory and inhibitory modulatory effects on MNs, activating skeletal muscles in a coordinated manner (Baldissera, 1981).

A more simplified approach for interneuronal classification is according to the location of their cell bodies, within the spinal segments: i) segmental/local, that project only to the same spinal segment, and ii) propriospinal/projection, whose axons reach distant spinal segments (Baldissera, 1981). Propriospinal fibers, lying very close to the GM, connect one spinal segment with another. The largest propriospinal pathway connects the cervical and lubrosarcal enlargements and coordinates limb

movements (Krakauer, 2000; Floeter, 2003). Even so, this dichotomous division doesn't seem accurate enough. Intracellular staining has shown that segmental spinal INs may extent their axons outside the GM and then may re-enter the GM at different segments (Shinoda et al., 2006).

The spatial organization of the different motor nuclei follows a proximal-distal rule. According to this rule, motor nuclei innervating the proximal muscles lie medially within the spinal cord, while those innervating distal muscles are located progressively more laterally. Thus, for the arm, the motor nuclei innervating the axial, shoulder girdle, wrist, and digit muscles are arrayed from medial to lateral positions-columns. The separation of motor neurons innervating axial and proximal muscles from those innervating distal muscles is maintained throughout the spinal cord (Ghez, 2000) (Figure1-1).

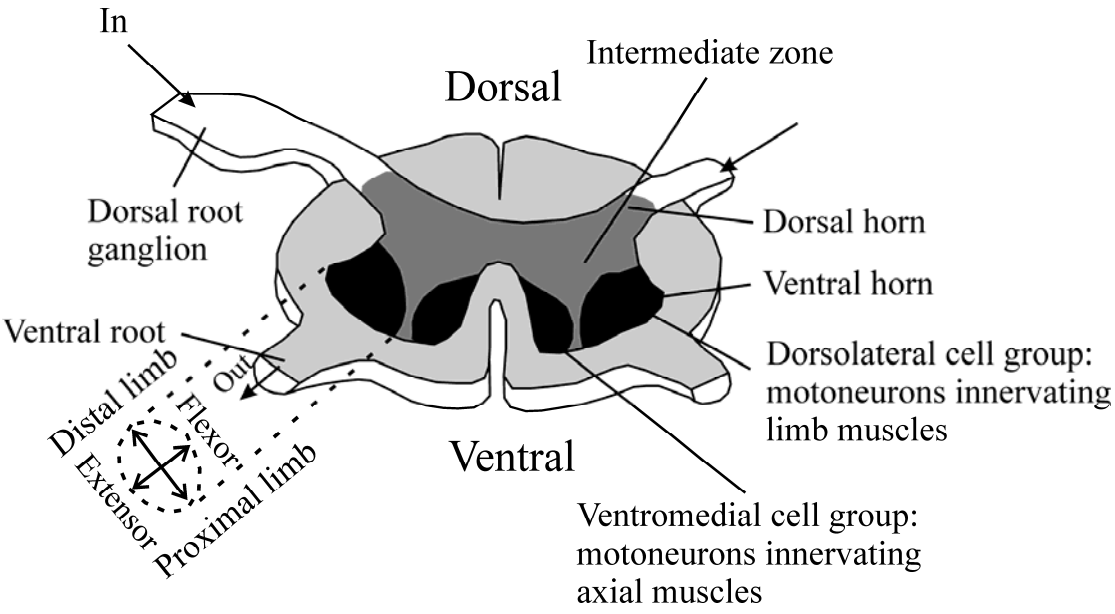


Figure 1-1: Arrangement of the motor nuclei of the cervical spinal cord. The medial nuclei contain the motor neurons innervating axial muscles of the neck and back. Among the lateral nuclei the most medial motor neurons innervate proximal muscles while the most lateral innervate distal muscles. (Modified from Kandel et al., 1995).

In the lateral motor column, cervical forelimb/hand motor-nuclei can be divided in a rostral and a caudal group, according to the muscles they innervate. The rostral group concerns spinal segments C4 to C6, including deltoid, supraspinatus, infraspinatus, and BB muscles. The caudal group extents from C7 to T1, including forearm flexors and extensors, triceps, pectoralis, and the intrinsic muscles of the hand (Sprague, 1948; Romanes, 1964). Therefore, upper segments primarily control proximal and axial musculature including shoulder and neck muscles, while MNs innervating hand/distal muscles are located predominantly in the lower cervical segments (Rapoport, 1978; Richmond et al., 1978; Karim and Nah, 1981; Kuypers, 1981; Jenny and Inukai, 1983; Augustine and White, 1986; Ueyama et al., 1990; Moritz et al., 2007). However, deviations on spinal musculotopic organization have been observed. For instance, overlaps have been reported in the lower (caudally) cervical segments including MNs that innervate more proximal muscles like triceps and pectoralis, (Jenny and Inukai, 1983).

Descending Motor Systems from the Brain to the Cervical-Spinal Cord

Many different systems, across numerous brain areas need to work together to ensure proper motor control. The cerebellum and the basal ganglia provide feedback that regulates brainstem and cortical motor areas (Ghez, 2000). Supraspinal information that arrives at defined spinal levels undergoes further processing (Figure 1-2) (Bizzi et al., 2000).

In primates and other vertebrates, the main descending pathways from the brain to the spinal cord can be defined according to their origin. The cerebral cortex modulates the action of MNs in the brain stem and the spinal cord. Two main systems originating from the brainstem have been described, the medial and the lateral one (Kuypers and Schadi, 1964; Kuypers, 1981) (Figure 1-3).

The medial pathways are phylogenetically old and consist of three major tracts, (i) the vestibulospinal (medial and lateral), (ii) the reticulospinal (medial and lateral) and (iii) the tectospinal one. These pathways descend in the ipsilateral ventral columns of the spinal cord and terminate predominantly on INs and long propriospinal neurons in the ventromedial part of the intermediate zone, influencing MNs that innervate axial and proximal muscles. The medial pathways provide the basic postural control system upon which the cortical motor areas can organize more differentiated movements (Figure 1-3).

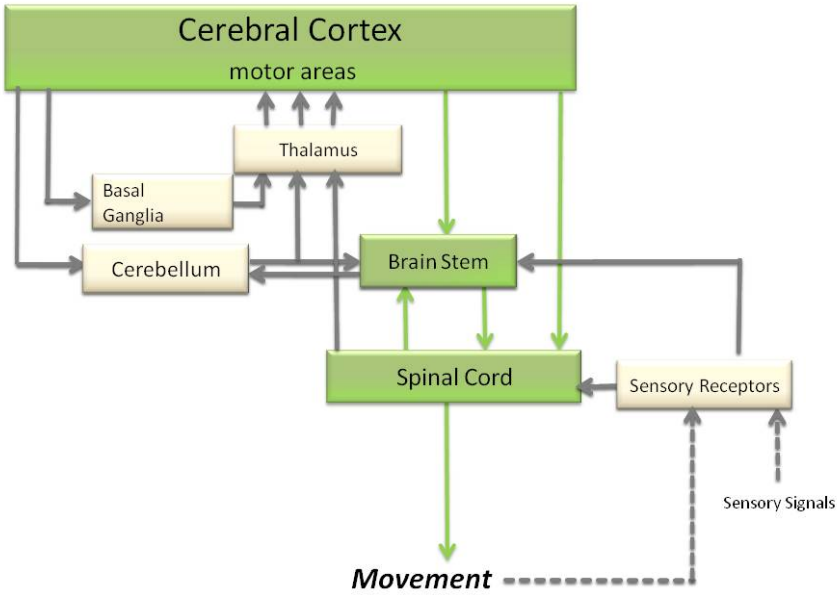


Figure 1-2: Organization of the motor system. Motor systems are organized hierarchically and in parallel. In contrast to the evolutionary older motor system that projects via the circuits of the brainstem, the corticospinal system projects directly to the spinal cord. Thus cortex direct controls the spinal MNs (α-MNs), activating appropriate muscles involved in skillful and fine voluntary movements. The corticospinal axons which are crossing at the level of the brainstem descend to the contralateral spinal ventral horn. A major source of the descending corticospinal tract is the MI. Sensory ascending signals from the basal ganglia and the cerebellum, via the thalamus, modulate the descending corticospinal inputs. (Modified from Kandel et al., 1995).

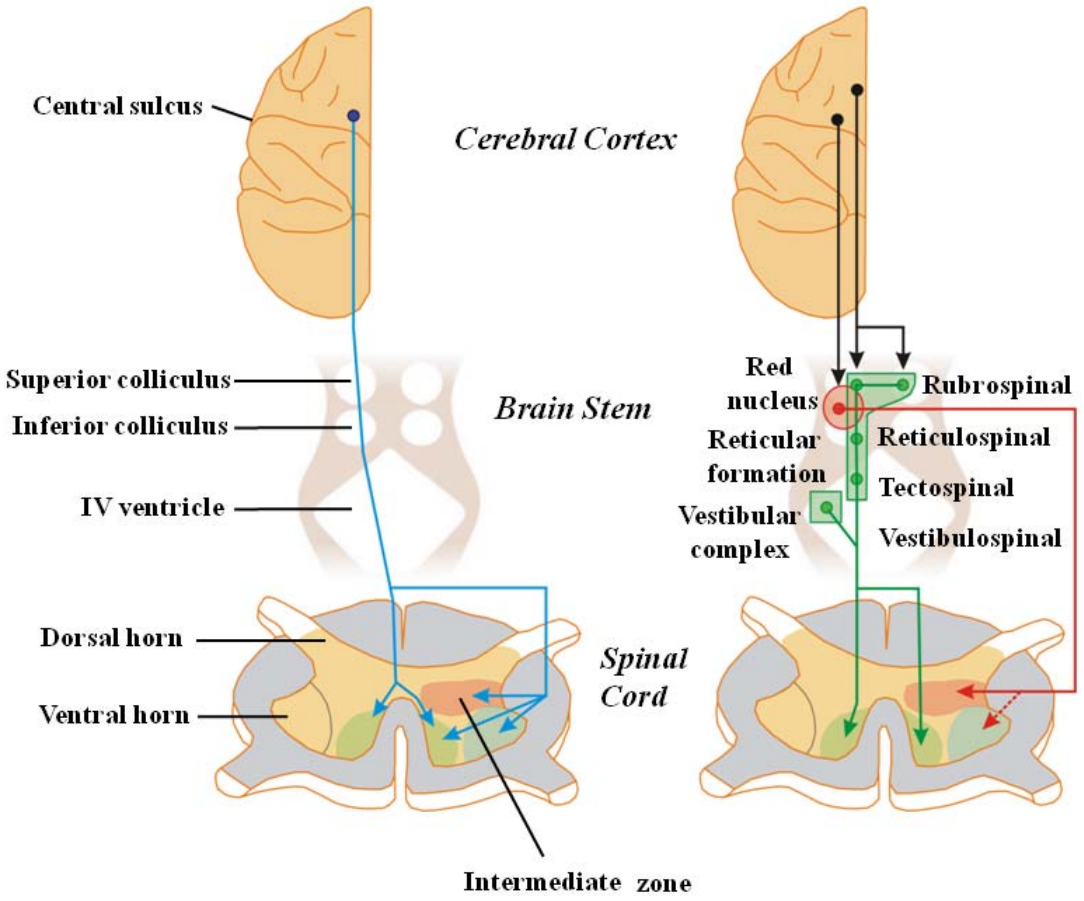


Figure 1-3: Schematic representation of the distribution of the corticospinal and cortico-brainstem fibers, according to Kuypers (1981). On the left, the corticospinal tract, terminating sites withinin the spinal cord is color coded blue. On the right, the **medial** descending pathway consisting of the reticulospinal, the tectospinal and the vestibulospinal descending tracts, which arise from the reticular formation, superior culliculus and vestibular complex, respectively, are in green. These fibers terminate bilaterally in the ventromedial part of the intermediate zone (the green area). Fibers in red, arise from the red nucleus and comprise the **lateral** descending pathway, which terminates in the contralateral dorsolateral region of the intermediate zone (red area in section). Some of these fibers (dashed red arrow) terminate to the lateral group of motor nuclei (light blue area,). Line in black corresponds to cortical projections (black arrows) to the midbrain. (Modified from Lemon, 2008).

The lateral pathways provide terminals to INs in the dorsolateral part of the spinal GM, influencing MNs that control distal muscles of the limbs. The rubrospinal tract comprise the main lateral descending pathway, which originates in the magnocellular portion of the red nucleus in the midbrain. Rubrospinal fibers descend

through the medulla to the dorsal part of the lateral column of the spinal cord. In primates the rubrospinal tract is important for the control of distal limb muscles (Figure 1-3) (Ghez, 2000).

The Corticospinal Tract

Cortical motor fibers descend in two tracts, the corticobulbar and the corticospinal one (Ghez, 2000). The corticobulbar tract terminates on facial muscles, whereas corticospinal fibers terminate in spinal MNs that innervate limb and trunk muscles. In addition, cortical motor areas indirectly influence the spinal motor activity, by acting on the descending brainstem pathways (Figure 1-3).

The corticospinal tract originates from several cortical areas, as revealed by retrogradely transported tracer injections (WGA-HRP) in the cervical segments of the spinal cord and by lesion studies. The cortical distribution of efferent termination to the spinal cord provides a clear indication of its potential influences on spinal mechanisms. In the monkey, multiple areas of the frontal lobe project to the cervical spinal cord, e;g; areas *MI/FI*, *PMd/F2*, *PMv/F5* and *F4* (Martino and Strick, 1987; Dum and Strick, 1991; He et al., 1993), areas *SMA/F3* and cingulate motor areas (*CMAv*, *CMAd* and *CMAr*) (Dum and Strick, 1991; He et al., 1995; Dum and Strick, 1996), areas of the parietal lobe: *SI*, posterior parietal cortex, and parietal operculum (SII), (Lemon and Griffiths, 2005).

The corticospinal fibers sequentially pass through the internal capsule, the cerebral penduncle, the longitudinal fibers of the pons and the medullary pyramid, to reach the caudal end of the brainstem, where most of them cross to the opposite side in the pyramidal decussation (Watson and Harvey, 2009). In the forebrain, mesencephalon and rombencephalon, the corticospinal fibers descend in parallel with

corticopontine and corticobulbar tracts (Kuypers, 1981). In primates and other mammals (carnivores and rodents), the crossed fibers of the corticospinal tract are found mostly in the dorsolateral part of the lateral column, (Petras, 1968; Nudo and Masterton, 1990). Corticospinal projections, also, reach all levels of the spinal cord- cervical, thoracic, lumbar and sacral- innervating all regions of the spinal GM. Furthermore, corticospinal targets overlap with those originating from the brainstem, terminating bilaterally within the ventromedial intermediate zone and contralaterally within the dorsolateral intermediate zone.

Early studies (Tower, 1940; Lawrence and Kuypers, 1968b, 1968a) have demonstrated that complete bilateral lesions of the medullary pyramid in the macaque monkey, keeping intact the direct cortical projection to the lower brain stem, produced a permanent deficit in skilled hand control, whereas other motor functions, such as walking or climbing, recovered rapidly. However, partial lesions of the medullary pyramid, did not induce permanent deficits, due to subsequent plastic changes (Kucera and Wiesendanger, 1985; Aoki et al., 1986; Raineteau and Schwab, 2001).

According to comparative studies (Heffner and Masterton, 1975; Nudo and Masterton, 1988; Bortoff and Strick, 1993), the corticospinal tract has been evolved to serve manual-digital dexterity, as in phylogenetically older mammals is less developed (Verhaart, 1962; Watson and Harvey, 2009). Specifically, direct projections from MI to the spinal MNs, have been correlated with the generation of relatively independent finger movements and precision grips (Lawrence and Kuypers, 1968b; Heffner and Masterton, 1975; Kuypers, 1981; Bortoff and Strick, 1993). The significance of the evolution of the corticospinal tract among species is based both, on direct corticospinal connections and indirect oligosynaptic pathways (Lemon and Griffiths, 2005).

The corticospinal cells may project also directly to dorsal laminae of the spinal cord, contributing in the selection of the somatosensory afferent signals. However, the majority of corticospinal terminals are not distributed directly on spinal MNs spinal nuclei. They reach widely spinal INs in the intermediate zone (Kuypers, 1981; Ralston and Ralston, 1985; Bortoff and Strick, 1993).

Each cortical motor area contains at least one representation of the contralateral face and limbs. The MI-corticospinal neural terminals, as well as, SMA and CMAd, target predominantly the intermediate zone (laminae V-VIII). The MI displays more intense projections to the lamina VI, and dense terminations among the lateral motor nuclei supplying the muscles of the arm, hand and digits (Kuypers, 1981; Armand et al., 1997). The MI-corticospinal projections are much more dense than those from any other cortical-frontal area (Dum and Strick, 1996; Maier et al., 2002).

Moreover, lamina IX terminal buttons, deriving from area MI and the motor areas of the medial wall, indicate monosynaptic cortico-motoneuronal connections. Thus medial wall areas act upon forelimb motoneurons directly, independent of the MI predominant monosynaptic output, (Liu and Chambers, 1964; Kuypers and Brinkman, 1970; Catsman-Berrevoets and Kuypers, 1976; Cheema et al., 1984; Ralston and Ralston, 1985; Dum and Strick, 1991; Bortoff and Strick, 1993; He et al., 1993; 1995; Dum and Strick, 1996). Area PMd sends more corticospinal terminals than PMv (He et al., 1993). Two frontal motor areas, F6 and F7, are virtually devoid of corticospinal neurons, while their main descending projections terminate in the brainstem (Keizer and Kuypers, 1989).

Furthermore, the organization of the corticospinal projections is correlated with the cortico-cortical connections among the motor areas. In particular, areas F6

and F7 do not send corticospinal projections and do not project to the MI. On the contrary, areas F6 and F7 are connected with motor areas rostrally to MI, i.e., F2, F3, F4, and F5 (Barbas and Pandya, 1987; Luppino et al., 1993).

Aim of the Study

Recent findings in the primate cerebral cortex demonstrate that several somatosensory-motor and association cortical areas are activated for both action-execution and action-observation (Raos et al., 2004 ; 2007; Evangeliou et al., 2009; Kilintari et al., 2010). These findings indicate that action-perception and actual performance share an extensive overlapping neuronal substratum.

If the activation of the motor system during observation of an action does not result in overt movements, the question is where in the motor system the execution is blocked? It has been proposed that the inhibitory mechanism of an observed action may be localized downstream to motor cortex, possibly at the level of the spinal cord (Jeannerod, 2004; Jeannerod, 2006). Moreover there is a debate in the literature, regarding the contribution of the spinal neural circuits in the corticospinal excitability.

The aim of the present study was to examine whether and how the spinal cord is implicated, during action observation. To this end, we analyzed the cervical spinal cord of the *rhesus* monkey, employing the quantitative 2-DG imaging technique. Monkeys were trained to either execute grasping actions or observe the same action performed by the experimenter. Our study is the first one, providing direct evidence for the actual involvement of the spinal cord in action-observation.

METHODS

Subjects

Experiments were performed on 8 adult female *Rhesus* monkeys (*Macaca mulatta*) weighting between 4 and 5 Kg. All animals were purpose-bred by authorized suppliers within the European Union (Deutches Primatenzentum and R.C. Hartelust BV). Housing, surgical and experimental procedures were approved by institutional (FO.R.T.H.) animal use ethical committee, in accordance with European Union (directive 86/609 and its amendments) and national regulations.

Monkeys were accommodated in their cages (Crist Instrument Co, Hagerstown, MD, USA) and were allowed to adapt to their new environment for a month before any intervention. During this period, they had free access to food (Mucedola, Milan, Italy) and water. Appropriate handling (music stimulation and baby toys presentation) and monitoring for normal behavioral and eating habits were provided by the laboratory personnel. Thereafter, monkeys underwent a surgical implantation of a stainless steel bolt on the skull (Crist Instrument Co, Hagertsown, MD, USA) for head restraint purposes. Bolt anchored with the use of dental cement (Resivy, Vence, France) and mandibular plates, secured on the bone by titanium screws (Synthes, Bettlach, Switzerland).

Surgical procedures were performed under aseptic conditions and general anesthesia (ketamine hydrochloride, Imalgene 1000, Merial, France, 20mg/kg, i.m and sodium pentobarbital 25mg/kg, i.m). Systemic antibiotics (Rocephin, Roche, Switzerland, 60-70 mg/kg/day i.m) and analgesics (Apotel, Uni-Pharma, Hellas), to minimize pain or discomfort, were administered pre- and post-operatively. After the

recovery period (at least three weeks) monkeys started to participate in training sessions.

Experimental Set-Up and Tasks

During the training session, monkeys were seated in a primate chair (Crist Instrument Co, Hagerstown, MD, USA), with their head fixed, hindlimbs immobilized and one (execution monkeys) or both forelimbs (observation and biological-motion monkeys) restrained with *velcro* tapes. The behavioral apparatus was placed in front of the monkeys, at shoulder height (Figure 2-1), at a distance of 50 or 25cm depending on whether the experimenter or the monkey had to perform the reaching-to-grasp movement. An electronically monitored circular shutter at the front side of the behavioral apparatus provided access to an object. The object had to be grasped by either the monkey or the experimenter. Eye position was recorded with an infrared oculometer (Dr Bouis devices, Karlsruhe, Germany). Electromyograms (EMGs) were recorded from the biceps and wrist extensor muscles (gain X 2000, bandpass filter 0.3-3000 kHz), using Ag-AgCl surface electrodes. EMG records were aligned at the end of the movement, rectified and averaged over 150 movements in each case. Monkeys were trained using operant conditioning. Successful completion of each trial was rewarded with water, which was delivered through a tube placed close to the animal's mouth. Training lasted from 6 to 11 months, depending on the demands of each task. Monkeys were trained 5 days/week for at least an hour per day. Training was completed when monkeys employed their tasks with a success rate higher than 90%. Behavioral apparatus, eye position, data acquisition and reward were controlled by a computer running custom developed software.

The Three Behavioral Tasks

Grasping-Execution (E)

E monkeys were trained to reach and grasp an object with their left forelimb, whereas the right one was restricted (Figure 2-1). One of the monkeys performed the task under visual guidance and the other two in complete darkness. In the first case the monkey was required to fixate the illuminated object for 0.7-1 s, until a dimming of the light would signal reaching, grasping and pulling a ring with the left forelimb using the hook grip (insertion of the index finger into the ring with pronated hand) while maintaining fixation. In the second case, a low-frequency auditory cue (90 Hz), delivered from a speaker placed 25cm in front of the monkey in the median sagittal plane below the behavioral apparatus, instructed the monkey to look straight ahead toward the memorized location of the object for 0.7-1 s, until a high frequency auditory cue (180 Hz) signaled the generation of the learned action while maintaining its gaze straight ahead. The movement was usually completed within 500-600 ms. The E monkeys were allowed to move their eyes outside a circular window (8^o diameter) only during the intertrial intervals (ranging between 2 and 2.5 s).

Grasping-Observation (O)

Three grasping-observation monkeys (O) were trained to maintain their gaze within the 8^o diameter circular window while observing the experimenter grasping the ring with the hook grip (Figure 2-1). The experimenter was standing on the right side of the monkey and performed the reaching-to-grasp movements with the right hand. The monkey could see the hand approaching the object, the preshaping of the hand, the interaction of the hand with the object and subsequently the object grasping and holding. Observation task parameters were similar to the ones described for the

execution task. During the training and the experiments both hands of the observing monkeys were restricted. These monkeys were initially trained to grasp under visual guidance and then to observe the grasping movements of the experimenter. To avoid possible influence of this earlier grasping-training on the observation effects, one of these monkeys was trained to grasp with its left hand, the second one with its right hand and the third one with both hands consecutively. This way, any side-to side difference due to the earlier grasping-training would be cancelled out when the average quantitative map of the three left sides was compared with the average quantitative map of the three right sides of the spinal cord.

Arm-Motion (Cm)

Two arm-motion monkeys (Cm) were used as the control group (Figure 2-1). The Cm monkeys were trained to maintain their gaze straight ahead (within the 8^{o} diameter circular window), while (i) the shutter of the behavioural apparatus opened and the object appeared and (ii) the shutter closed and the experimenter approached towards it with the hand extended. Accordingly, the task of the Cm monkeys contained neither the hand preshaping nor its interaction with the object. During the training and the experiments both hands of the Cm monkeys were restricted. Intertrial intervals ranged between 2 and 2.5 s.

The [^{14}C] Deoxyglucose Technique

Energy metabolism (EM) within the CNS reflects its working load. Consequently, EM corresponds to neuronal synaptic activity. In the adult mammals CNS demands of energy are among the greatest of all body tissues, as directly reflected in its relatively enormous rate of oxygen consumption (~20% of the resting total body oxygen consumption in adults). Oxygen is normally utilized in the neural

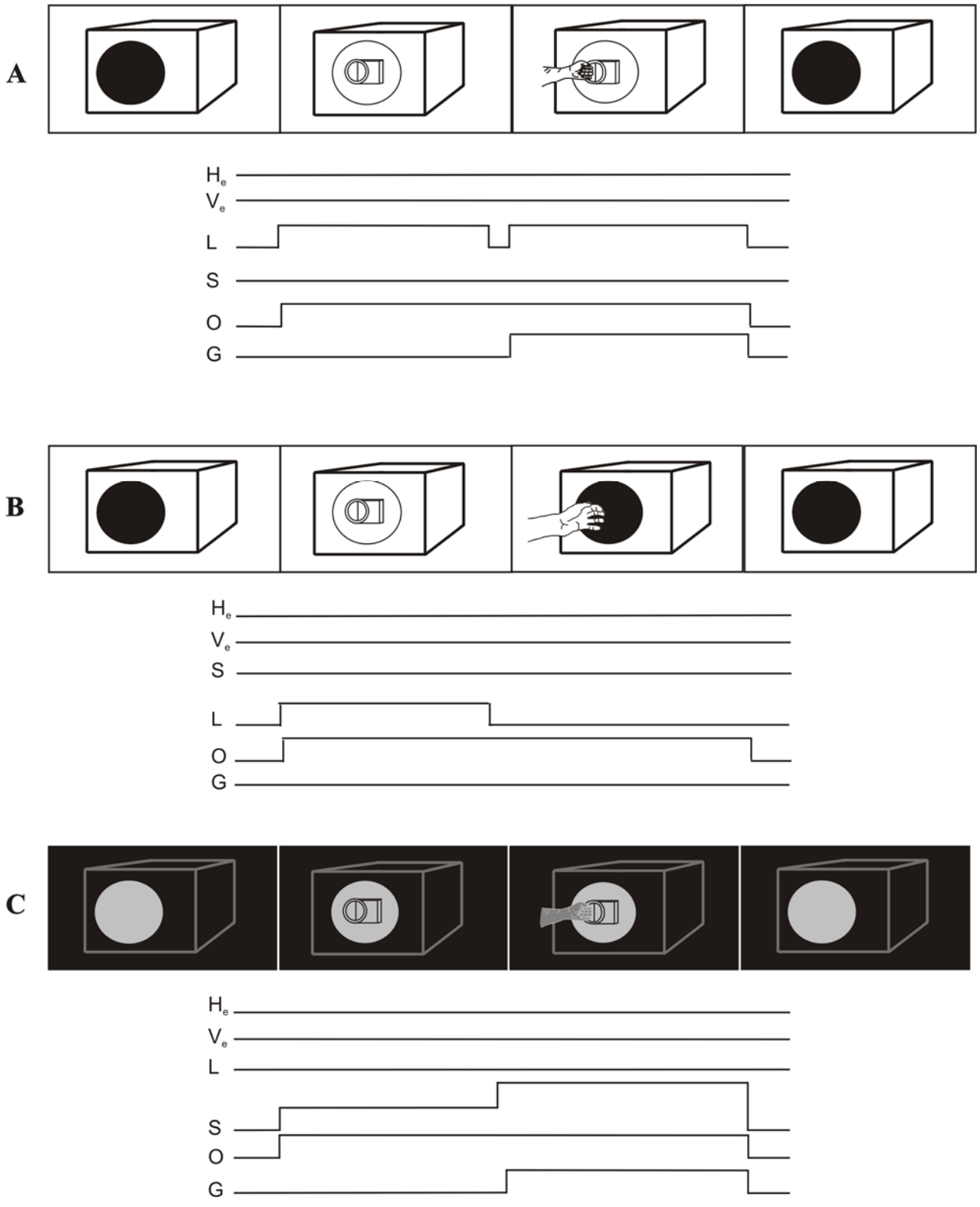


Figure 2-1: Drawings of the behavioral paradigms and the task events (line graphs). (A) grasping-execution in the light; (B) arm-motion control task; (C) grasping execution in the dark. *He,Ve:* horizontal and vertical component of eye position, respectively; *L*: illumination of object; *S*: auditory cues; *O*: object presentation; *G:* grasping action.

tissue, almost entirely for the oxidation of carbohydrates. Normally, the main metabolic source of the CNS is the monosaccharide glucose, which is taken up from the arterial blood (Sokoloff, 1977).

In 1977, Louis Sokoloff and co-workers developed the 2-DG method to measure the local rates of energy metabolism simultaneously in all brain structures of awake animals, based on the fact that functionally active neurons use glucose as their basic source of energy in normal condition (Sokoloff et al., 1977). The 2-DG differs from glucose only in the replacement of the hydroxyl group (HO) of the second carbon atom, by a hydrogen atom (H) (Figure 2-2). It is transported bidirectionally between the blood and the CNS tissue, by the same saturable carrier that transports glucose. Thus, the 2-DG competes with glucose for its phosphorylation by hexokinase.

The hexokinase phosphorylates 2-DG and glucose to 2-DG-6-phosphate (2-DG-6-P) and glucose-6-phosphate (G-6-P), respectively (Sols and Crane, 1954; Wree et al., 1988). G-6-P is further metabolized to F-6-P (fructose-6-phosphate) and eventually to carbon dioxide (CO_2) and water (H_2O). However, further metabolism of 2-DG-6-P is negligible and it accumulates in the neural tissue (Sokoloff et al., 1977; Nelson et al., 1984; Nelson et al., 1986). The use of either oxygen or glucose, as radioactive tracers, is not suitable for autoradiography,

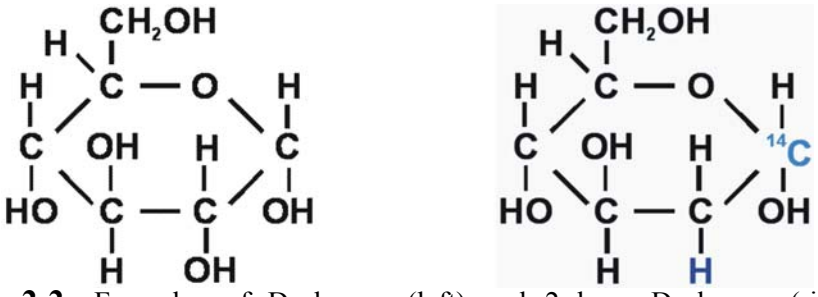


Figure 2-2: Formulae of D-glucose (left) and 2-deoxy-D-glucose (right): ^{14}C marks radioactive isotope in the 2-deoxyglucose, while H (in blue) replaces OH.

metabolic products are volatile with short physical half life; whereas the labeled products of glucose are lost rapidly from the neural tissues (Sokoloff et al., 1977).

Theoretical Model of 2-DG

The theoretical basis of the 2-DG method is as follows (Figure 2-3). Given that the interval of time following the administration of the 2-DG is kept short (45min), then the amount of the formed DG-6-P remains practically unaltered. Therefore, the estimation of the quantity of DG-6-P accumulated in the neural tissue at any given time, after the introduction of 2-DG into the circulation, is equal to the integral of the rate of the 2-DG phosphorylation by hexokinase in that tissue, during that interval of time. Also, the rate of 2-DG phosphorylation is related to the amount of glucose that has been phosphorylated, over the same interval of time, depending on the time courses of the relative concentrations of 2-DG and glucose in the precursor pool and the *Michaelis-Menten* kinetic constants for hexokinase (for both 2-DG and glucose). When glucose in the CNS is in a steady-state, the amount of phosphorylated glucose, during a given interval of time equals the steady state flux of glucose through the hexokinase-catalyzed step times the duration of the interval. The net rate of flux of glucose through this step equals the rate of glucose utilization (see model in Figure 2-3). In the operational equation of the method the numerator represents the amount of radioactive product formed in any given interval of time (Figure 2-4). This is equal to Ci*, which is the combined concentrations of 2-DG and 2DG-6-P in the tissue at time, T, (measured by quantitative autoradiography) minus a term that represents the free (non-metabolized) 2-DG still remaining in the tissue. The fraction denominator represents the integrated specific activity (the ratio of labeled to total molecules) in the precursor pool as measured in plasma, corrected for the lag in the equilibration of the tissue precursor pool with the plasma, times a factor that corrects for kinetic

differences between the labeled and natural compound (isotope effect) (see figures 2-3 & 2-4) (Sokoloff et al., 1977; Sokoloff et al., 1989). The rate constants (k^*_1, k^*_2 and k^*_3), as well as, the lumped constant ($\lambda V^*_m k_m/ \Phi$, V_m, k^*_m) are not measured in each experiment; the values for these constants are species specific and have been determined separately for different animals (rat (Sokoloff et al., 1977), monkey (Kennedy et al., 1978)).

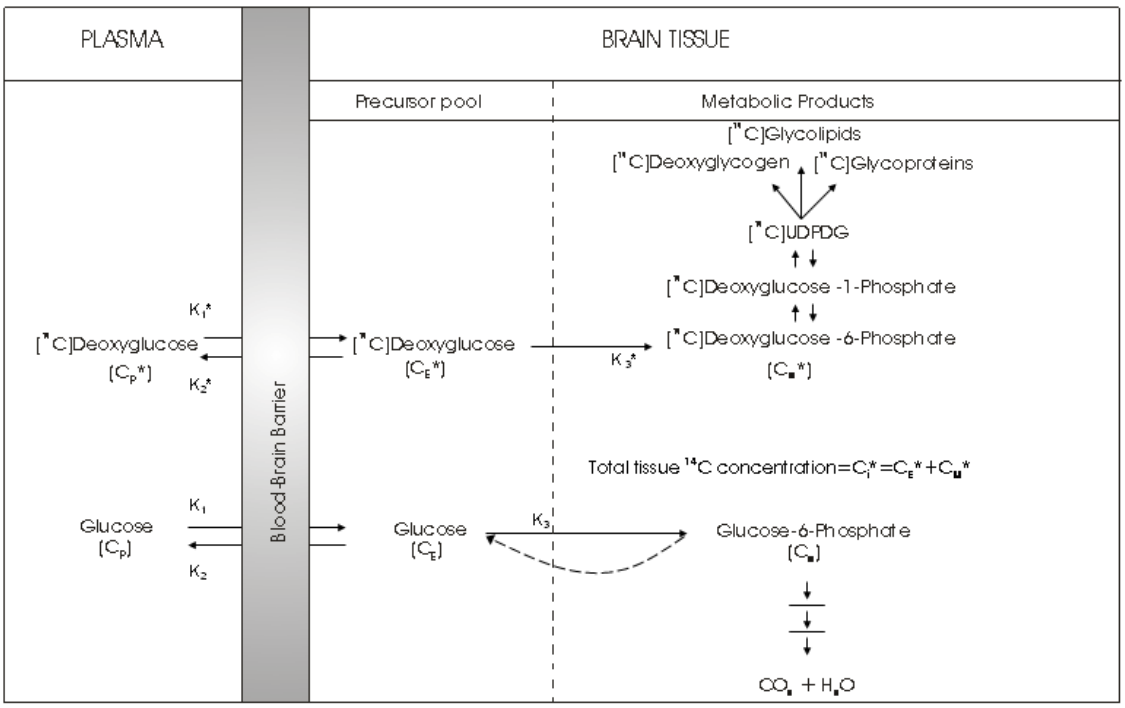


Figure 2-3: Diagrammatic representation of the theoretical basis of [^{14}C]-deoxyglucose method for measurement of local cerebral glucose utilization (Sokoloff et al, 1977). C_i^* represents the total 2DG concentration in a single homogeneous tissue of the brain. C_P^* and C_P represent the concentrations of 2DG and glucose in the arterial plasma respectively; C_E^* and C_E represent their respective concentrations in the tissue pools that serve as substrates for hexokinase. C_M^* represents the concentration of 2DG-6-phosphate and C_M the concentration of glucose-6-phosphate in the tissue. The constants K_1^*, K_2^* and K_3^* represent the rate constants for carrier-mediated transport of 2DG from plasma to tissue, for carrier-mediated transport back from tissue to plasma and for phosphorylation by hexokinase respectively; K_1, K_2 and K_3 are the equivalent glucose rate constants. The dashed arrow represents the glucose-6-phosphate hydrolysis by glucose-6-phosphatase activity, which is low.

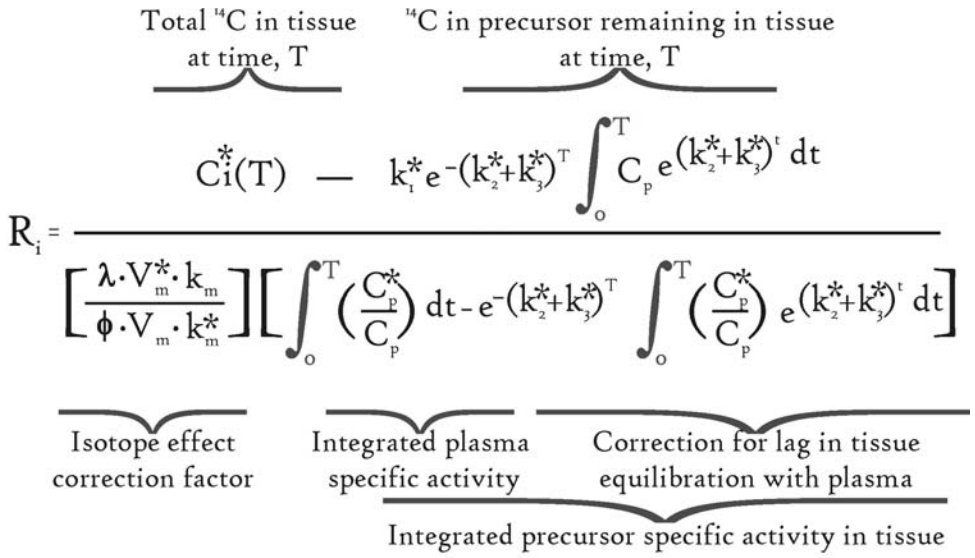
^{14}C-deoxyglucose Experimental Procedures

On the experimental day, monkeys were catheterized through femoral vein and artery under general anesthesia (ketamine hydrochloride, 20mg/kg, im.). The catheters were filled with diluted heparin solution (100 U/ml) before insertion. Both catheters were 45 cm long, to minimize extensive flushing of dead space during sampling. The animals were allowed to recover from anesthesia for 4-5 hours before the beginning of the experiment.

The experiment was initiated by the intravenous injection of 2-DG as a pulse of 100 μCi/kg (specific activity 55 mCi/ml, ARC, St. Louis, MO, USA). Because the 2-DG is supplied in ethanol solution, it was evaporated to dryness and then re-dissolved in 1ml of saline. To monitor the plasma, glucose and 2DG concentrations, arterial blood samples were collected in heparinized tubes during predetermined time intervals: at time zero (start of infusion), after 15, 30, 45 s, and after 1, 2, 3, 5, 7.5, 10, 15, 25, 35 and 45 min. Care was taken to clear the dead space of the arterial catheter prior to the collection of each sample to avoid contamination. The samples were immediately centrifuged in a high speed Beckman centrifuge and kept on ice for analysis. After the collection of the last sample, the animal was killed by an intravenous infusion of thiopental (10 mg/ml) followed by a saturated potassium chloride (KCl) solution to stop the heart. Plasma glucose levels, blood pressure, hematocrit and blood gases ranged within normal values in all monkeys and remained constant throughout the duration of the experiment.

The concentration of deoxyglucose was calculated by the arterial plasma ^{14}C content. Twenty μl of plasma and 3 ml of scintillation liquid (Insta-Gel, Packard co., Illinois, USA) were introduced in a counting vial and assayed in a liquid scintillation counter (Beckmann Coulerton Inc., Foullerton, CA, USA). The efficiency (Ef) of the

counting was estimated by internal standardization (calibrated [^{14}C]-toluene). The obtained counts (cpm) were transformed into disintegrations per minute (dpm) according to the equation dpm=cpm/Ef. The plasma glucose concentration was assayed in a dry glucose analyzer (Spotchem, Menarini, Italy) to establish the required steady state for plasma glucose levels.



$$R_i = \frac{C_i^*(T) - k_1^* e^{-(k_2^*+k_3^*)T} \int_0^T C_p e^{(k_2^*+k_3^*)t}\,dt}{\left[\frac{\lambda \cdot V_m^* \cdot k_m}{\phi \cdot V_m \cdot k_m^*}\right]\left[\int_0^T \left(\frac{C_p^*}{C_p}\right) dt - e^{-(k_2^*+k_3^*)T} \int_0^T \left(\frac{C_p^*}{C_p}\right) e^{(k_2^*+k_3^*)t}\,dt\right]}$$

Figure 2-4: Operational equation of the radioactive deoxyglucose method. (From Sokoloff et al., 1977).

Explanation of symbols:

R_i : rate of glucose utilization per unit mass of tissue.

Ci*: total ^{14}C concentration in a single homogenous tissue of the brain.

Cp*, **Cp**: concentrations of [^{14}]deoxyglucose and glucose in the arterial plasma, respectively.

Ce*, **Ce**: [^{14}]deoxyglucose and glucose concentrations in the arterial plasma, respectively.

Cm*: concentration of [^{14}]deoxyglucose-6-phosphate in the tissue.

k_1*, **k_2***, **k_3***: rate constants for carrier-mediated transport of [^{14}] deoxyglucose from plasma to tissue (**k_1***), for carrier mediated transport back from tissue to plasma (**k_2***), and for phosphorylation by hexokinase (**k_3***).

k_1, **k_2**, **k_3**: equivalent rate constants for glucose.

T: time of termination of the experimental period.

o: fraction of glucose which, once phosphorylated, continues down the glycolytic pathway.

λ: ratio of the distribution space of deoxyglucose in the tissue to that of glucose.

φ: the fraction of glucose that, once phosphorylated, continues down the glycolytic pathway.

k_m*, **V_m***, **k_m**, **V_m**: Michaelis-Menten kinetic constants of hexokinase for deoxyglucose (**k_m***, **V_m***) and glucose (**k_m**, **V_m**), respectively.

Immediately after the end of the experiment the cerebral hemispheres, the cerebellum and the spinal cord were removed, frozen by immersion in isopentane maintained at -45^{o}C (± 5^{o}C) with dry ice, for at least 15 min, to ensure full and even freezing of the tissue mass. The frozen tissue was covered with embedding medium (Lipshaw Manufacturing Co, Detroit, MI, USA). Tissues were stored at -80^{o}C, until sectioning for autoradiography. Serial 20 μm thick horizontal sections were cut in a cryostat at -20^{o}C.

Sections were thaw-mounted on coverslips and immediately transferred on a hot plate (maintained at 60^{o}C) for drying, to prevent diffusion of the label. Coverslips were glued on cardboards and exposed, together with precalibrated ^{14}C–standards (Amersham plc, Little Chalfont, Buckinghamshire, UK) on X-ray medical films (Kodak EMC1 or Biomax MR) in X-ray cassettes for 3-14 days, depending on the time sensitivity of the films and the measured plasma glucose levels. Films were developed in a Kodak-X-OMAT 1000 automatic processor.

Developed autoradiographic X-ray films depict the relative ^{14}C concentrations, within the autoradiographic tissue sections, so that the darker the region the higher the rate of glucose utilization. Quantitative densitometric analysis (DA) of the areas of interest in autoradiograms was performed with a computerized image-processing system (MCID, Imaging Research, Ontario Canada). A calibration curve of the relationship between optical density and tissue ^{14}C concentration for each film was obtained by measurements of the optical density corresponding to all different precalibrated standards of the same film. The resulting calibration curve, together with the optical densities of the spinal grey matter, determined the LCGU, according

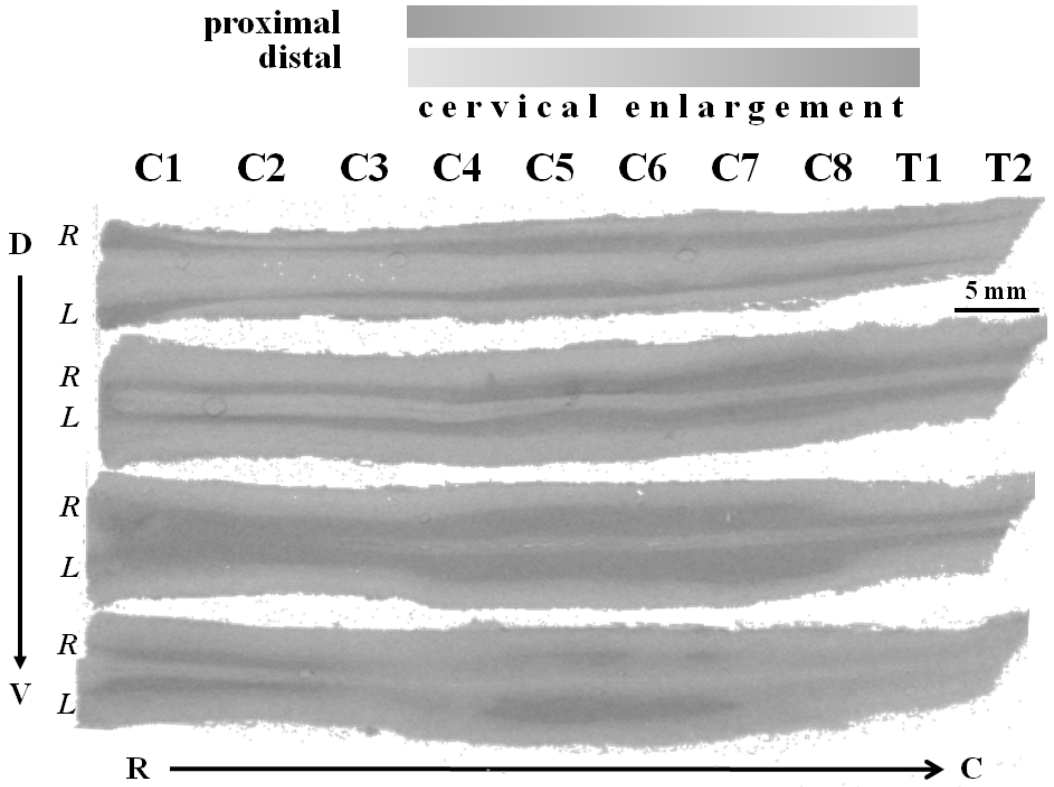


Figure 2-5: Autoradiograms of 4 horizontal sections at different dorsoventral levels. Each section extends from the first cervical to the first thoracic segments. C, caudal; D, dorsal; R, rostral; V, ventral; *R,* right; *L,* left. C1-C8, T1, T2: cervical and thoracic spinal segments. The grey bars at the top of the figure denote the distribution of the spinal motoneurons which control the forelimb muscles.

to the operational equation of the method (Sokoloff et al., 1977). The densitometric data obtained from the autoradiograms of the spinal tissue were further processed to construct 2-dimensional maps (2-D) of metabolic activity.

Data Analysis, Reconstruction of Quantitative Spinal Maps and their Geometrical Normalization

2-D maps of the spatio-intensive pattern of metabolic activity, within the rostrocaudal (from the first cervical to the first thoracic segments) and the dorsoventral (from the dorsal to the ventral horn) extent of the grey matter were generated separately for the left and the right side of the spinal cord (Figures 2-5 & 2-

6). Each section contributed a data array (sampling resolution 50 μm/pixel) which was aligned with the arrays obtained from adjacent sections, the total of ~200 serial sections of 20 μm thickness for each spinal cord. In the illustrated average 2D-maps, the spatial resolution in both the rostro-caudal and dorso-ventral dimensions is 100 μm. The alignment of the data arrays was based on marks made on the WM during the dissection of the spinal cords at the locations of the dorsal roots, thus demarcating the spinal segments. The size of the spinal cord varied from animal to animal. To compensate for this variability, individual glucograms were geometrically normalized along with the spinal demarcation points, thus allowing for the direct comparison of the different maps. For this reason, averages of the lengths and heights of the spinal segments were separately estimated from all spinal cords to construct a reference map. The glucogram of each side of each spinal cord was then fit to this reference map using linear transformations of the plane (Moschovakis et al., 2001) with the help of Transform (Fortner Software LLC, Sterling, VA) and custom-designed routines in the Matlab environment (Mathworks, Natick, MA). With this procedure, we created geometrically normalized maps containing a standard number of pixels. Data from different geometrically normalized maps were combined to obtain the average-LCGU maps we illustrate here. To generate average maps, the LCGU value found in a certain pixel in one of the geometrically normalized maps was added to the value found in the pixel occupying the same position in one or more other similar maps and the result was divided by the number of maps used.

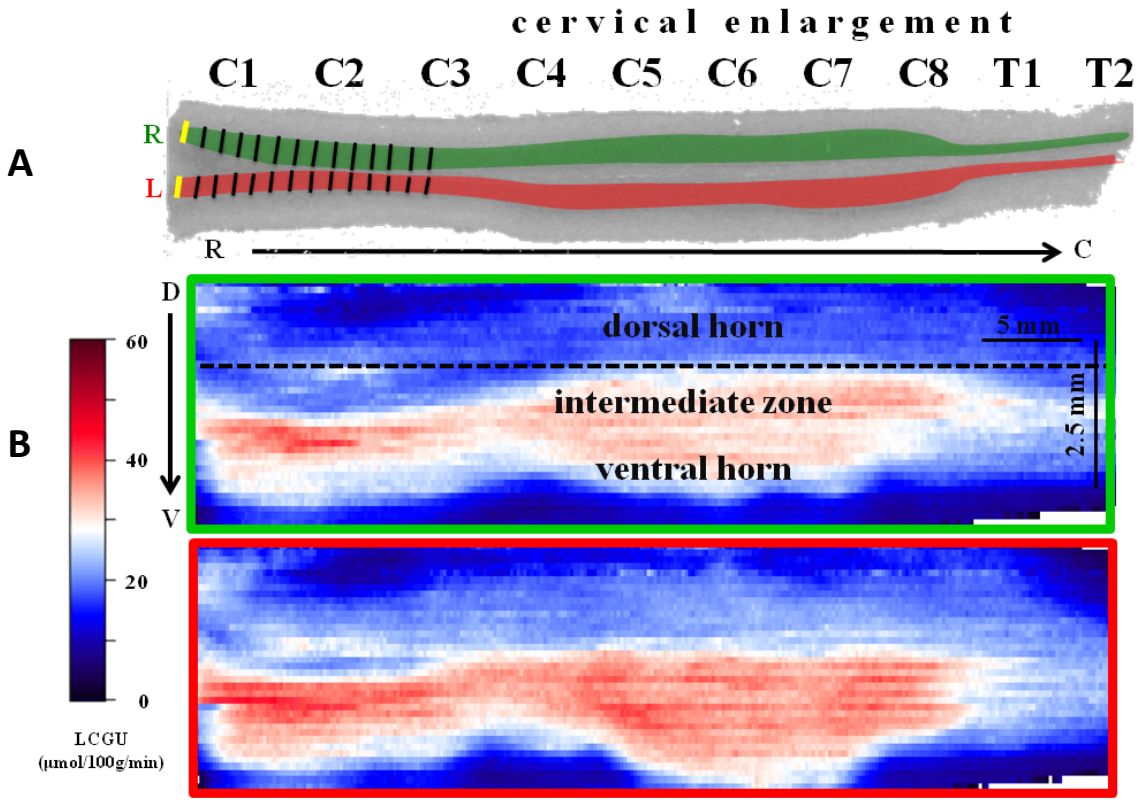


Figure 2-6: Reconstruction of the 2-D map of metabolic activity. **A)** Autoradiogram of a horizontal section of the spinal cord. GM of the left (L) and the right (R) side of the spinal cord is marked in red and green respectively. Thin black lines represent LCGU measurements made every 50µm, along lines running through the mediolateral extent of the GM of each side, covering the rostrocaudal extent from C1-T2, to obtain a data array. Data arrays obtained from 5 consecutive sections were aligned at the rostralmost edge of the GM of each side (represented by the black conjoint rectangles), and averaged to produce a line at the 2-D map of activity shown in (B). **B)** The metabolic map of the R (framed by the green rectangle) and L (framed by the red rectangle) side of the spinal cord of an E monkey. The upper half in each LCGU map corresponds to the dorsal horns, whereas the lower half corresponds to the ventral horns. Metabolic activity in the ventral horn ipsilateral to the moving forelimb (L) is elevated. Color bar represents the LCGU (in µmol/100g/min). Other conventions as in figure 2-5.

Statistical Analysis

Normalization of LCGU values was based on the averaged unaffected area of the spinal gray matter value pooled across all monkeys (Raos et al., 2004; 2007; Evangeliou et al., 2009). To normalize metabolic activity, LCGU values were

multiplied with a factor that was separately determined for each spinal cord. This factor is equal to the ratio of the mean LCGU value found in an unaffected spinal grey matter region of the spinal cord in question over the mean LCGU value obtained from the same region after pooling all spinal cords. Percent LCGU differences between the affected left (ipsilateral to the moving hand) and the control right (contralateral to the moving hand) side of the spinal cord of the E monkeys were calculated as follows: (Eleft-Eright)/Eright*100. Percent LCGU differences between the O and the Cm monkeys were calculated as follows: (O-Cm)/Cm*100. Because side-to-side differences in normal monkeys rise up to 7% (Kennedy et al., 1978), only differences higher than 7% were considered for statistical treatment. To determine statistical significant differences (Table 1, bold values), we relied on Student's unpaired *t* test (Raos et al., 2004; 2007; Evangeliou et al., 2009).

The baseline glucose consumption values found here are in accordance to those reported by other studies. According to Kennedy et al. (1978) the glucose consumption in the cervical spinal gray matter of conscious control monkeys is 21±2 μmoles/100g/min. Similarly, Schwartzman et al. (1983) have demonstrated LCGU values ranging from 20.1±1.1 to 23.5±0.4 μmoles/100g/min for the intermediate and the ventral laminae of the cervical spinal cord.

RESULTS

On the day of the ^{14}C-DG experiment, all monkeys executed their tasks for the entire experimental period (45 min). Success rate remained roughly the same (>90%) throughout the experiment. Since the concentration of ^{14}C-DG in the blood decays with a time constant equal to about 4 min, only 15% of the tracer is available after the 10 first min of the experiment, and therefore the monkeys' behavior after this initial period influences LCGU values insignificantly (Reivich et al., 1977). For this reason, we provide quantitative description of the behavior of the monkeys during the critical period of the first 10 min. The oculomotor performance of the monkeys, as three-dimensional histograms of the dwell time of the line of sight as a function of eye position, during the critical 10 first min of the ^{14}C-DG experiment is presented (Figure 3-1). Because the rate of responses may influence the LCGU values, the mean rate of movements for the execution, the observation and the arm-motion tasks was set to be similar.

The three E monkeys executed an average of 11 arm-movements per min and kept their eyes within the window of the behavioural apparatus for 7 min during the critical 10 first min of the ^{14}C-DG experiment. We generated glucograms of the E monkeys by averaging the three geometrically normalized quantitative spinal maps of metabolic activity in each side of the spinal cord, separately. When the average spinal-map of the left side (figure 3-2b, E left) is compared with the corresponding map of the right side (figure 3-2c, E right), increased metabolic activity is apparent in the intermediate zone and the ventral horn (lower half of the map) of the left side, ipsilateral to the moving hand, as expected (see also Table-1). EMG activity recorded from the biceps and wrist extensor was increased in the left forelimb executing the

movements, whereas it was at baseline levels in the right non-performing forelimb (Figure 3-3).

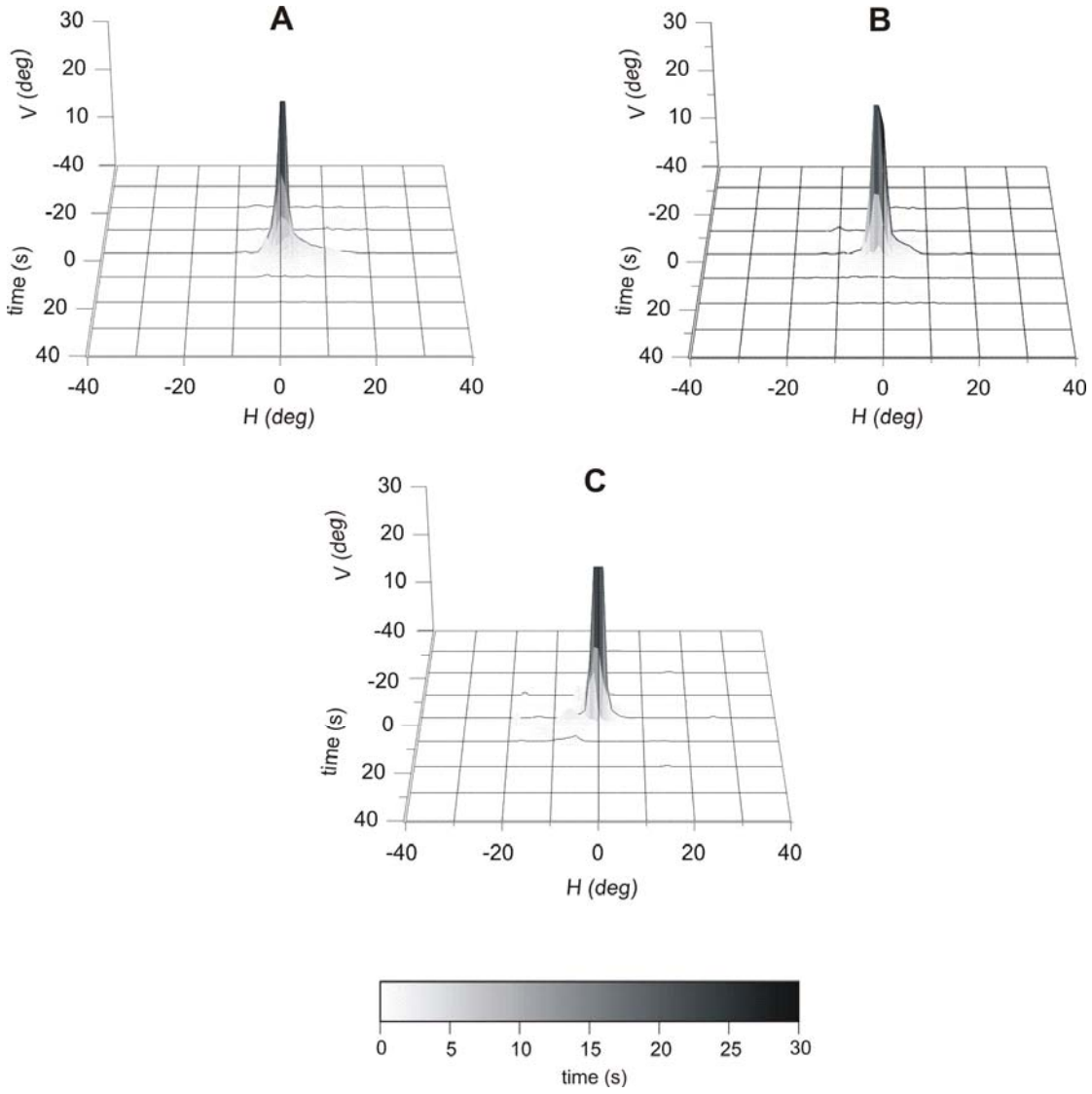


Figure 3-1: Three-dimensional histograms of the dwell time of the line of sight as a function of eye position during the critical ten first min of the 2-DG experiment. **A**: averaged oculomotor behavior from grasping-execution monkeys. **B**: averaged oculomotor behaviour from grasping-observation monkeys. **C**: averaged oculomotor behaviour from the motion-control monkeys. Horizontal axis (H; x) and vertical axis (V; y) in degrees, z-axis in seconds. Gray-scale bar indicates time in seconds.

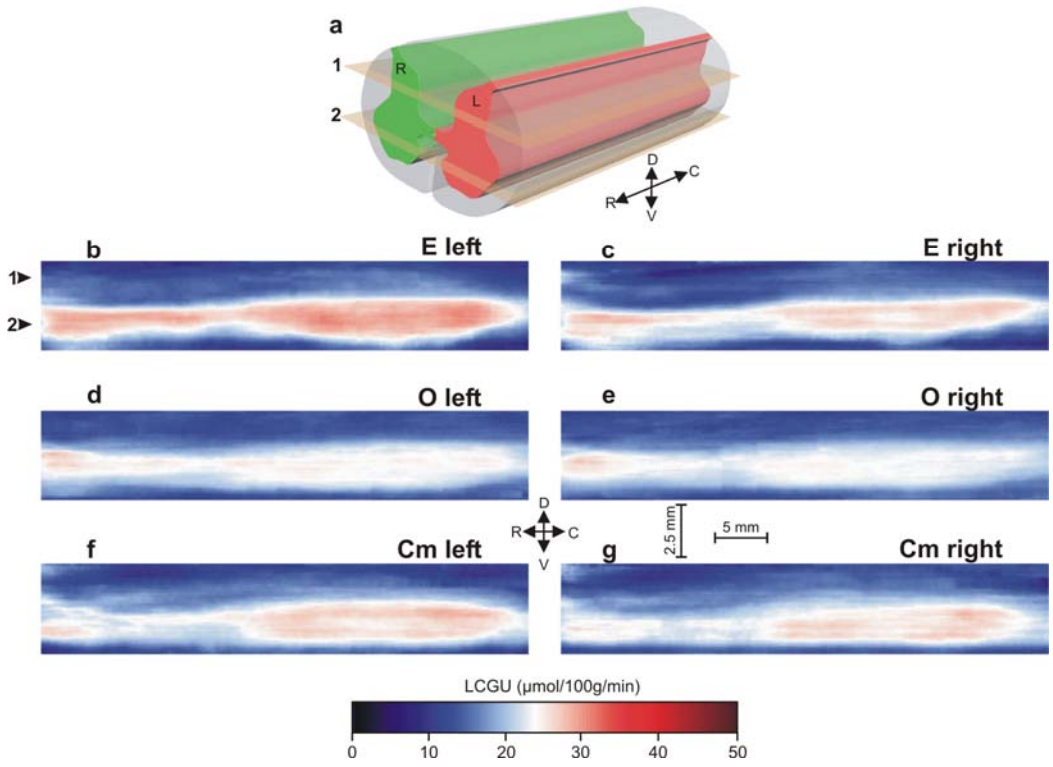


Figure 3-2: Metabolic effects induced by execution and observation of grasping movements in the cervical and first thoracic segments of the spinal cord. Each colour-coded map represents the average of the geometrically normalized, quantitative maps (glucograms) in each side of the spinal cord. a: Three dimensional schematic representation of the spinal cord (anterolateral view). The spinal grey matter in the right and the left side of the spinal cord is illustrated by the green- and the red-painted volumes, respectively. Horizontal planes 1 and 2 correspond to two different dorsoventral levels of sectioning, traversing the spinal cord through the dorsal and ventral horns, respectively. b, c: Average spinal-maps in the left (b, E left) and right (c, E right) sides of the three monkeys executing grasping movements with their left forelimb (E). Arrows 1, 2 indicate the dorsoventral levels of the corresponding planes in panel a. A pronounced activation in the intermediate zone and the ventral horn is apparent in the side ipsilateral to the grasping forelimb (E left), as compared with the contralateral side (E right); d, e: Average spinal-maps in the left (d, O left) and the right (e, O right) sides of the three monkeys observing grasping movements executed with the right hand of the experimenter (O). A significant suppression in the intermediate zone and the ventral horn in both O left and O right is apparent, as compared with the arm-motion control monkeys (Cm), and with the control side of the executing monkeys (E right); f, g: Average spinal- maps in the left (f, Cm left) and right (g, Cm right) sides of the two Cm monkeys. Colour-bar represents the LCGU values in µmol/100g/min. C, caudal; D, dorsal; R, rostral: V, ventral. (From Stamos et al., 2010).

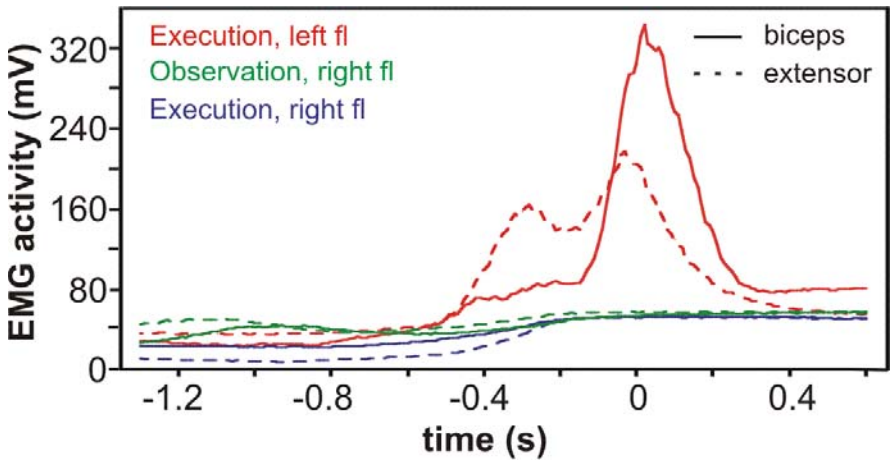


Figure 3-3: Averaged rectified elecrtomyographic records from the biceps (continuous lines) and wrist extensor (interrupted lines). The electromyographic activity recorded from the muscles of the performing (left) and non-performing (right) forelimbs, during action execution, is represented by the red and blue lines, respectively. The EMG activity from the right forelimb, during action observation is represented by the green lines. The records are aligned on the onset of movement. (Copied from Raos et al., 2004).

The three O monkeys observed an average of 12 arm-movements per min and fixated within the window of the behavioural apparatus for 7 min during the critical 10 first min of the ^{14}C-DG experiment. When the average spinal-map of the left side (Figure 3-2d, O left) is compared with the average spinal-map of the right side (Figure 3-2e, O right), no side-to-side difference is apparent (see also Table-1). Both sides of the O monkeys (Figure 3-2d, e) display decreased metabolic activity in the lower cervical segment when compared, not only with the map of the activated left side (Figure 3-2b), but also with the map of the control right side of the E monkeys (Figure-3-2c). EMG activity in the biceps and wrist extensor was unaffected in both forelimbs of the monkeys observing grasping movements, same way as in the non-performing forelimb of the monkeys executing grasping movements (Figure-3-3).

intermediate zone & ventral horn rostrocaudal extent	**Cml** LCGU ±SD	**Cmr** LCGU ±SD	**Cm** LCGU	**El** LCGU ±SD	**Er** LCGU ±SD	**Ol** LCGU ±SD	**Or** LCGU ±SD	**O** LCGU	**Er/El** (%)	**O/Cm** (%)
1st quarter	23.0±1	23.5±1	23.3	26.5±2	24.5±2	24.0±1	23.5±1	23.8	**8**	2
2nd quarter	23.5±1	23.5±1	23.5	26.0±2	24.0±1	23.5±1	23.0±1	23.3	**8**	-1
3rd quarter	25.0±1	25.0±1	25.0	27.5±2	25.0±1	23.5±1	23.5±1	23.5	**10**	-6
4th quarter	25.5±2	25.0±1	25.3	27.5±2	25.0±1	23.5±1	23.0±1	23.3	**10**	**-8**

Table-1: Metabolic effects in the spinal cord of the monkey brain. Cml and Cmr values represent the average local cerebral glucose utilization (LCGU) values (in µmol/100g/min) from the two left and the two right sides of the spinal grey of the motion-control monkeys, respectively. Cm is the average of the Cml and Cmr. El and Er values represent the average LCGU values from the three left and the three right sides of the spinal grey of the grasping-execution monkeys, respectively. Ol and Or values represent the average LCGU values from the three left and the three right sides of the spinal grey of the grasping-observation monkeys, respectively. O is the average of Ol and Or. SD, standard deviation of the mean. El/Er, O/Cm, percent differences between El and Er, as well as O and Cm, respectively, calculated as (experimental-control)/control*100. Values in bold indicate statistically significant differences by the Student's unpaired t test at the level of $P < 0.001$. (From Stamos et al., 2010).

The Cm monkeys were used to exclude potential effects induced by the observation of the objects, the eye movements while scanning these objects and the biological movement of the experimenter's arm. The difference between the O and the Cm monkeys is that, unlike the former, the Cm monkeys observe neither the preshaping of the approaching hand nor the interaction of the hand with the object. The two Cm monkeys observed an average of 12 movements per min and maintained their gaze within the window of the behavioural apparatus for 7 min during the critical 10 first min of the ^{14}C-DG experiment. When the average spinal-map of the left side (Figure 3-2 f, Cm left) is compared with the average spinal-map of the right side (Figure 3-2 g, Cm right), no side-to-side difference is apparent (see also Table-1). The

metabolic activity in both sides of the Cm monkeys (Figure 3-2 f, g) is similar to that in the control right side of the E monkeys (Figure 3-2 c) and higher than that in the spinal-maps of the O monkeys (Figure 3-2 d, e; Table-1).

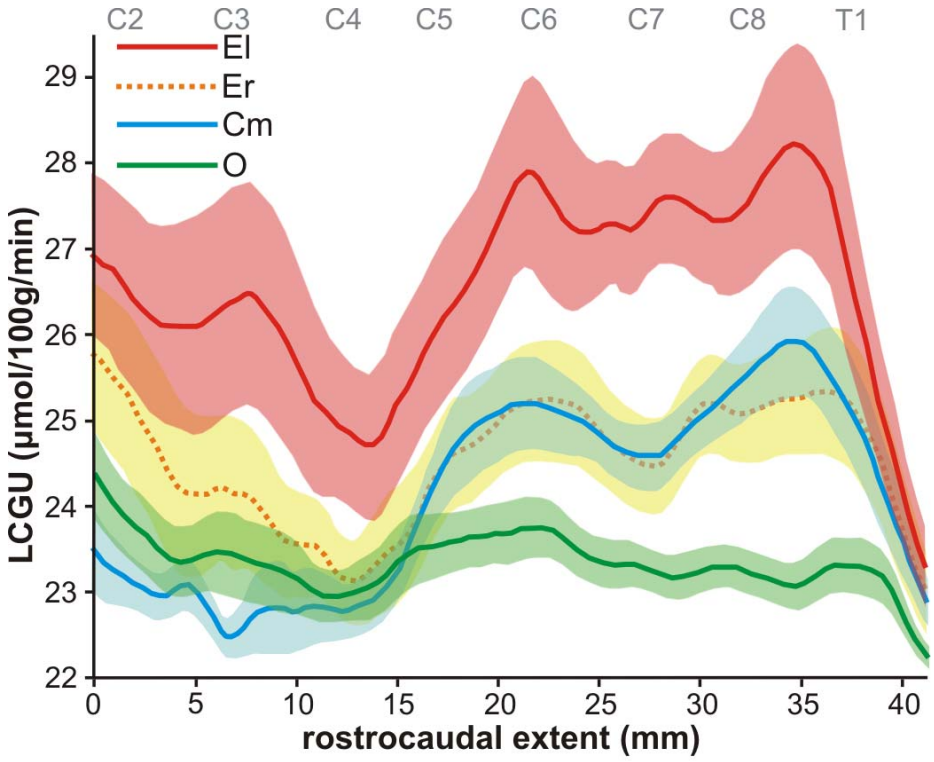


Figure 3-4: Plot of the LCGU values in the intermediate zone and the ventral horn of the spinal cord. Each plot represents average LCGU values (µmol/100g/min) and 95% confidence intervals per 100 µm, along the cervical and first thoracic segments of the spinal cord. Average in the left (El) and the right (Er) sides of the three monkeys executing grasping movements with their left forelimb are represented by the solid and dotted red lines, respectively. Average in both sides, pooled together, of the three monkeys observing grasping movements executed with the right hand of the experimenter (O) is represented by the green line. Average in both sides, pooled together, of the two arm-motion control monkeys (Cm) is represented by the blue line. (From Stamos et al., 2010).

To illustrate graphically the spatial distribution of metabolic activity, we plotted the LCGU values in the intermediate zone and the ventral horn (Figure 3-4). Each plot represents the average LCGU values and 95% confidence intervals (per 100

μm length) along the rostrocaudal extent of the spinal cord (from the first cervical to the first thoracic spinal segment). Because the left to right LCGU values along this rostrocaudal extent in the Cm and O monkeys did not differ significantly (Table-1), we averaged the left and the right sides of each one of these groups to obtain single

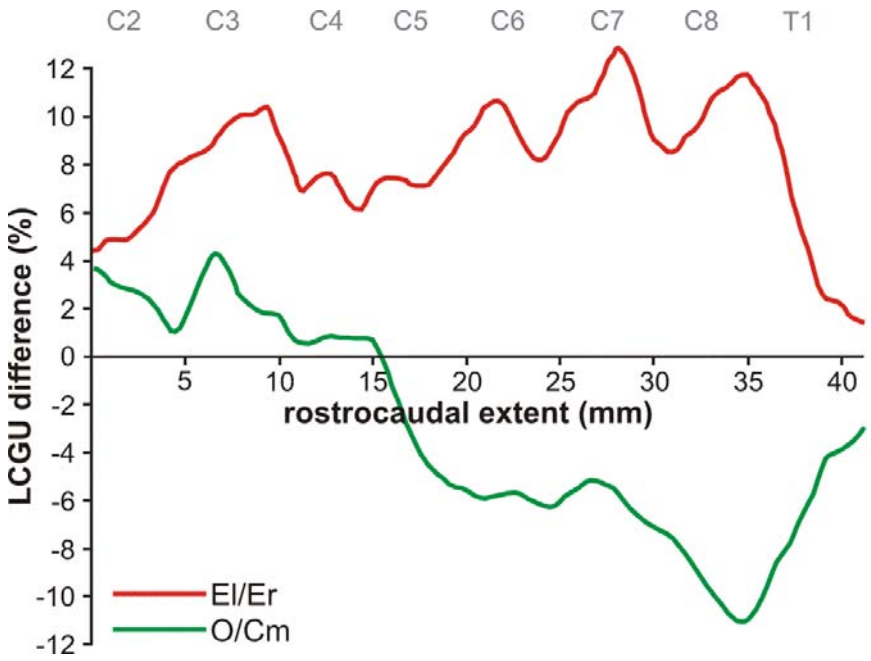


Figure 3-5: Plots of the percent LCGU differences along the cervical and first thoracic segments of the intermediate zone and the ventral horn of the spinal cord. Red plot illustrates the differences in the intermediate zone and the ventral horn between the left and the right side in the three execution monkeys (El/Er). Green plot illustrates the differences between the three observation monkeys and the arm-motion control (O/Cm). Baseline corresponds to 0% LCGU difference from the reference. The intermediate zone and the ventral horn of the left side are activated along all cervical and thoracic segments in the monkeys executing grasping movements. In contrast, the intermediate zone and the ventral horn are suppressed along the lower cervical and thoracic segments in the monkeys observing the same grasping movements performed by the experimenter. (From Stamos et al., 2010).

graphs for the Cm and O monkeys. The plots manifest that (i) the activity in the right, control spinal side of the grasping monkeys (Figure 3-3, Er) is similar to that in the Cm monkeys (Figure 3-3, Cm), (ii) the activity in the intermediate zone and the ventral horn of the left spinal side (Figure 3-3, El), ipsilateral to the moving forelimb, of the monkeys executing grasping movements is increased along the entire rostrocaudal extent, (iii) the activity in the intermediate zone and the ventral horn of both spinal sides in the monkeys observing the same grasping movements performed by the experimenter (Figure 3-3, O) is suppressed along the lower segments of the cervical enlargement.

To graphically represent these effects, the percent LCGU differences in the intermediate zone and the ventral horn have been plotted along the cervical and first thoracic segments, for the El values in reference to the Er ones, and for the O values in reference to the Cm ones (Figure 3-5). The activation induced by grasping-execution is evident along the entire examined anteroposterior extent of the spinal cord (Figure 3-5, El/Er). In contrast, the suppression induced by grasping-observation is more prominent along the lower cervical and first thoracic segments (Figure 3-5, O/Cm), where the motorneurons controlling the distal musculature of the forelimb are located (Jenny and Inukai, 1983; Chiken et al., 2001).

DISCUSSION

The present results demonstrate that the metabolic activity in the ventral horns of the cervical enlargement of the spinal cord is suppressed bilaterally in the grasping-observation monkeys, whereas it is activated ipsilaterally to the grasping hand in the grasping-execution monkeys as expected. The effect induced by grasping-observation was localized in the lower segments of the cervical enlargement where the MNs innervating the hand muscles reside (Jenny and Inukai, 1983; Chiken et al., 2001). A similar somatotopic pattern for the grasping-observation effects was also demonstrated in the cerebral cortex. Raos et al. (2007) reported that the effects induced by grasping-observation were localized in the anterior bank of the Cs, where the distal forelimb is represented. Furthermore, the effects induced by grasping-observation were bilateral in the spinal cord same way as in the cerebral cortex (Figure 4-1). The bilateral metabolic depression found in the spinal cord, may explain the absence of EMG activity in both forelimbs and the suppression of overt movements, during grasping-observation (Raos et al., 2004; 2007). This finding explains why actions are not elicited by the spectator, even though the spectator's motor cortex is activated.

Several TMS studies demonstrated the modulation of the corticospinal excitability during action-simulation. Specifically, action-observation (Fadiga et al., 1995; Clark et al., 2004; Patuzzo et al., 2003; Borroni et al., 2005; Montagna et al., 2005; Leonard and Tremblay, 2007; Borroni et al., 2008), as well as motor imagery

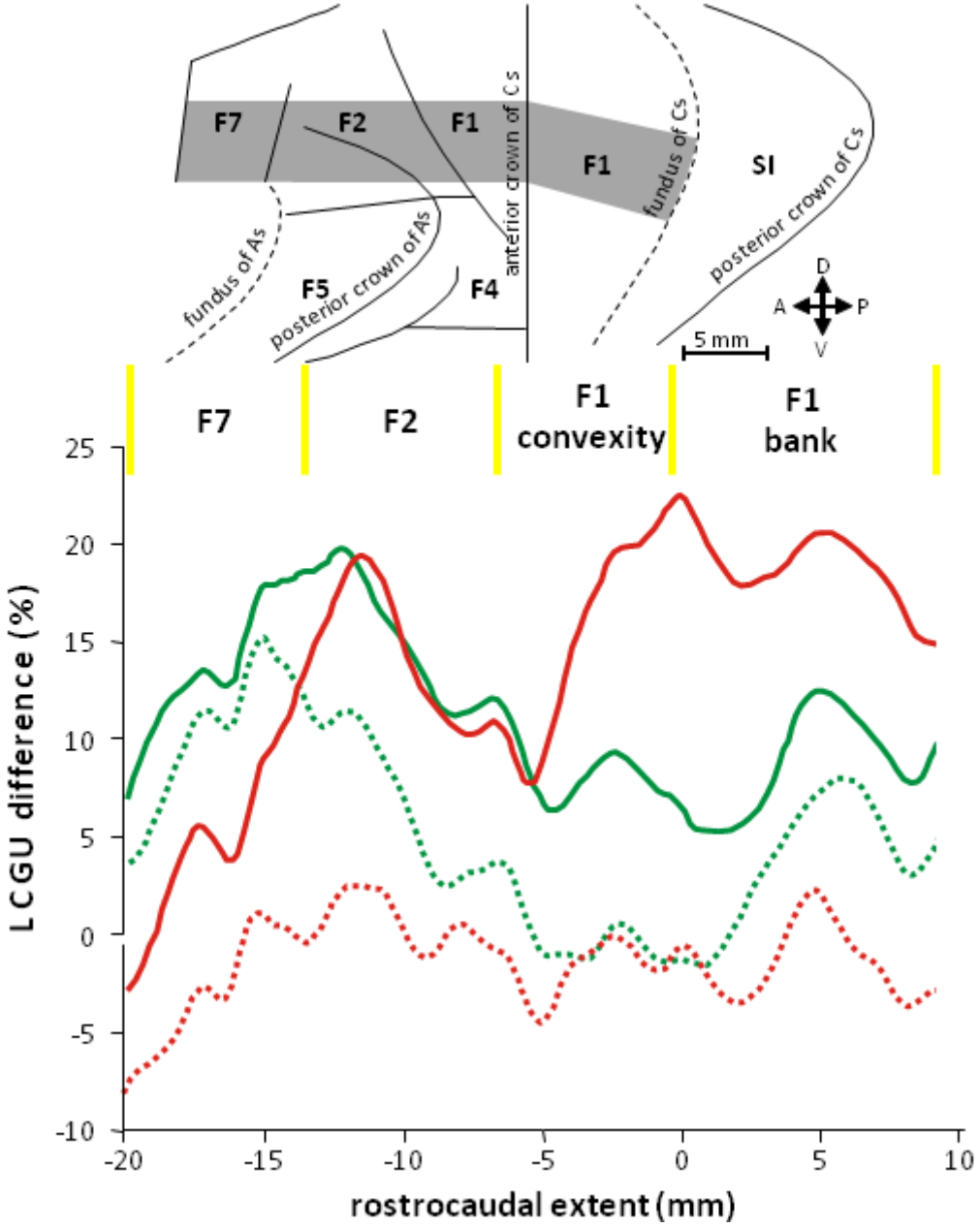


Figure 4-1: Plots of percentage LCGU differences along the rostrocaudal extent of the forelimb representations in the dorsal premotor (F7, F2) and the primary motor (F1/MI) cortices (along the ribbon highlighted in the drawing above the plots). Red plots illustrate the differences between the two execution monkeys and the Cm. Green plots illustrate the differences between the three observation monkeys and the Cm. Plots with solid and dotted lines correspond to the right and the left hemispheres, respectively. Red and green shaded areas indicate 95% confidence intervals. Baseline corresponds to 0% LCGU difference from the Cm. Zero rostrocaudal extent represents the point of alignment of the horizontal brain sections in the lateral-frontal reconstructed maps, i.e., the anterior crown of the Cs. Areas rostral and caudal to the anterior crown of the Cs are represented by negative and positive values, respectively. (Modified from Raos et al., 2007).

(Izumi et al., 1995; Abbruzzese et al., 1996; Yahagi et al., 1996; Kasai et al., 1997; Kiers et al., 1997; Yahagi and Kasai, 1998; Fadiga et al., 1999; Rossini et al., 1999; Facchini et al., 2002; Clark et al., 2004; Patuzzo et al., 2003; Leonard and Tremblay, 2007) enhance corticospinal excitability, as estimated by the amplitude of MEPs. It has been shown that TMS induced facilitatory effects are elicited in a somatotopic manner, i.e., only the muscles that would be recruited for the execution of an overt action are facilitated during the simulation of that action. For instance, during simulation of finger flexion there is a significant increase of MEPs in the flexor muscles involved in a finger flexion action, whereas no such increase can be seen in the antagonistic extensor muscles (Fadiga et al., 1999; Hashimoto and Rothwell, 1999; Rossini et al., 1999).

Facilitatory effects have been correlated with several other factors, such as (i) the complexity of the observed/imagined task: the more complex the action the higher the induced MEPs (Brighina et al., 2000; Roosink and Zijdewind, 2010); (ii) the agent to whom the observed action is attributed to: elevated MEPs for the other, subthreshold MEPs for the self [(Schutz-Bosbach et al., 2006), although Patuzzo et al (2003)] failed to detect such differences; (iii) the orientation of observed actions: i.e subject's own hand facing out from the subject evoked higher amplitudes than subject's own hand facing towards the subject (Maeda et al., 2002); (iv) the lateralization of the observed action, e.g. observed hand actions performed with the hand contralateral to the hemisphere in which the TMS was employed induced higher MEPs (Aziz-Zadeh et al., 2002); and (v) the distinct phases of grasping actions, e.g. the more expanded the finger aperture of the observed actions the bigger the induced MEPs (Gangitano et al., 2001; 2004). TMS facilitatory effects are independent of the posture of the observed hand (Urgesi et al., 2006), are comparable for bio-

mechanically possible and impossible movements (Romani et al., 2005), as well as for intransitive body movements and goal-directed actions (Cattaneo et al., 2009). MEP amplitude variations represent an end point measurement which may be due to excitability changes either at the cortex or the spinal cord or both (Siebner and Rothwell, 2003; Fadiga et al., 2005). Studies aiming to evaluate whether the modulation of the MEP amplitude during action-simulation is accompanied by changes in the spinal excitability provided conflicting results. Some authors have reported modulation of the corticospinal excitability (Bonnet et al., 1997; Rossini et al., 1999; Baldissera et al., 2001), while other studies failed to detect significant spinal excitability changes during action-simulation (Abbruzzese et al., 1996; Kiers et al., 1997; Patuzzo et al., 2003; Borroni et al., 2005; Montagna et al., 2005; Borroni and Baldissera, 2008; Borroni et al., 2008). Furthermore, the majority of the studies reporting spinal modulation during action-observation and motor-imagery concluded that the changes in the amplitude of the MEPs are mainly due to excitability changes in the motor cortex (Kiers et al., 1997; Rossini et al., 1999; Borroni and Baldissera, 2008). Only Li and colleagues (2004).provided evidence for changes in the excitability of the spinal segmental circuitry during motor imagery, independently of the modulation of the motor cortex

Our results provide direct evidence for the involvement of the spinal cord in action-observation. The fact that the spinal forelimb representation was activated for action-execution and depressed for action-observation, although the forelimb representation of the MI/FI was activated in both cases (Raos et al., 2004; 2007), indicates that most probably an inhibition of the tonically active spinal INs underlies the metabolic depression we detect in the spinal cord. The descending pathways from

the cortex and brainstem terminate on the INs of the intermediate zone (laminae V-VIII) rather than on spinal MNs (Kuypers, 1981; Rathelot and Strick, 2006; 2009).

Our 2-DG studies demonstrated that the MI/FI forelimb representation in the anterior bank was activated for both action-execution and action-observation, with the latter eliciting smaller effects (approximately 50% weaker intensity). In contrast, premotor area F5 was more activated for action-observation than for action-execution and premotor area F7 was activated only for action-observation (Raos et al., 2004; see also fig. 4-1). Premotor cortical areas can influence spinal activity through either the MI/FI (Dum and Strick, 2005) or the corticospinal (Dum and Strick, 1991; He et al., 1993) and cortico-brainstem (Kuypers, 1981; Keizer and Kuypers, 1989) spinal projections. Moreover, premotor cortical areas have proven to facilitate the MI/FI (Shimazu et al., 2004; Schmidlin et al., 2008) and inhibit the spinal cord (Moll and Kuypers, 1977; Sawaguchi et al., 1996). The reticulospinal tract, which controls spinal MNs innervating axial and proximal muscles (Kuypers, 1981), was recently demonstrated to influence also the spinal MNs projecting to distal limb muscles, forming a pathway parallel to the corticospinal tract (Riddle et al., 2009). Therefore, we propose that premotor cortical areas F5 and F7, which were more affected by action-observation than by action-execution (Raos et al., 2007), may provide an inhibitory input to the spinal cord of the observing monkeys, which suppresses the metabolic activity at the level of spinal INs. In parallel, MI/FI which was less affected by observation than by execution may provide an excitatory input, although smaller than that induced by execution of the same action.

It has been proposed by Jeannerod (2001; 2004; 2006) that a *dual mechanism* may operate at the spinal level, involving a subthreshold excitatory corticospinal input and a parallel inhibitory influence suppressing the overt movement. It is known that

within the CNS both excitation and inhibition are energy consuming processes (Sokoloff, 1977; Ackermann et al., 1984; Nudo and Masterton, 1986; Savaki et al., 1992). It is reasonable to ask: why the metabolic activity is suppressed during action observation? We believe that the metabolic suppression reflects the net effect of the supraspinal excitatory and the local-spinal inhibitory events, i.e., the cessation of the spontaneous tonic activity of spinal INs. It has been shown that spinal INs operate at high firing level at rest (Maier et al., 1998; Prut and Perlmutter, 2003; Harel et al., 2008), in contrast to cortical neurons which are mainly silent during rest (Cheney and Fetz, 1980). It is evident that the ability of the descending projections to drive spinal MNs may be considerably influenced by the activity of spinal INs (Kuypers, 1981).

The inhibition of the monkey spinal INs during withholding of movement has been reported (Prut and Fetz, 1999). A significant proportion of spinal INs exhibiting early premovement activity was inhibited during the delay, in the absence of any somatosensory input or motor output. It was suggested that the response pattern of these spinal INs may reflect a “braking” mechanism, which prevents movement execution. The impact of the inhibitory effect exerted on spinal INs is so powerful that it is manifested at the population level. Spinal multiunit activity responses to a cue signaling the onset of an instructed delay period consisted of an early inhibition that was locked to the cue event (Asher et al., 2010). Pyramidal tract neurons with elevated spontaneous activity that was completely suppressed during action-observation (suppression type) have been described recently in the premotor area F5. The suppression type neurons were as common as the facilitation type neurons, which displayed the opposite firing pattern: low or absent background activity and increased firing rate during action-observation. It was proposed that the activity profile of the suppression type neurons might evoke a disfacilitation of the spinal MNs (Kraskov et

al., 2009). The decreased metabolic activity in the spinal cord during action-observation could partly be due to the F5 suppression type neurons.

Evidence for the existence of an inhibitory mechanism acting at the spinal level to prevent overt movements during action observation has been provided in the past. Baldissera and colleagues (2001) reported that the modulation pattern of the H-reflex during action-observation was opposite to that occurring during the actual execution of the observed action, and proposed that this finding might reflect the mechanism preventing the overt replica of the seen action (Baldissera et al., 2001). However, subsequent studies reported that the modulation pattern of the H-reflex and the facilitated motor evoked potentials elicited by observation matches the temporal pattern of the muscle recruitment, during action-execution, thus undermining the results and the interpretation of the earlier study (Borroni et al., 2005; Montagna et al., 2005; Borroni and Baldissera, 2008).

The hypothesis that the suppression of overt actions during action-simulation may be mediated through a cortico-cortical inhibitory mechanism has been suggested previously. Brass and colleagues (2001) instructed subjects to perform predefined finger movements, while they were observing another person executing either congruent or incongruent movements. They found a strong co-activation of the dorsolateral and frontopolar areas of the prefrontal cortex during the observation period of incongruent movements. Authors proposed that the focal prefrontal hyper-activations could prevent subjects to replicate overtly the represented action. Neuropsychological observations conducted by Lhermitte and colleagues (Lhermitte, 1986; 1986) in patients with prefrontal lesions, who compulsively imitated actions performed in front of them, are in agreement with the results of Brass et al Nevertheless, in Lhermitte's early clinical studies, brain lesions and pathology were

not the same in all patients (due to stroke, tumor etc.) although symptomatology was similar. Actually, the brain damages were extending from anterior to posterior regions of the entire frontal cortex.

However, the aforementioned interpretation of a cortico-cortical "disconnection" is not compatible with the results of brain imaging studies, due to the simple fact that the motor cortex is activated during action-simulation (Jeannerod, 2004). Therefore, the best explanation for the prefrontal activations in the above described cases is that prefrontal circuits are recruited in order to select the appropriate, non distracting behavioral program. In other words, subjects who are executing a pre-instructed action, which is incompatible with the observed one, must ignore the irrelevant stimulus in order to prevent distraction by the external event. The frontal lobe patients of Lhermitte appear to ignore disruptive stimuli (Jeannerod, 2004; Jeannerod, 2006).

Our results support the theoretical model of the *dual mechanism*, which presupposes the parallel-excitatory/inhibitory- influence upon the spinal circuitry, during action-simulation. More specifically, the MI activation induced by grasping-observation could result in a subthreshold MI-corticospinal activation, which may descend in parallel with a PM-corticospinal inhibition. Indeed, the PM area F7, which were activated for grasping-observation but not for execution, and area F5, which was more activated for grasping-observation than for execution (Raos et al., 2007) may mediate the inhibition in the spinal cord during grasping-observation.

In conclusion, here we demonstrate for the first time the depression of overall activity in the spinal segments of forelimb representation during observation of reaching-to-grasp movements. This finding explains the suppression of overt

movement during action-observation, despite the activation of the primary motor cortex in the observer's brain (Raos et al., 2004; 2007).

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