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Behavioral And Muscular Deficits Induced By Muscimol Injection Into The Primate Primary Motor Cortex During A Reach-To-Grasp Task

Par

Eléonore Serrano

Département de Neurosciences, Faculté de Médecine

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Présenté par

Eléonore Serrano

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Dr Trevor Drew
Président-rapporteur

Dr Numa Dancause
Directeur de recherche

Dr Daniel Bourbonnais
Membre du jury

Résumé

Le contrôle moteur fin et précis des doigts est une habileté importante dans la vie quotidienne pour écrire ou manger par exemple. Ce contrôle moteur est pris en charge par le cortex moteur primaire (M1) qui transmet le signal neuronal à la moelle épinière via la voie corticospinale. Le macaque rhésus est un excellent modèle pour étudier ce système moteur car, comme chez l'humain, il possède cette voie cortico-motoneuronale directe. Bien que les déficits du contrôle moteur de la main suite à des inactivations de M1 aient été étudiés sur des modèles de singes, peu d'études ont décrit les changements musculaires sous-tendant ces déficits. Le but de cette étude était d'évaluer les effets d'une inactivation partielle de M1 sur le comportement et l'activation du patron musculaire du membre supérieur chez le macaque rhésus. Pour ce faire, nous avons effectué des injections intra-corticales de Muscimol, un agoniste du GABA, pour inactiver temporairement l'aire de représentation de la main de M1. Des singes ont été entrainés à réaliser une tâche d'atteinte et de préhension qui requière l'utilisation du pouce et de l'index pour attraper une pastille de nourriture. En parallèle, les activités électromyographiques (EMG) des muscles proximaux et distaux du membre supérieur contralatéral aux sites d'injections ont été enregistrées. L'inactivation partielle de M1 entraine différents déficits moteurs comme une diminution du taux de succès, une perte des mouvements indépendants des doigts, une première flexion de l'index plus lente, et l'apparition de nouvelles stratégies de préhension pour attraper la pastille. Dans le cas de trouble sévère, les singes ont présentés tous ces déficits comportementaux. Ces troubles moteurs étaient sous-tendus par des activités musculaires anormales. En effet, les analyses EMG ont mis en évidence des changements dans les latences et les patrons d'activations musculaires des muscles proximaux et distaux au cours de la phase d'atteinte, d'ajustement et de préhension. Dans le cas de trouble modéré, les patrons d'activations musculaires étaient préservés malgré certain déficits visibles. Cependant, les patrons d'activations musculaires étaient altérés si la tâche demandait une rotation de l'avant-bras et de la main. Ces résultats montrent que les déficits comportementaux et les changements musculaires dépendent de la sévérité des troubles moteurs et/ou de la difficulté de la tâche (i.e. une rotation de l'avant-bras).

Mots-clés : cortex moteur primaire, Muscimol, contrôle fin des doigts, modèle de macaque, électromyographie

Abstract

Fine digit movements contribute to many different aspects of our daily life and require appropriate muscle coordination. The main pathway through which M1 sends motor commands to spinal motor neurons is via the corticospinal tract. The rhesus macaque, like humans, have this direct corticomotoneuronal pathway of M1, making it a useful model to study this system. Although the effect of M1 inactivation on the control of the hand in term of behavioral changes has been studied in monkeys, little is known of how muscle activation patterns of the upper limb during reaching and grasping in monkeys becomes altered. The goal of this study was to evaluate the effect of a partial inactivation of the primary motor cortex (M1) in rhesus macaques on both behavioral performance and muscle activations. To do so we performed intra-cortical injections of Muscimol, a GABA agonist, to inactivate the hand area of M1. Monkeys performed a reach-to-grasp task that required a precision grip to retrieve a food pellet from a well. Electromyographic (EMG) activity of the proximal and distal muscles of the contralateral upper limb were recorded and quantified relative to the behavioral performance. We found that depending on the severity of the impairment, the Muscimol injection could induce several different movement abnormalities, such as decrease in the success rate, loss of independent finger movements, longer duration of the first flexion of the index finger, and use of alternate types of grasp to retrieve the food pellet. In cases of severe impairment, monkeys displayed all these movement abnormalities concurrently. In addition, we observed that behavioral deficits were associated with muscle discoordination. Indeed, EMG analysis revealed that the latencies and the muscle activation patterns were altered during the reach, hand preshaping and the grasp phases of the movement. These inappropriate EMG activities were visible on both proximal and distal muscles of the upper limb. In cases of mild impairment, monkeys had fewer behavioral deficits, but still showed some changes in the temporal muscle activation patterns. In contrast to the severe cases, the muscle activation patterns were more preserved. Interestingly, in the mild cases, the muscle activation patterns were altered if a rotation of the forearm was required by the task. Thus, we found that behavioral and muscular activation changes were dependent on the severity of the impairment and/or the difficulty of the task (i.e. required a rotation of the forearm).

Keywords: primary motor cortex, Muscimol, precision grip, macaque model, electromyography

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List of abreviations

CM: corticomotoneuron

CMA: cingulate motor area

CNS: central nervous system

CS: corticospinal

CST: corticospinal tract

EMG: electromyography

GABA: gamma-aminobutyric acid

HRP: horseradish peroxidase

ICMS: intra-cortical micro-stimulation

LED: light-emitting diode

M1: primary motor cortex

MCP: metacarpophalangeal joint

NNMF: non-negative matrix factorization

PMD: dorsal premotor area

PMV: ventral premotor area

PTN: pyramidal tract neuron

S1: primary sensory cortex

SMA: supplementary motor area

STA: spiked trigger-averaging

TIA: transient ischemic attack

« Ce qu'on s'éclate au Canada»

John McClane, Die hard 3 : Une journée en enfer

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Chapter 1 – General Introduction

1. Stroke, a burden on the health system

Stroke injuries pose a heavy burden on the Canadian health care system. It is one of the leading causes of persistent physical disability and at least 405 000 Canadians are currently living with the consequences of stroke. In addition, the incidence of stroke is expected to increase by 80% by 2038 (Krueger et al. 2015).

A stroke happens when blood stops flowing to any part of the brain, damaging brain cells. There are two types of strokes, hemorrhagic and ischemic. Hemorrhagic stroke is caused by the rupture of a blood vessel. This type of stroke accounts for 20% of all stroke cases. In contrast, ischemic stroke is caused by a blockage or clot in a blood vessel. This either significantly slows down the blood flow or stops it completely, interrupting vital oxygen and nutrient supply to the brain. This type of stroke is much more common and accounts for approximately 80% of all stroke cases (Heart & Stroke Foundation). Finally, there is also a related condition called a transient ischemic attack (TIA), where the blood supply to the brain is temporarily interrupted. This causes what's known as a mini-stroke. It can last a few minutes or persist up to 24 hours.

In the stroke cases due to blockage of the middle cerebral artery occlusion, there is often damage to M1 which leads to a loss of fine motor control mainly in the contralateral limb (Bamford et al. 1991). In addition, unilateral stroke affects the upper limb more than the lower limb, and recovery of the upper limb is worse (Twitchell 1951). Stroke survivors live with motor impairments in the upper limb that cause decrease of productivity, autonomy and quality of life (Olsen 1990).

Thus, the study of the functional role of M1 in terms of hand control is important not just for a better understanding of the role of M1 in the control of movements, but also for a larger clinical importance.

2. The motor system

2.1 Organization of primary motor cortex

The ability to organize complex motor acts and execute fine movements with precision depends on control signals from the motor areas in the cerebral cortex. The motor areas of the cerebral cortex are subdivided into a M1 and several premotor areas. Brain mapping studies have shown that M1 is organized somatotopically in humans (Penfield and Boldrey 1937) and monkey (Woolsey et al. 1952). These studies used surface stimulation to evoke movements of different segments of the body such as leg, trunk, arm, neck, face and mouth. The amount of cortical space devoted to any particular body part represents the amount of control that M1 has over that body part. Therefore, a large part of the motor cortex is dedicated to move the muscles of the fingers and the muscles related to speech (Becker 1953).

In 1975, Asanuma proposed an organization based on cortical columns in the motor cortex. These studies used intracortical microstimulation (ICMS) in monkeys, an invasive technique that delivered a train of cathodal current pulses that evoked muscle contractions. They observed that stimulation at threshold current induced contractions to a single muscle. In this point of view, each cortical column in M1 would project to a single muscle (Asanuma 1975).

However, this columnar organization described by Asanuma was challenged. In a study of Fetz and Cheney (1980), M1 neuron's discharge timing were correlated to muscles contractions while monkeys executed a ramp-and-hold wrist movements. They used spike-triggered averaging (SpTA) to assess the influence of a single M1 neuron on a population of spinal cord motoneurons. The results showed that a given M1 neuron could directly facilitate a number of different muscles, usually two-three muscles per M1 neuron. In addition, an anatomical study with horseradish peroxidase (HRP) showed that identified corticospinal (CS) axons spread collaterals at several levels of the spinal cord and individual CS axons made connections with motoneurons of different muscles (Shinoda, Yokota, and Futami 1981). These studies demonstrated the existence of divergent projection and connectivity of motor cortical neurons.

The divergent projection of motor cortical neurons was also tested during fine control movement. In a study of Schiber and Hibbard (1993), isolated M1 neurons were recorded while

monkeys executed independent finger movements. They observed that neuronal populations were active with movement of different fingers and with extensive overlap. Therefore, control of finger movements involved population of neurons distributed throughout the M1 hand area rather than a somatotopically segregated population (Schieber and Hibbard 1993).

2.2 Premotor areas

In addition to M1, six premotor areas are involved in the production of motor outputs. These premotor areas are defined as areas from the frontal lobe with a direct access to M1 and the spinal cord (Dum and Strick 2002). These 6 premotor areas are: the dorsal (PMd) and ventral (PMv) premotor area, the supplementary motor area (SMA), and the three cingulate (CMA) motor areas (Morecraft and Van Hoesen 1992; Picard and Strick 1996; Barbas and Pandya 1987; Matelli, Luppino, and Rizzolatti 1985). These premotor areas are the main source of inputs to M1 (Dancause et al. 2006; Dum and Strick 2002; Stepniewska, Preuss, and Kaas 1993). Premotor areas are also a major source of corticospinal fibers (~44%) (Dum and Strick 1991).

Further subdivisions were identified in the PMd and the SMA premotor areas. These premotor areas contain a rostral portion called pre-PMd and pre-SMA, and a caudal portion called PMd proper and SMA proper (Geyer et al. 2000; Picard and Strick 2001). These caudal subdivisions have more connections with M1 and more corticospinal projections than their rostral counterparts (Dum and Strick 1991; He, Dum, and Strick 1993). In contrast, the pre-PMd and the pre-SMA have more connections with frontal and prefrontal areas (Bates and Goldman-Rakic 1993; Lu, Preston, and Strick 1994; Luppino et al. 1993).

2.3 The corticospinal pathway

The corticospinal fibers originate from the layer V of the motor cortex, the axons descend through the internal capsule, the cerebral peduncle, and then form the medullary pyramids where these projection is called the pyramidal tract. In humans, most of those axons (75-90%) (Davidoff 1990; Nathan, Smith, and Deacon 1990; Jang 2014) cross the midline in the pyramidal decussation at the junction of the medulla and spinal cord. The crossed fibers descend in the dorsolateral columns of the spinal cord, forming the lateral corticospinal tract. The lateral CST project to motoneurons in the lateral part of the ventral horn of the spinal cord and to interneurons of the

intermediate zone. In contrast, the uncrossed axons (~10%) descend in the ventral columns of the spinal cord, forming the ventral corticospinal tract. The ventral CST project bilaterally to motoneurons that innervate axial muscles in the intermediate zone of the spinal cord (Kuypers 2011; Kuypers 1962).

In seminal studies using retrograde transneuronal transport of rabies virus, Rathelot and Strick examined the distribution of corticomotoneuronal (CM) cells in M1 that make monosynaptic connections with the motoneurons of the hand muscles. In these experiments, virus was injected in a single digit muscle of macaque monkeys and then was transported retrogradely across one synapse to label M1 cells. The distribution of CM cells projecting to motoneurons of the hand muscles was mainly located in the caudal part of M1 and the sulcus. In addition, these CM cells of one digit muscle were widely distributed and overlapped extensively with the representation of other muscles in M1 (Rathelot and Strick 2006, 2009).

It was demonstrated there was a causal relationship between CM cells and muscle activity, with CM cells that directly facilitate different distal muscles (Fetz and Cheney 1980). Moreover, approximately half of these cells could facilitate proximal and distal muscles (McKiernan et al. 1998). In the study of McKiernan et al. (1998), they tested whether the CM cells produce postspike effects in both proximal and distal muscles. They used SpTA while macaque monkeys performed a reach-and-grasp task that required multijoint coordination of the forelimb. The results showed that 45.5% of the CM cells produced postspike effects in both proximal and distal forelimb muscles. In addition, 44.7% of those produced postspike effects in distal muscle only, while 9.8% produced postspike effects in proximal muscle only. Therefore, CM cells make more frequent and potent connection with motoneuron pools of distal compared with proximal muscles. Interestingly, the results also showed that while 70.7% of the CM cells induced facilitation effects, 29.3% of those induced suppression effects, on mostly distal muscles. Accordingly, those CM cells involved in coordinated forelimb movements facilitate and/or suppress muscles activities at multiple joints. Indeed, the extensive representation of a single hand muscle in M1 was close to different muscles of elbow and shoulder movement as shown by stimulus-triggered averaging (StTA) (Park et al. 2001). This proximity could provide neural substrate to favor a broader range of functional synergies involving multijoint movement (McKiernan et al. 1998).

This direct CM system of M1 is crucial for the control of complex finger movements such as precision grip. It is well developed in humans, great apes, and some non-human primates including the macaque (Porter R 1985).

Indeed, lesion of the descending pathway lead to a permanent loss of precision movement (Lawrence and Kuypers 1968a, 1968b). Lawrence and Kuypers (1968a) made the first systematic investigation of bilateral pyramidal lesions and tested the motor skills of eight macaque monkeys. They observed that monkeys recovered part of the general motor behavior (run, jump, climb), but lost the ability to execute independent finger movements. These lesions studies support the importance of M1 and the corticospinal projections in the control of hand movements.

3. Motor cortical control and synergy

Successful execution of voluntary movements depend on the integrity of M1 but also many different part of the central nervous system (CNS). These cortical areas produce neural signals descending to the spinal cord to innervate the spinal interneurons and motoneurons, and finally activate different muscles into motor behaviors (Dum and Strick 1996; Rathelot and Strick 2009).

The role of M1 in the control of arm and hand movements has been extensively studied and highlights a diversity of functions. The discharge of M1 neurons in a variety of experimental paradigms correlates with the direction, speed, and movement, as well as to joint posture and muscle force (Georgopoulos et al. 1982; Georgopoulos and Stefanis 2007; Evarts 1968; Kalaska et al. 1989; Scott and Kalaska 1997). In effect, almost every movement parameter that has been tested has been found to be encoded by motor cortex neurons. This plethora of correlations make it difficult to understand how the spinal circuitries interpret descending cortical signals for a variety of motor tasks. In addition, motor cortical areas need to assure the proper coordination between a large number of muscles and multiple joint motions even for simple movements. Presumable, the CNS simplify the control of goal directed movement by combining discrete elements. Recent work suggest that these discrete elements are muscle synergies (Bizzi, Mussa-Ivaldi, and Giszter 1991; d'Avella, Saltiel, and Bizzi 2003).

The idea that complex patterns of muscle activity may be constructed from a few synergies produced by a limited number of spinal modules was first detailed by Bizzi and his collaborators (Bizzi, Mussa-Ivaldi, and Giszter 1991; Tresch, Cheung, and d'Avella 2006; Tresch, Saltiel, and Bizzi 1999; Tresch et al. 2002; d'Avella and Bizzi 2005). In this approach, a mathematical decomposition methods, like non-negative matrix factorization (NNMF), used on EMG data identified a small number of muscle synergies that was sufficient to explain a wide range of movements. The NNMF was used as a weighting matrix that differentially activates *all* the muscles recorded during a movement, and linearly combined with a time-varying activation coefficient. Therefore a variety of movement can be produced by modifying few muscle synergies (4-6 synergies in most studies). Such synergies have been described in normal reaching movements in primates and humans (d'Avella et al. 2008; d'Avella et al. 2006; Overduin et al. 2008). Other studies used it to evaluate the deficits in movement and changes in synergies after stroke (Cheung et al. 2009; Cheung et al. 2012; Clark et al. 2010).

Another approach to identify synergies was adopted by Drew and his collaborators (Krouchev and Drew 2013; Krouchev, Kalaska, and Drew 2006; Drew, Kalaska, and Krouchev 2008; Yakovenko and Drew 2015). This approach used a more restricted definition of a synergy: a group of muscles that are temporally co-activated and in which muscle activity begins and ends synchronously. Indeed, based on their works on the contribution of the motor cortex to the control of locomotion, they showed that pyramidal tract neurons (PTNs) in the cat were activated sequentially during locomotion. Most of PTNs discharge during only a restricted part of the step cycle, some discharged at the onset of swing and others discharged later in the swing phase (Drew 1993; Lavoie and Drew 2002). Other studies have demonstrated that PTNs discharge sequentially during reaching movements in cats (Yakovenko, Krouchev, and Drew 2011), and M1 neurons also discharge sequentially to coordinate reaching movement in primates (Murphy, Wong, and Kwan 1985). On the basis of these studies, Yakovenko and collaborators (2011) suggested that that the sequential activation of PTNs would modify the muscle synergies by specifying the changes in the spatiotemporal pattern of EMG activity needed to coordinate the movement of the limb. In this approach, a novel cluster analysis identified a greater number of synergies (up to 11) (Krouhev 2006). Each synergy contained only a small number of muscle bursts recorded, and was active during only a small part of the phase movement (i.e. swing or stance phase during locomotion, or reach phase). These synergies could provide a flexible substrate by which descending commands

can regulate and modify limb movements. In a more recent study (Yakovenko 2015), they demonstrated that PTNs cells make a similar motor control to muscle activity during reaching and during stepping over an obstacle.

Muscles synergies have been proposed as building blocks that could simplify the construction of motor behaviors. The muscle synergies represent a repertoire of motor subtasks that can be differently recruited by the nervous system to produce diverse complex movements. Analysis in hemiparetic stroke patients showed differences in the number of muscles synergies, which reflect disruptions in descending neural commands and correlated to motor deficits (Clark et al. 2010). Therefore muscle synergies patterns could be used as physiological markers by clinicians, and elucidate which interventions work best for certain patients and not for others.

4. Motor impairments following stroke

Hemiparetic stroke is accompanied by abnormalities of muscle tone, muscle weakness, and disturbances of muscular coordination. However, in many patients, when weakness and spasticity are treated effectively or resolved spontaneously, motor dysfunction remains severe. Therefore, the primary source of movement dysfunction or global disability in many hemiparetic stroke patients is likely neither spasticity nor muscular weakness, but abnormal movement coordination (Dewald et al. 2001; Hesse et al. 1996). Accordingly, understanding the impaired motor movement and muscle coordination following stroke is essential for the design of effective rehabilitation.

Different research groups have shown alterations of muscle activation patterns following stroke. One such change is inappropriate muscles co-activation (Kamper and Rymer 2001).
Indeed, numerous clinical studies have shown inappropriate co-activation of agonist muscles (Hammond et al. 1988), frequently accompanied by co-contraction of antagonist muscles (Bourbonnais et al. 1989; Kamper and Rymer 2001). These alterations were observed in flexors and extensors of the shoulder and elbow (Dewald et al. 1995), wrist (Hammond et al. 1988) and digits (Kamper and Rymer 2001) in hemiparetic patients. Kamper and Rymer assessed the extent of co-activation during voluntary extension of the metacarpophalangeal (MCP) joint through three

sets of experiments: isometric, isokinetic and free contractions. The torque, position and EMG analysis were compared between stroke and control subjects. The results confirmed the presence of chronic motor dysfunction in the fingers that involved improper co-activation of finger flexors and extensors across the three sets of experiments. Indeed, an extension torque to the MCP joint resulted in the production of an inappropriate flexion torque instead. This inappropriate co-activation was also observed with the flexors and extensors of the wrist (Hammond et al. 1988). Other studies among stroke survivors confirmed the presence of muscle co-activation patterns in the paretic limb, and that abnormal coordination correlated with the severity of upper limb impairment (Dewald et al. 1995; Lee et al. 2013).

Another change in muscle activation patterns following stroke is a loss of selective activation of sets of muscles needed to perform skilled movement tasks (Lang and Schieber 2004). Agonist-antagonist co-activation may explain the reduced ability to selectively recruit upper arm and forearm muscles. This is consistent with the results of experiments performed by Lang and Schieber using humans with pure motor hemiparesis. Kinematics analysis of individuated finger movements (i.e., the ability to move each finger while keeping the other fingers still) were compared between patients and controls subjects. They first showed independent finger movements were differentially impaired. In the affected hands, the independence of the thumb was normal, the independence of the index finger was slightly impaired, while the independence of the middle, ring and little fingers was substantially impaired. The hemiparetic subjects received an extensive rehabilitation training that could explain this differential impairments. The rehabilitation process required independent movements of the index finger and the thumb that could lead to plastic changes in M1, compensation by other cortical motor areas, and/or compensation by other descending pathways (Lang and Schieber 2003). Second, they showed that the loss of independent finger movement was due to a reduced selectivity of finger muscle activation during an individuate finger movement task (Lang and Schieber 2004). Furthermore, this study showed that the loss was beyond the agonist or antagonist muscle activation. Indeed, they observed that a muscle of the little finger (the ADQ, *abductor digit quinti*) was also activated during movement of the thumb and index fingers in hemiparetic subjects (Lang and Schieber 2004).

Electromyographic (EMG) studies have shown delayed initiation and termination of muscle activity as another alteration of muscle control observed in hemiparetic subjects (Chae et al. 2002; Dewald et al. 1995; Hammond et al. 1988). These alterations also correlated significantly with clinical measures of motor impairment, suggesting there is relationship between muscle timing and motor impairment. In Chae and collaborators (2002), stroke survivors were instructed to contract the wrist flexor or extensor as forcefully and quickly as possible against the confinement of an apparatus in response to an audible sound, and to relax the muscle as quickly as possible as soon as the end of this sound. The data showed significantly greater delay in initiation or termination of muscle contraction in the paretic and non-paretic limbs.

Interestingly, clinical studies also revealed more subtle ipsilateral motor deficits that emerged acutely (Sunderland et al. 1999) and persist chronically (Yarosh, Hoffman, and Strick 2004; Wetter, Poole, and Haaland 2005). In the study of Yarosh and collaborators (2004), abnormalities in the temporal activation of the muscles were observed in wrist muscles ipsilateral to a stroke in hemiparetic subjects during a reaching task. Step-tracking movements of the wrist and associated EMG activity (*extensor carpi radialis longus* i.e. ECRL, *extensor carpi radialis brevis* i.e. ECRB, *extensor carpi ulnaris* i.e. ECU, and *flexor carpi radialis* i.e. FCR) of stroke subjects were examined and compared to control subjects. Stroke subjects showed great difficulty or were unable to perform the task with the contralateral wrist, as expected. The results showed that patients had also marked impairments in the ipsilesional step-tracking movement of the wrist with abnormal EMG patterns. First, these abnormalities in the EMG patterns consisted in movement initiated by only the onset times of agonist muscle (i.e. ECU) without the co-activation of the onset times of synergist muscles (i.e. ECRB and ECRL) during wrist extension and ulnar deviation movements. Second, the activity was inappropriately timed if the activity with synergist muscles was observed. The onset times of synergist muscles, ECRB and ECRL, occurred well before or after the onset times of the agonist muscle ECU. Third, the alternation of agonist and antagonist activity was disrupted as observed during a flexion and ulnar deviation of the wrist. Either patients initiated the movement using the onset times of antagonist ECU instead of the agonist FCR, or the onset times of antagonist ECU occurred well later or did not occurred. These abnormal activations of antagonist muscle bursts produces misdirected and irregular trajectories due to inappropriate temporal coordination of muscle activity. This study demonstrated that unilateral lesion can produce bilateral effect on the generation and control of distal movement.

Changes in the temporal activation of muscles are visible from days to months after a stroke, and can change with time. Wagner and associates (2007) explored how the muscle activations in the arm contralateral to a stroke in hemiparetic subjects evolved from the "acute phase" (mean= 9 days post-stroke) to the "subacute phase" (mean=109 days post-stroke) compared to healthy subjects by looking at the changes in the muscles' activation onset times during a reaching task. The muscle onset time was used to reflect the temporal activation of the muscles during recovery. In the acute phase, the muscle onset times occurred later and were more variable relative to healthy subjects. In contrast to healthy subjects, the most distal muscles (wrist *extensor carpi radialis* and wrist *flexor carpi radialis*) and the triceps onset times occurred after the start of the movement. Subsequently, the onset times decreased in the subacute phase relative to the acute phase, such that they became more similar to those observed in the control subjects. Furthermore, muscle onsets occurred prior to the start of movement for all muscles except the distal muscle of wrist *flexor carpi radialis*. Nonetheless in both time points, the hemi-paretic group had considerably more variability in muscle onset times compared to controls. The changes observed in the muscle activation were differentially related to changes in reaching performance. Their results suggest that improvement in muscle timing and a better level of volitional activation might underlie improvement in reaching performance following a stroke (Wagner et al. 2007).

5. Muscimol: a tool to evaluate cortical motor control

Stroke often disrupts descending motor commands due to a loss of cortical/subcortical input to muscles, contributing to impaired movements and coordination (Twitchell 1951). In addition, stroke could also induced damages in brain regions that have synaptic connections with the primary lesion site. For example, following cerebral infarction in the middle cerebral artery, neuronal death, gliosis, and axonal degeneration have been observed in the thalamus, substantia nigra, and distal pyramidal tract all of which lie outside of the middle cerebral artery territory (Ogawa et al. 1997; Forno 1983; Buss et al. 2004; Buss et al. 2005; Schmitt et al. 1998; Schmitt et al. 2000).

The use of animal models helped to evaluate the solely role of M1 in the generation of voluntary movements without the induced damage in other structures observed after a stroke. In the literature, monkey models have been used only occasionally to measure the behavioral deficits and the impact

on muscles activities induced by lesions to the motor cortex (Hoffman and Strick 1995) or corticospinal tract (Hepp-Reymond and Wiesedanger 1972, Lawrence and Kuyper 1968). Large lesions of the primary motor cortex (M1) make voluntary movements of the contralateral side weaker and slower. For example, it has been shown that after a lesion of the M1 arm area there was a marked change in the movement kinematics and the patterns of activity in agonist, antagonist and synergist wrist muscles during step-tracking movements (Hoffman and Strick 1995). Fine motor control deficits were also visible after small lesions of the M1 hand area (Friel and Nudo 1998). Following 1 month of rehabilitative training, skilled use fingers appeared to recover. Despite the apparent recovery of skilled fingers dexterity, the study provide evidence of compensatory strategies. This study demonstrated that even after small infarct that produce mild and transient motor deficits, the monkeys used compensatory movement patterns as a recovery of motor function. These studies highlight the contribution of M1 in the generation of appropriate muscle activation patterns and fine motor control of the finger.

However, to evaluate the contribution of M1 in the motor control of the hand, without the secondary effects of lesion that could involve mechanical damage and tissue inflammation, animal studies used Muscimol injection. Indeed, reversible inactivation of cortical areas by using drug injections has been a powerful tool to elucidate the differential functional contributions of cortical forelimb representations to limb movement control. Muscimol, a pharmacological GABA-A agonist agent, was used to temporarily inactivate different areas of the cortex. Intra-cortical injections of Muscimol have been realized in different animal models such as the rat, cat and monkey (Martin 1991; Martin and Ghez 1993; Matsumura et al. 1991; Brochier et al. 1999; Schieber and Poliakov 1998).

Monkey studies showed that the injection of Muscimol in M1 induced impairments such as decreased speed, weakness of muscles, and loss of independent finger movement (Kubota 1996; Matsumura et al. 1991; Schieber and Poliakov 1998; Brochier et al. 1999; Fogassi et al. 2001). During a reach-to-grasp task, a reduction of movement speed, of movement time, and reaction time was observed after injection of Muscimol (Kubota 1996; Fogassi et al. 2001). Based on the video analysis, the movement time almost double with respect to the control condition (Fogassi et al. 2001) and the reaction time was significantly longer (Kubota 1996). Another study with a visual reaction-time task, where the animal need to press a lever and release it after a go signal to obtain

a reward, confirmed the significant increase in reaction time after Muscimol injection (Matsumura 1991).

Moreover, muscle weakness was also often observed together with the slowness of movement (Matsumura et al. 1991; Kubota 1996; Schieber and Poliakov 1998; Brochier et al. 1999; Fogassi et al. 2001). The weakness in the finger muscles was measured by Brochier et al. 1999. In this study they evaluated the effects of a small inactivation (10 µg) of the primary somatosensory (S1) and motor (M1) cortex on the control of fingers forces in a precision grip task. The animal needed to grasp a metal tab between the thumb and the index finger, lift it into a vertical position window and hold it stationary for two seconds to obtain a reward. In addition, the monkey's performance was assessed with a modified Klüver board. The precision grip was differentially impaired by the injection either into S1 or M1. The M1 injections produced impairment in the ability to perform independent digit movements and a general weakness in the digit muscles. Indeed, all the fingers moved together in a whole-hand grasping configurations, and this loss of precision grip resulted in inappropriate digits positions on the metal tab surfaces. In addition, the monkey was unable to oppose the thumb and index digit to enter the food well of the Klüver board. The motor deficit was restricted to the most distal segments of the hand because the monkey could still execute the reach phase of the movement. In contrast to M1, the S1 injections induced a loss of manual coordination in order to position the thumb and index finger for grasping, and an increase in the grip force. In addition, during the Klüver board task, the monkey was able to execute some crude independent finger movements to retrieve and eat food morsels under visual control. These results showed that the main effect of small inactivation into the thumb and index representation area of M1 was the inability to perform independent finger movements in association with muscular weakness of the hand.

Similarly, Fogassi et al (2001) realized small and large injection (15 and 90 µg of total Muscimol injected respectively) of Muscimol in M1 and the ventral premotor cortex (PMv). The size of injection was an important factor that caused different impact with deficits restricted to the most distal segment and/or also more proximal segment. The small inactivations of M1 hand field area produced paresis in the fingers of the contralateral hand, in association with a reduction of the grip force, and hypotonia. The capacity to grasp was also strongly impaired; monkeys reached with a "flat" hand posture similar to a whole-hand grasping observed previously by Brochier et al (1999). Therefore, the precision grip couldn't be executed. The larger inactivation produced

deficits that were the same as those after a small and single injection, but more severe. The grasping was completely disrupted and one monkey showed impairment of more proximal movements (Fogassi et al. 2001).

The injection of Muscimol induced also specific deficits in the digits and was studied in detail with partial inactivation of M1 hand area during a visually cued individuated finger movement task (Schieber and Poliakov 1998). This study tested the existence of a somatotopical finger representations in M1. If the M1 hand area contains this somatotopic organization, then partial inactivation might produce weakness, slowness and loss of independence of one or two adjacent digits while the other fingers remained unaffected. Monkeys were trained to perform visually cued individuated finger movements. The right hand was placed in a pistol-grip manipulandum that separated each finger into a different slot. Monkeys were instructed with different light-emitting diodes (LEDs) which digit to flex or extend. By flexing or extending a digit, a ventral or dorsal switch was closed respectively. The monkey received a water reward if the instructed switch was closed and hold in a proper response time without closing any other switches. In addition, monkeys were trained to retrieve small food morsels from a large well that permitted entry of the whole hand and a small well that permit entry of only one finger. The results showed several deficits: (i) increased failure of instructed individuated finger movement, (ii) prolongation of response time (that included both the pre-movement reaction time and the movement time itself), and (iii) decrease of individuated finger movement. Interestingly, this study observed a wide variety of possible combinations of these deficits. Indeed in some inactivation sessions all these three deficits were observed. In other sessions, however, increase in movement failure (i) could be observed without significant prolongation of response time (ii) or decrease in individuation (iii). In still other cases, response time and/or decrease individuation could be observed without a significant increase in failure. Moreover, in some cases there was prolonged response time without decrease individuation and vice-versa. Based on these different combinations, the authors suggested that prolongation of response time and decrease in individuation could be to some extent dissociable. In addition, the injection impaired performance of retrieving food morsels from the two wells and the contralateral hand failed to preshape. They found little evidence that which finger movements were affected after each injection was related to the injection location along the central sulcus. These findings showed that the M1 hand area was not so somatotopically organized as implied by the homunculus, but suggest a distributed organization for control of the different fingers

One study, in awake cat, evaluated the changes in kinematics of reaching and grasping after single injection of Muscimol (1µg) (Martin and Ghez 1993). This study showed that local inactivation within the forelimb representation in the M1 motor cortex of the cat produced measurable deficits in reaching behavior with slower movements, reaching errors, and defect in kinematic planning or in trajectory adaptation to avoid an obstacle, and in grasping movements during food retrieval.

While the studies described above presented detailed descriptions of behavioral deficits following M1 inactivation with kinematics, forces, and response time, few studies have attempted to provide a description of electromyographic (EMG) muscle activity. Matsumura et al. (1991) performed an experiment looking not just at the behavioral deficits but also the change in EMG activity induced by injection of agonist (Muscimol) and antagonist (Bicuculline) of GABA in the primate motor and premotor cortex during a raisin pick-up test and a visual reaction-time task. They showed the local injection of Muscimol in either area caused deficits in hand and arm movements. Injection of Muscimol in M1 deteriorated the digit manipulation in the raisin pick up test, with an increase in retrieval attempt and in raisin drop. The movements were weaker and slower, with monkeys that spent more time for single trials. The EMG analysis were not possible with this task due to vigorous arm movement that created too much noise in the EMG recordings. As previously described, the results of the visual reaction-time task showed a significant increase of reaction time after Muscimol injection, and this deficit was in association with changes in muscle activities. The Muscimol caused a transient decrease in the total muscle activity of hand muscles like the *flexor carpi ulnaris* (FCU) and *extensor carpi radialis* (ECR) showed in the article. The EMG activity decreased with a similar time course of the manipulation deficits. This study demonstrated the behavioral deficits were associated with changes in muscle activity as probably a reflection of plastic changes in cortical motor area (Matsumura et al. 1991).

Taken together, these studies suggest the motor cortex is crucial for the regulation of spatiotemporally organized ensembles of muscles activity. In addition, inactivation of the M1 hand area could produce main effect as slowness and weakness of the movement, and/or a loss of independent finger movements. However, none of these studies have explored how the activation across a set of muscles was changed.

6. Objectives of the study

The general goal of the current work is to further our understanding of how the hand area of M1 contributes to the execution and coordination of movement of the upper limb during a reach-to-grasp task. As outlined in the previous sections, M1 plays a critical role in the motor of the hand and fine digit movements, and the main pathway through which M1 sends motor commands to spinal motor neurons is via the corticospinal tract. Clinical studies have shown that cortical damage, such as stroke, interferes with the descending signals to the spinal cord, resulting in abnormal movements. To explore the relationship between cortical damage and motor control, fundamental research has often used Muscimol to temporarily inactivate different areas of the cortex and measure the induced motor impairments in different animal models. In spite of extensive studies of behavioral deficits following Muscimol injections, the correlation of these motor deficits with changes in muscle activities of the upper limb have not been described. The current project set out to address this gap in our knowledge. The present experiments were conducted in macaques, a species in which, like humans, M1 has direct corticomotoneuronal projections to hand motoneurons. We used Muscimol to test the contributions of the M1 hand area to the production of a reach-to-grasp movement and the evoked motor patterns that constitute this movement.

We hypothesized that behavioral deficits in reach-to-grasp movements after partial inactivation of M1 would be associated with abnormal muscle co-activation patterns, and that these abnormalities would depend on the severity of the inactivation (Fogassi et al. 2001; Cheung et al. 2012). Our first objective was to investigate the behavioral performance of the paretic hand both prior to and after partial inactivation of the hand area of M1. The behavior was evaluated based on the monkeys' ability to retrieve a food pellet from a well using video recordings and task sensors. Two monkeys were trained to perform a reach-to-grasp task that culminated in a precision grip. The target could be oriented in a vertical or horizontal orientation and, as such, required monkeys to perform a rotation of the forearm or not. The rotation of the forearm added more difficulty to the motor control of the movement. Our second objective was to describe the muscle activation patterns relative to the behavioral performance. Muscles of different segments of the paretic limb, from proximal muscles such as the deltoid to distal muscles such as the first interosseous, had their electrical activity recorded from using chronic subcutaneously implanted wires (i.e. EMG). The behavior and the EMG activities were evaluated and compared before and after the Muscimol

injections. First, the behavior was assessed using the task success rate, the number of digit flexions required to retrieve the pellet, the duration of the first flexion of the index finger, the duration of the reach and the grasp phases of the movement, and the type of grasp used. These measures allowed us to test whether the partial inactivation of M1 could lead to deficits in one or both phases of the movement and whether there was an induced loss of manual dexterity. Second, the EMG activities were compared relative to the behavior to determine whether the execution of reach-to-grasp movements underwent changes in the coordination of voluntary muscle activities. The latencies and the muscle activation patterns was analyzed based on the onsets and offsets of proximal muscles (deltoid, biceps) and distal muscles (wrist flexors and extensors, and intrinsic hand muscles). The results will provide a better understanding of the contribution of M1 to the control of fine digit movements and how muscle patterns changed after partial inactivation of the M1 hand area.

Chapter 2 – Materials and methods

The experiments were conducted on 2 adult female monkeys (*Macaca mulatta*), Monkey 1 (5.5 kg) and Monkey 2 (5.7 kg). All surgical and experimental protocols were performed in accordance with the guidelines set forth by the Canadian council on Animal Care, and were approved by the Comité de Déontologie de l'Expérimentation sur les Animaux de l'Université de Montréal.

Training

The monkeys were trained for up to 6 months on the reach-to-grasp task (Fig. 1A). The monkeys were trained to be brought out of the cage, sit quietly in our custom-made primate chair, and brought back to the cage every day for 2 months. The custom-made chair had two removable panels for the arms and an opening near the mouth allowing the monkeys to eat the pellet rewards. After monkeys' habituation to sit in the chair, they were brought into our training room and installed in front of the task. Then, they were trained to realize a reach-to-grasp task.

The apparatus consisted of a box with a home plate and a precision target for each hand. The two home plates were placed on a plane surface at 10 cm in front of the monkey and contained two sensors each. The two targets were placed vertically to face monkeys at approximately 10 cm height above the home-plates. Each target contained a small well in the middle to hold a banana flavor food pellet as a reward (190mg Dustless Precision Pellets; BioServ, Flemington, NJ, USA), and a slot that permitted the monkeys to grasp pellets only by performing a precision grip. Sensors were installed at each extremity of slots. In addition, the slots could be oriented horizontally or vertically to retrieve the pellet from the well. All sensors were used to monitor the proper sequential execution of reach and grasp phases of the movement.

First, monkeys were trained every day for 2-3 months to place and keep their hands between the home plates sensors before subsequently performing a reach and grasp movement to grasp the pellet. The reward was remotely controlled and one pellet was given when monkeys placed the hand between the home plates sensors.

The second parts of the training started after monkeys learned to place their hands on the home plates and executed a sequential movement of reaching, grasping, and retrieve/eating the pellet. Then, monkeys were trained to perform the task with our automatic program that included

5 different delays before the pellet drop (from 800 ms to 2 s). Monkeys were trained daily to keep the hand on the home plate and execute the reach-to-grasp task with a high (<80%) and constant (<2 weeks) success rate.

Behavioral task

In the day-to-day experiment, the monkeys sat in a the chair installed in front of the behavioral task consisting of one sensor home plate and one precision well per arm (Fig. 1A *left panel*). The monkeys had to perform a reach-to-grasp movement culminating in a precision pinch with either hand. The precision grip was done using the thumb in opposition with the index digit. The trial sequence was as follows: (i) monkeys first placed their hands in the start position (Enter start). (ii) Then, after a variable delay (baseline) a food pellet dropped into the well. (iii) The sound of the pellet being dropped served as a signal to the monkey to move (Pellet drop; a "GO" signal). (iv) The monkey's hand left the start position (Exit start; reach start) to reach towards the food well, until its fingers crossed the sensors around the slot (Enter slot; grasp start). (v) After performing a successful precision pinch to grasp the food, the fingers left the slot (Exit slot; grasp end) and the animal brought its hand to its mouth to consume the food pellet (Return) (Fig. 1A *right panel*).

Surgical procedures

Once the training was completed, the monkeys were prepared for surgeries under aseptic conditions, and anesthesia was induced with an intramuscular injection of ketamine hydrochloride (Ketaset; Pfizer, Inc, New York, NY, USA). The animals were transitioned to 2-3% isoflurane (Furane; Baxter, Deerfield, IL, USA) with oxygen. To help prevent inflammation and swelling of the brain, the animal received an intramuscular injection of Dexamethasone 2 (Vetoquinol; 0.5 mg/kg), a sub-cutaneous injection of Atropine (0.04 mg/kg) and intravenous injection of Mannitol 20% (1500 mg/kg) at the beginning of the surgery. To keep the animal's body temperature near 36.5°C throughout the surgery a self-regulating heating blanket was installed under the animal (Harvard Apparatus, Holliston, MA). Blood oxygen saturation and heart rate were continuously monitored.

In a first surgery, the monkeys were implanted with a head post on the skull to allow fixation of the head during the experiment. The head posts were fixed to the skull with screws covered with dental acrylic (Palacos R, Heraeus Medical, Germany) that contained a mix of antibiotics (Vancomycin hydrochloride, Alfa Aesar A Johnson Matthey Compagny and Tobramycin, TCI America Thermo Fisher Scientific).

A second surgery was done 4 to 8 months later, allowing time for animals' habituation of the head post and for the skin around the implant to heal and thicken. Muscles were intramuscularly implanted with electromyographic (EMG) insulated, multistranded microwires (Cooner Wire, Chatsworth, CA, USA). Using various sizes of stainless steel tunneling needles, all the wires were passed subcutaneously from the head posts' acrylic/skin junction at the back of the head with a small incision (Fig. 1B). Pairs of wires were then tunneled subcutaneously to the appropriate target muscles and each wire was then inserted intramuscularly (Park, Belhaj-Saif et al. 2000). Accurate placement of the EMG wires was tested by electrical stimulation of the muscles through implanted wires and observation of the evoked movements both during the surgery and in later sessions in the awake monkey. A total of 16 muscles per arm was implanted: Deltoid (*musculus deltoideus*), BB (*biceps brachii*), BR (*brachioradialis*), PT (*pronator teres*), PL (*palmaris longus*), FCU (*flexor carpi ulnaris*), FCR (*flexor carpi radialis*), FDC (*flexor digit communis)*, FDS (*flexor digit superficialis)*, ECU (*extensor carpi ulnaris*), ECR (*extensor carpi radialis*), EDC (*extensor digit communis)*, ED23 (*extensor digitorum 2 and 3*), ADD1 (*adductor of the thumb)*, ABD1 (*abductor of the thumb)*, intD1 (*first dorsal interosseus),* and FPB (*flexor pollicis brevis superficialis*).

A third surgery was conducted to implant intra-cortical multi-electrode arrays in several cortical areas in both hemispheres and one chronic inactivation chamber (Fig. 1B) one month following the EMG implantation. Multi-electrodes arrays (Utah Array, Blackrock microsystems; and Floating Microelectrode Array, MicroProbes for Life Science) were implanted in the ventral and dorsal premotor cortex (PMv, PMd) in both hemispheres and the primary motor cortex (M1) in the right hemisphere. These neural data will not be presented in this study. In the left hemisphere, the dura was left intact over the central sulcus and a chronic chamber (2x2 cm opening; plexiglass) was positioned to provide access to the dura over the M1 hand representation. The chamber allowed us to perform Muscimol reversible inactivation experiments in the left hemisphere, which we refer to as the "ipsilesional" hemisphere. By extension the right hemisphere is referred to as the "contralesional" hemisphere in both monkeys.

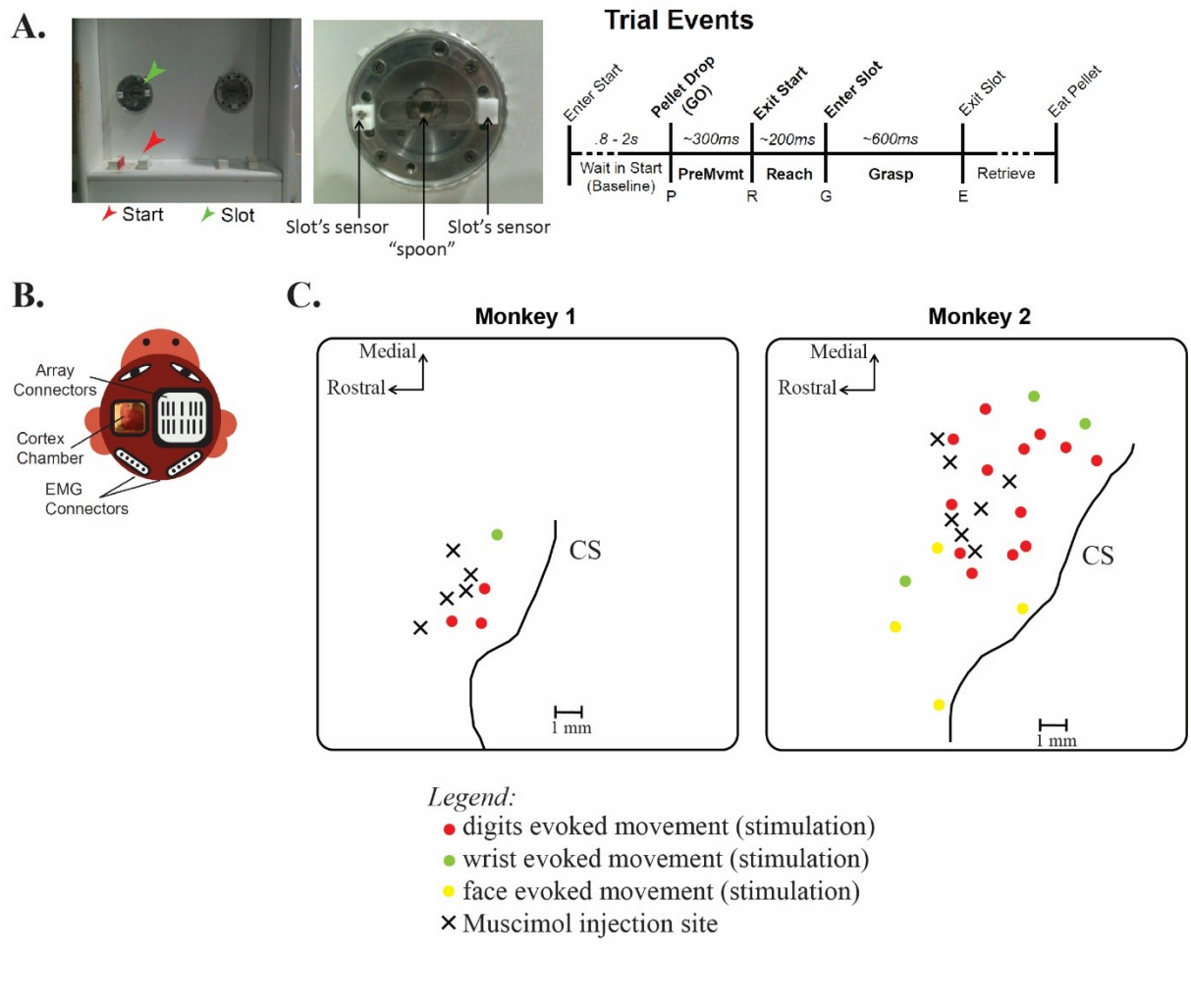


Figure 1: Overview of the experimental setup. A, photos of the experimental task (left panel) and a zoom on the target with the well and slot (middle panel). The different events during one trial as measured by the sensors' target (right panel): Enter start; Pellet drop; Exit start; Enter slot; Exit slot; Eat pellet. **B**, Illustration of the EMG connectors and cortex chamber on the monkey's head. **C**, cartoon of the chamber of Monkey 1 (left) and Monkey 2 (right) with injections sites in the M1 hand area (black cross) and stimuli sites (red dot: digit, green dot: wrist, yellow dot: face evoked movement). A total of 4 and 20 sites were mapped in Monkey 1 and 2, respectively. For each session, 1 x 0.75 µL of Muscimol was delivered using a Hamilton syringe. *CS=Central Sulcus.*

Mapping of the motor cortex and sites of injection

A brief topographic motor map of M1 was constructed inside the inactivation chamber by using standard intra-cortical micro-stimulation trains (i.e. ICMS), while the awake animal sat quietly in the primate chair in order to confirm the location of the hand representation area (Fig. 1C). ICMS stimulation consisted of a 40 ms train of 13 monophasic cathodal pulses of 200 µs delivered at 350 Hz from an electrically isolated, constant current stimulator (Mansoori, Jean-Charles et al. 2014). A glass coated tungsten microelectrode was placed on a micromanipulator mounted on a stereotaxic frame fixed to the primate chair and descend in the cortex through the chamber of inactivation for electrical stimulation. In the chamber, stimulations were done medio-laterally to locate the face and proximal representation and antero-posteriorly to locate the central sulcus (CS) and primary somatosensory cortex (S1). All cortical sites retained for the Muscimol inactivation protocols evoked digit or wrist movements in the contralateral arm with ICMS trains. For every site, the type of evoked movement, the depth and the lowest stimulus intensity was noted. There were 4 sites mapped in Monkey 1 and 20 sites mapped in Monkey 2 (Fig. 1C, *dots*). This allowed us to identify digit and wrist representation area for both monkeys, and face representation area for Monkey 2 (Fig. 1C). A total of 5 Muscimol injections were done in Monkey 1, and 7 Muscimol injections were done in Monkey 2 (Fig. 1C, *black crosses*).

Protocol

Data were collected in a sequential order with the two hands and slot orientations presented in separate blocks of 25 trials (in order: Left arm – Vertical orientation = LV; Right arm – Vertical orientation = RV; Left arm- Horizontal orientation = LH; Right arm – Horizontal orientation = RH). These blocks (LV-RV-LH-RH) of data were recorded at 5 different time points: before the inactivation (Pre-injection block, i.e. Pre), 15 to 40 min after the inactivation (i.e. Post), 3 hours after the inactivation (i.e. T03), 10 hours (i.e. T10), and 24 hours after inactivation (i.e. T24). Only data in Pre and POST were analyzed and presented in this study.

Prior to the Muscimol inactivation protocol, we first recorded baseline behavioral, and EMG (Pre). Then, based on our motor map of M1, Muscimol was intra-cortically injected in M1 hand representation. The drug was loaded in a 5 µL Hamilton syringe with a bevelled tip 26 gauge needle (Hamilton Robotics, Reno, NV, USA). The syringe was placed on a micromanipulator

mounted on a stereotaxic frame fixed to the primate chair. That allowed us to descend the syringe in the cortex accessible within the chamber of inactivation. One injection of 0.75µL of Muscimol solution (concentration 5µg/µL) was injected in all individual experiments presented in this study (Fig. 1C). This concentration of Muscimol (5 µg/µL) has been already successfully used to temporally inactivate cortical motor areas on Macaque models (Schieber and Poliakov 1998, Brochier 1999, Fogasse 2001). Each individual injection of 0.75µL Muscimol was delivered at a constant rate of 4 nl/s with a micro-injector (Harvard Apparatus, Holliston, MA, USA). The spread of one injection of Muscimol had an estimated maximum radius of 1.5 mm (Martin and Ghez 1999) and therefore was restricted to the M1 hand area.

Behavioral data analysis

BEHAVIORAL ASSESSMENT: A frame-by-frame video analysis was made to extract behavioral data. We calculated the percentage of successful trials to obtain a success rate per block of trials. A trial was considered successful if the animal was able to retrieve the pellet and eat it. The numbers of trials in which the monkey successfully retrieved pellets were counted. If during the retrieval the pellet fell or was not retrieved successfully, the trial was considered a failure.

The number of flexions executed with the index finger to retrieve the pellet was also assessed. A total mean number of flexions were calculated based on trials from each block of data. For each trial, the number of flexions executed by the monkey while their finger was inside the well until the retrieval or falling of the pellet was counted.

The duration of the first flexion of the index finger during the grasp phase of the movement was also calculated. This parameter was defined as the time beginning when the distal tip of the index finger was in contact with the pellet and started to flex until the time the hand moved to bring the pellet towards the mouth (i.e. in the case of successful trials), or until the time the distal tip of the index finger stopped to be in contact with the pellet (i.e. in the case of several flexions or unsuccessful trials). For each trial, the duration of the first flexion of the index finger was calculated based on the number of frames between the start and the end of the first flexion (1 second = 30 frames).

DESCRIPTION OF GRASPING CONFIGURATION: Different grasping configuration could be identified after inactivation in the vertical and horizontal slot orientation (Macfarlane and Graziano 2009, Castiello and Dadda 2019). The movement pattern to retrieve the pellet was reported based on the video analysis. The categorization was defined by the shape of the grasp used to successfully dislodge pellets from the slot based on the frame-by-frame video analysis for each trial. A total of 4 categories were identified in percentage: (i) Normal precision grip ("N-PG"): the pellet is pinched between the distal pad of the first digit (D1) and the index digit (D2). (ii) Abnormal precision grip ("AN-PG"): the pellet is grasped between the thumb and the index digit but no longer between the distal pad of D1 and D2. The monkey does the precision grip but the pellet is between the distal pad of D1 and along the radial side of D2, or between the distal pad of D2 and another part of D1. (iii) Abnormal precision grasp ("AN-PGrasp"): the pellet is grasped with the collaboration of the second and third digits in opposition to the thumb. The pellet is held between these three digits (tri-digital) or between D1-D2 and the palm of the hand. Occasionally the pellet was lodged between lateral surfaces of two adjacent fingers. (iv) Abnormal power grip ("AN-PoG"): the pellet is grasped by several digits or into the palm (see Fig. 6B).

DURATION OF REACHING AND GRASPING: To assess the impact of the inactivation on the reaching and grasping components of the movement we compared the duration of the reaching and the grasping phases before and after Muscimol injection. The duration of the phases was calculated from all trials based on the signals from the sensors of the task. The reach duration was defined as the time between the hand leaving the platform (Home plate sensors off) and the digits entering the slot (enter of the slot sensors on). The grasp duration, i.e. Contact time, was defined as the time between the digits entering the slot (enter of the slot sensors on) and the digits leaving the slot (enter of the slot sensors off). The Contact time did not take into account the number of flexions inside the slot, only the time spent by the monkeys' digits inside the slot to retrieve the pellet. Some Contact time trial onsets and offsets were misplaced by the task because of multiple activations of the sensors. All trials were inspected, and either corrected or excluded based on visual inspection. This included trials for which the reach duration was abnormal, for example if the animal was distracted by some noises and interrupted the movement.

EMG signals

Data were analyzed for reach and grasp phases of the movement, and all muscle activity presented were recorded from the contralateral arm (i.e. impaired). We analyzed the data in two complementary ways. First, we used averaged EMG data to examine the general pattern of burst activity. Second, we analyzed EMG data on a trial by trial basis to examine the relationship between muscle activity before and after inactivation. Only trials that passed the visual inspection (see above) were retained for these analyses. EMG signals were rectified and low-pass filtered at 30Hz (1^{st}-order Butterworth). These rectified-filtered EMGs were aligned on the start of the Grasp (i.e. slots' sensors ON).

The average EMGs were used to assess the consistency of the EMGs' signals across experiments as well as to identify bursts of activities for each phase of the movement (see Fig. 6). For each muscle we identified one or two bursts of activity involved during the reach phase of the movement (reach#1 and reach#2), and/or one or two bursts of activity involved during the grasping phase of the movement (grasp#1 around 0 ms, and grasp#2).

The EMGs data for the trial by trial analysis were subsequently used to determine the onsets and offsets of each EMG bursts. All the analysis of EMG during reach-to-grasp was restricted to the EMG signal that was collected from 1000 ms prior to and following the start of the Grasp (i.e. slot sensors' ON).

Muscles onsets and offsets criteria and selection

The EMG signals had to fulfill two criteria: to show burst of activity with a significant peak during the reach and/or grasp phases of the movement, and to show abrupt decrease of EMG activity from which we could determine the onset and offset of activity in single trials. Therefore, for each burst identified in the average EMG, we identified each corresponding burst in individual trials. Then, for the trial by trial EMG analysis, a custom program was written in MATLAB (MATHWORKS, Natick, MA) to select the onset and offset of each burst. We manually marked the onset and offset of each burst of EMG activity and classified them as reach or grasp burst. All bursts onsets and offsets were measured with respect to synchronization with Grasp.

Selected bursts were classified as reach or grasp depending on the burst onset time and peak time. Reach-related burst exhibited onset that occurred before the start of the Reach (i.e. home-

plate sensors' OFF), with a peak during the Reach phase. An exception was made for FDC of Monkey 2 which had a second reach-related burst (R.2), with an onset that occurred around the start of the Reach (i.e. home-plate sensors' OFF) and a peak between the reach start and the start of the grasp. This second reach-related burst showed a consistent peak across trials.

Grasp-related bursts were classified as pre-shaping burst (G.1) with onset that occurred before 0 ms (i.e. slot sensors' ON), and with a peak around 0 ms. Finally, bursts were classified as grasping burst (G.2) with onset that occurred after 0 ms and with a later peak.

Muscles latencies

As previously described, the analysis of EMG during reach-to-grasp was restricted to the EMG signal that was collected from 1000 ms prior to and following the start of the Grasp (i.e. slot's sensors ON = 0 ms). Therefore, all selected burst onset times were expressed in relation to the start of the Grasp, such that negative values represented onsets prior to the activation of the slot's sensors. The grasp-related bursts were kept aligned on the start of the Grasp. However, in order to compare bursts activities that occurred during the reach, reach-related bursts were re-aligned on the start of the Reach (i.e., home-plate sensors OFF). For each trial the reach-related burst onset (On. = -X ms) was aligned on the start of reach event (R= -Y ms) using the reach duration (abs (-Y)) (Fig. 2A):

$$Reach\ related\ Burst\ Onset\ Time\ Aligned\ = -X + abs(-Y)$$

Phase-plot

Burst onset and offset times were expressed as a proportion of the normalized reach and grasp phase of the movement. The grasp phase of the movement was defined as the time between the slot sensors' ON event and the burst offset of a selected muscle. The burst value was selected instead of the slot sensors' OFF to insure that the analyses were focused on the EMG pattern associated to the first precision grip for each trial. This was particularly important after inactivation, when monkeys often could use several finger flexions per retrieval. The intD1, and FDC were

chosen for Monkey 1 and 2, respectively for that purpose. Both muscles are involved in the control of digit movements and presented clear bursts of activity during the grasping of the pellet.

Reach phase of the movement was normalized from the start of the reach defined by the home-plate sensors' offsets (phase 0.0) until the end of the reach defined by the slot sensors' onsets (phase 1.0). For example, a reach muscle onset (R.1 on.) was normalized between the start (R= -Y ms) and the end of the reach phase defined by the slot's sensors, corresponding to the reach duration (= abs (-Y)) (Fig. 2B):

$$Normalized\ Reach\ Onset\ burst = \frac{R.1\ on. + abs\ (-Y)}{abs\ (-Y)}$$

Similarly, Grasp phase was normalized from the start of the grasp defined by the slot sensor onset (1.0) until the end of the first digit flexion defined by selected muscle burst offset (2.0) (intD1 for Monkey 1; FDC for Monkey 2). For example, grasp muscle onset (G.1 on.) was normalized between the start (G= 0 ms) and the end of the grasp phase defined by the offset of selected muscle (Z. off. = Z ms), corresponding to the grasp duration (= Z ms) (Fig. 2B):

$$Normalized\ Grasp\ Onset\ burst = \frac{G.1\ on. + Z}{Z}$$

After burst onset and offset times were normalized, data were plotted in phase space in which the phase offset of a given burst was plotted as a function of its onset. Each data point was formed pairwise in the phase space (x axis = onset, y axis = offset) and each burst formed a cluster. In addition, each burst was described by its centroid. The centroid of the encompassing rectangle is defined by the mean (onset = A, offset = B) phase vector, and the associated pair of standard deviations (Std's: stdA, stdB). Hence, each burst is assumed equivalent to the encompassing rectangle [- stdA, stdA] × [- stdB, stdB] around the centroid mean values [A, B] (Krouchev et al., 2013). Each recorded muscle exhibited one or more burst of activity during the movement. A rectangle showing the mean onset and offset of the activity was drawn around each burst. These rectangles will allow us to use an associative clustering analysis in the near future.

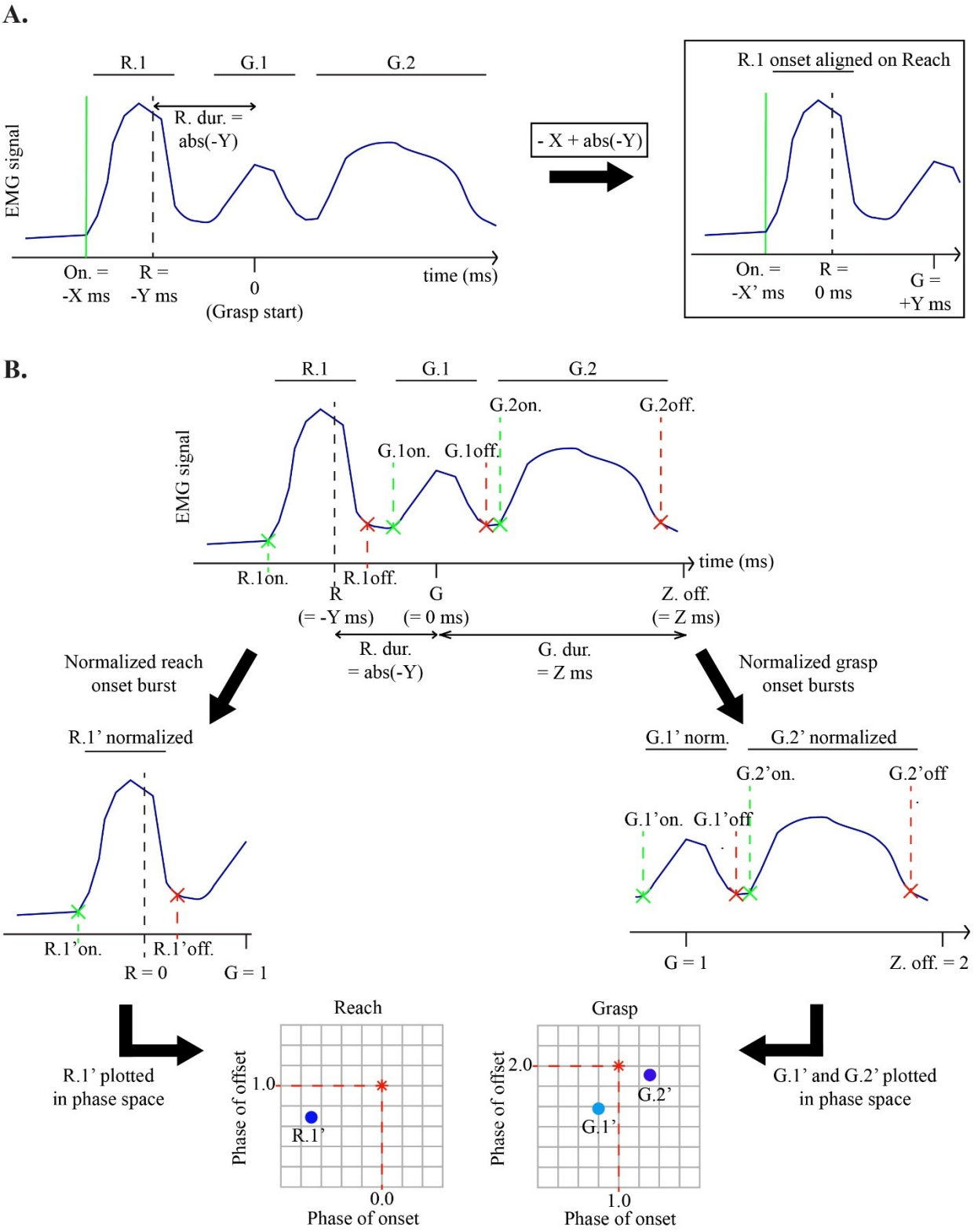


Figure 2: A schematic illustrating how burst onset and offset times for latencies and phase-plot were calculated. A, The start of the reach event (R) and reach-related burst onset (On.) occurred prior to the start of the grasp (G) and were expressed in millisecond with negative values. For each trial, the reach-related burst onset was re-aligned on the start of the reach using the reach duration (abs (-Y)). **B,** Onsets and offsets of measured periods of EMG activity were expressed as a proportion of the normalized period. For each trial, reach-related burst onset and offset (R.1 on., and R.1 off.) were normalized between the start (R) and the end (G) of the reach phase of the movement. The grasp-related onsets and offsets (G.1 on., G.1 off., G.2 on., and G.2 off.) were normalized between the start (G) and the end (Z. off.) of the grasp phase of the movement. Then burst of EMG activities were plotted in phase space.

Chapter 3 – Results

This project was focused on the rapid behavioral and muscular changes that occurred after single injection of Muscimol that produced partial inactivation of the M1 hand area. Behavior and EMG activities in both condition of the contralateral arm (RV, RH) from 12 experiments were analyzed and compared before (i.e. Pre) and minutes following the inactivation (i.e. Post).

The execution of the movement before inactivation

After training, the monkeys became highly stereotyped in performing the reach-to-grasp movement cumulating in a precision pinch with either arm. The size of the well's forced the monkeys to grasp the pellets with a precision grip, generally using the thumb in opposition with the index. Retrieval from the horizontal orientation was more difficult than the vertical ones for Monkey 2, because it involved a forearm supination. But in the case of Monkey 1, the start hand was in neutral position (Fig. 6A *left-top photo*). Therefore, to retrieve a pellet from the vertical orientation required a forearm pronation. Since they had a different start positions they were not doing the same movement depending on the conditions (Vertical, Horizontal) and the data were analyzed and presented separately.

After unilateral M1 inactivation, both monkeys showed impairments in the reach-to-grasp task. Depending on the severity of the impairments the monkeys showed from one up to several behavioral changes, in (i) the success rate, (ii) the number of flexion per retrieval, (iii) the duration of the first flexion, (iv) the type of grasping configuration per trial, (v) the duration in reach and grasp phase of the movement.

1. Evaluation of the success rate of the reach-to-grasp task

The intact animal in Pre showed a high success rate above 90% in both conditions. Monkey 1 had a mean success rate of 98.49% and 93.6% for RV and RH condition respectively. Monkey 2 had a mean success rate of 100% and 97.71% for RV and RH condition respectively. Therefore, monkeys can successfully retrieve and eat the pellet with a stereotyped movement.

We made single injection of Muscimol that produced only partial inactivation of the M1 hand area. In general, for all injection sites, we collected our data between 13 to 45 minutes after the offset of the injection. Animals were able to perform at least some trials and up to a complete block of 25 trials with the contralesional hand, albeit with some difficulties. Altogether, data showed a spectrum of deficits from no visible deficits in Post with movements similar to those observed in Pre, to greater deficits with clear struggles to retrieve the pellet. With this variability between experiments we subdivided our post-inactivation data in two groups based on the success rate: the "mild group" that included high success rate experiments > 80% with no visible deficit or only a moderate one deficit, and the "severe group" that included experiments with success rate <80% and clear deficits. For Monkey 1 in the RV condition, all experiments in Post stayed above 80% of success rate and therefore were categorized as one mild group (Fig. 3A). In the case of Monkey 2, a clear separation was visible between experiments with high and low success rate in Post (Fig. 3B). Experiments with a success rate higher than 80% in Post were included in the mild group, and experiments with lower success rate were included in the severe group (Fig. 3D, E). Finally, in the case of Monkey 1 in the RH condition, experiments above 80% of success rate were included in the mild group. The experiments below this limit were included in the severe group (Fig. 3C).

The success rate of the contralesional arm decreased following inactivation for Monkey 1 in horizontal condition and for Monkey 2 in both conditions. Monkey 1 showed no significant change in the mean success rate (98.4% +/- 2.19) in the vertical condition (14.83 minutes +/- 2.86 min after the offset injection, Fig. 3A) after inactivation. In the horizontal condition, the overall result showed a non-significant decrease of successful trials in Post (20.50 minutes +/- 3.78 min after the offset of the injection, Fig. 3B, $p=0.1073$) from 94% to 65.69%. With a success rate at 0% for one experiment (brown square) the animal performed a complete different movement and this

data was excluded from further analysis. Only the severe group showed a significant decrease in the mean success rate in Post with 68% of success (p=0.0014), and the mild group shows a success rate of 86.23% in Post (Fig. 3C).

The success rate of the paretic arm decreased significantly for Monkey 2 in both conditions. There was a significant drop in the mean success rate in the RV condition from 100% to 61.71% (25.29 minutes +/- 1.89 min after the offset of the injection, Fig. 3B, p=0.0267). There was a significant decrease in the mean success rate for the severe group with 20% of success (n=3 experiments, p=2.2489e-07, Fig. 3D) and a slight decrease for the "mild" group with 93% of success (n=4 experiments, p=0.0104, Fig. 3D). This was the only mild group that showed a significant decrease with a high success rate in Post.

In the horizontal condition, the overall result showed a significant decrease from 97.71% to 38.44% (39.43 minutes +/- 4.79 min after the offset of the injection, Fig. 3B, p=0.0017). With a success rate at 0% for one experiment (orange circle) and one experiment (blue star) with a complete different movement executed by Monkey 2, lead to data that couldn't be comparable and were excluded from further analysis. The severe group showed a significant and biggest drop of success rate with a mean of 28% of success (n=3 experiments with square, circle and cross symbol, p=3.7980e-06, Fig. 3E), but there was no significant change for the mild group with 91% of success in Post (n=2 experiments, Fig. 3E).

Our results demonstrated that partial inactivation of M1 could impair the reach-to-grasp movement as measured by the significant decrease of the success rate of the severe group. There was deficits in the contralateral hand therefore we assessed the motor impairments in the following sections.

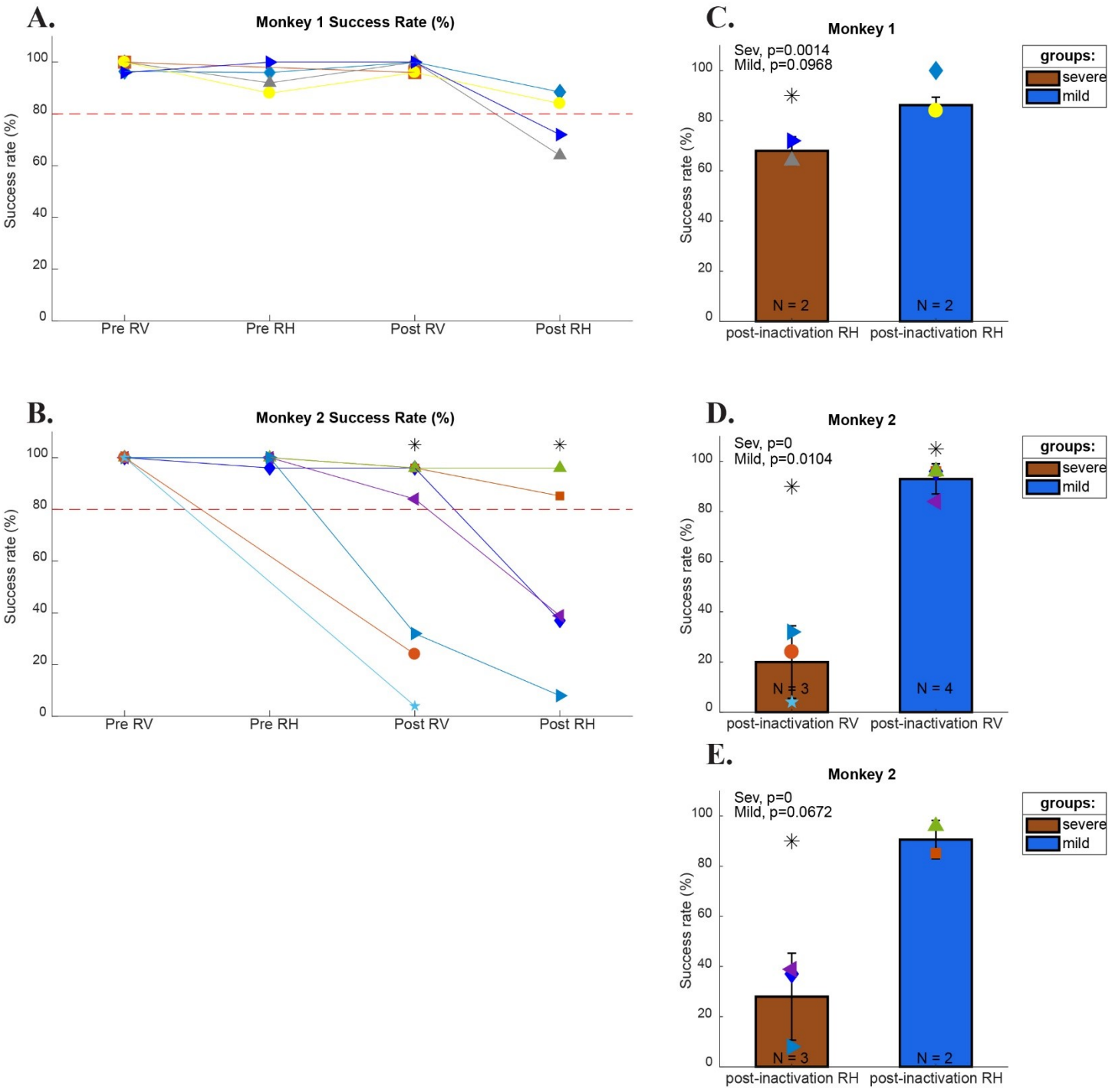


Figure 3: Monkeys Success Rate before and after inactivation. A, B, The curves represent the percentage of success rate of each step of our protocol. The data were represented in the same order than our protocol: Pre RV, Pre RH, Post RV, and Post RH. Each curve with their symbols represents one experiment. **C, D, E,** Comparisons of the Success rate (%) between Pre and Post data from the two different post-inactivation groups. The red bar represented the mean Success rate of the severe group, the blue bar the mean success rate of the mild group. Each symbol represented the mean Success rate of an individual experiment. A successful trial was defined as the retrieve of the pellet and successfully ate it by the monkey.

2. Number of flexions executed to grasp the pellet

The dexterity of the digits can be impacted by the M1 inactivation, leading to the monkey performing an increased number of finger flexions in order to grasp the pellet. Therefore, we counted the number of flexions executed by the monkey to retrieve the pellet from the well for both completed trials successfully and unsuccessfully. Significant increases in the number of flexions to retrieve the pellet demonstrate that there were deficits in the fine motor control of the hand following the inactivation.

Prior to inactivation (Pre), both monkeys mostly used one flexion of the index to grasp the pellet (Fig. 4). The only exception was Monkey 1 in the horizontal condition who most often than not executed 2 flexions (Fig. 4B *white bar*). The animal executed 2 flexions to retrieve the pellet in 81 trials, and 1 flexion in 32 trials (Fig. 4B *right panel, white bar*).

Following the inactivation both monkeys showed significant changes in the number of flexions. The Monkey 1, in the vertical condition, showed a small but significant increase from 1.38 in Pre to 1.58 in Post ($p=0.0356$, Fig. 4A *left panel*). Indeed, less trials were executed with 1 flexion and more trials were executed with 3 to 5 flexions (Fig. 4A *right panel, black bars*). In parallel, Monkey 2 showed a significant increase in the mean number of flexions per retrieval in the vertical condition with 1.19 mean flexion of the index in Pre to 1.58 in Post ($p=2.6805e-05$, Fig. 4C *left panel, white and black bars*). Less trials were executed with 1 flexion, and more trials were executed with 2 to 9 flexions to retrieve the pellet (Fig. 4C *right panel, black bars*).

These changes were more pronounced in the Horizontal condition. For Monkey 1, overall the results show a significant increase with 1.85 in Pre to 2.36 mean flexion per retrieval in Post ($p=5.2132e-05$, Fig. 4B *left panel, white and black bars*). This was due to fewer trials being executed with 1 or 2 flexions, and more trials executed with 3 to 5 flexions to retrieve the pellet (Fig. 4B *right panel, white and black* bars). Furthermore, overall there was also a large increase in the mean number of flexions per retrieval in Post for the horizontal condition, with 1.28 in Pre to 2.84 in Post ($p=1.5471e-17$, Fig. 4D *left panel, white and black bars*). In this case fewer trials were executed with 1 flexion, and more trials were executed with 2 to 10 flexions to retrieve the pellet (Fig. 4D *right panel, white and black* bars).

These changes were driven by the severe group, not the mild group, in all cases except for the Monkey 2 in the horizontal condition. For Monkey 1, in the horizontal condition, changes were

explained by the increased flexions observed in the severe group with 1.85 in Pre to 2.66 mean flexions of the index in Post (p=3.7139e-08, Fig. 4B *left panel, white and red bars*); there was an equivalent amount of trials executed with 1 to 4 flexions, and 3 trials executed with 5 flexions to retrieve the pellet (Fig. 4B *right panel, red bars*). In contrast, there was no significant change in number of flexions for the mild group (Fig. 4B *left panel, white and blue bars*), as most of the trials were executed with 1 or 2 flexions like in Pre (Fig. 4B *right panel, blue* bars). For Monkey 2 in the vertical condition, similar to Monkey 1, changes were due to the significant increase of the severe group with 1.19 in Pre to 1.97 flexions of the index in Post (p= 5.2608e-10, Fig. 4C *left panel, white and red bars*). In this condition only 37 trials were executed with 1 flexion, whereas more trials were executed with 2 to 9 flexions to retrieve the pellet (Fig. 4C *right panel, red bars*). In contrast, there was no change in flexions for the mild group (Fig. 4C *left panel, white and blue bars*) and most of the trials executed with 1 flexion to retrieve the pellet like in Pre (Fig. 4C *right panel, blue* bars). These results demonstrate that there is a loss in fine digit control for the severe group. Furthermore, in the case of Monkey 2 in the horizontal condition, the changes were significant both for the severe group with 1.28 in Pre to 3.36 flexions (p=1.6527e-20, Fig. 4D *left panel, white and red bars*), and for the mild group, with 1.28 in Pre to 2.12 flexions of the index in Post (p=7.858e-11, Fig. 4D *left panel, white and red bars*). Indeed there were less trials executed with 1 flexion to retrieve the pellet compared to Pre. The severe group showed more trials with 2 to 10 flexions, and the mild group showed more trials with 2 to 5 flexions to retrieve the pellet (Fig. 4D *right panel, red and blue bars respectively*).

In summary, the severe groups of Monkey 1 and 2 and the mild group of Monkey 2 RH showed an increase of the number of flexions after inactivation, with this change being greater in the horizontal than the vertical conditions. These increases indicated a loss of manual dexterity. To assess if this was associated with slowness of the hand, we evaluated the duration of the first index flexion.

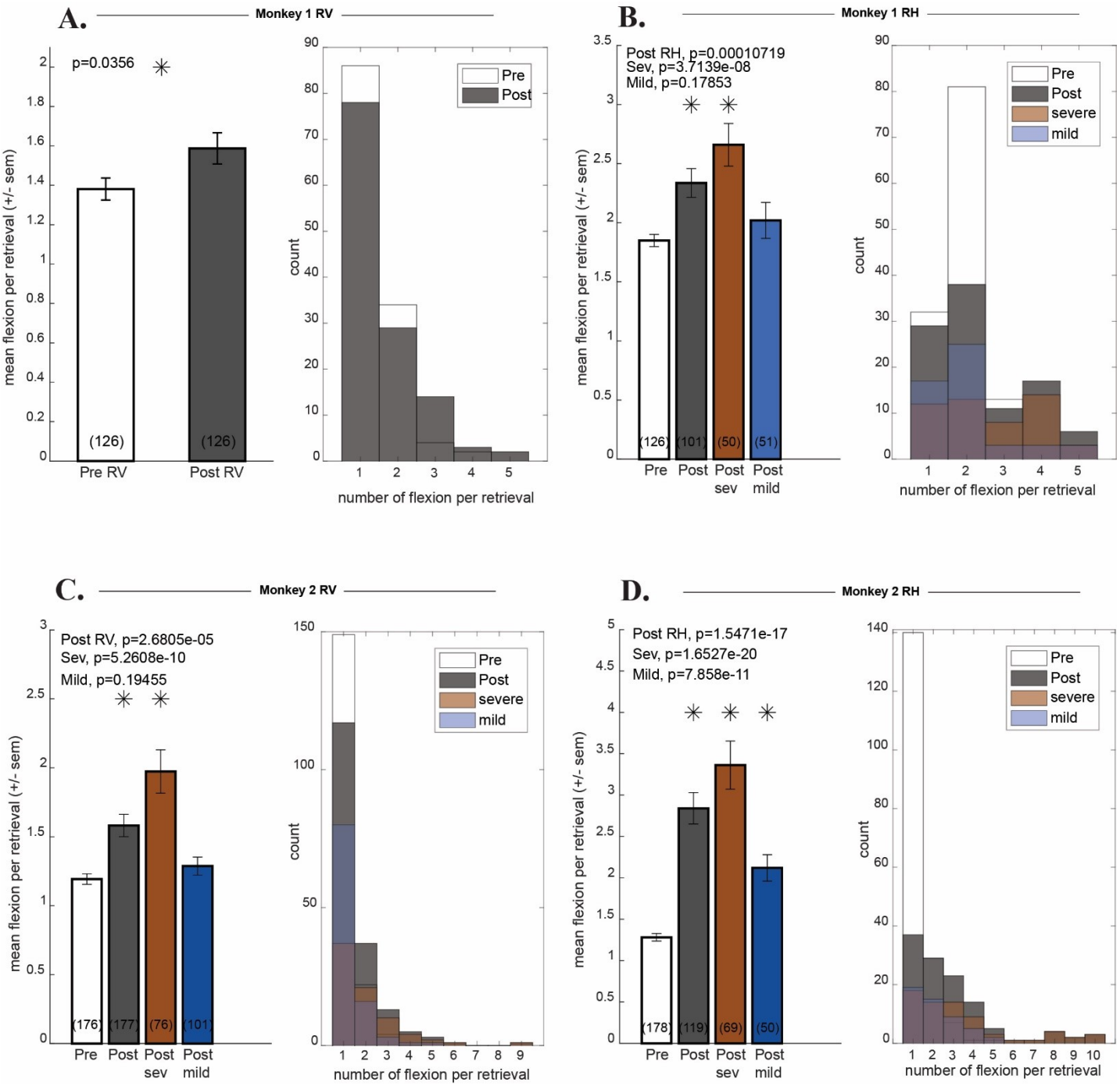


Figure 4: Monkeys' number of flexions before and after inactivation. A frame-by-frame video analysis was performed to count the number of flexions made by the monkeys while their fingers were inside the slot per trial. The bars in white represent the mean number of flexions in Pre, the black ones represent all experiments together in Post, the red ones represent only data from severe experiments, the blue ones represent only data from mild experiments. **A, B, C, D *left panels,*** the number of flexions are expressed in mean +/- standard error. Comparisons between Pre and Post were performed using unpaired t-tests. **A, B, C, D *right panels,*** histograms that represent the count of trials depending on the number of flexion per retrieval.

3. Evaluation of the duration of the first flexion to grasp the pellet

The M1 inactivation affected the duration of the first flexion of the index finger exerted by animals to retrieve the pellet. While this increased duration was observed in all experiments, these changes were most pronounced for the severe group and in the horizontal condition, where flexion durations were almost double what was observed in most experiments.

Prior to the inactivation (Pre), the mean duration of the first flexion was different between monkeys. In the vertical condition, it lasted 280.69 ms for Monkey 1 and 429.36 ms for Monkey 2 on average (Fig. 5A and C *left panel, white bar* respectively). In the horizontal condition both monkeys took longer to perform the first flexion. The mean duration of the first flexion was 336.96 ms for Monkey 1 and 544.53 ms for Monkey 2 (Fig. 5B and D *left panel, white bar* respectively).

Regardless of the overall differences in movement duration between monkeys, after the inactivation they both showed significant increases in the mean duration of the index's first flexion. In the vertical condition Monkey 1 showed a small but still significant increase from 280.69 ms in Pre to 319.58 ms in Post (p=0.0251, Fig. 5A *left panel, white and black bar respectively*). This was due to an increase in Post of trials lasting between 300-500 ms, with a concomitant decrease in the number of trials with a duration between 200-300 ms (Fig. 5A *right panel, black bars*).

Monkey 2 also showed a significant increase in the mean flexion duration in the vertical condition, from 429.36 ms in Pre to 550 ms in Post (p=3.3183e-07, Fig. 5C *white and black bars respectively*). There were less trials between 300-500 ms and more trials between 700-1200 ms (Fig. 5B *right panel, black bars*). When looking at the different groups of deficits, for the severe group there was a mean of 601.75 ms (p=1.1931e-08, Fig. 5C *red bar*) which was 172.40 ms longer than what was seen in Pre. A majority of trials were between 700-800 ms (Fig. 5C *right panel, red bars*). In the mild group, there was a mean of 511.44 ms (p=4.0431e-04, Fig. 5C *blue bar*) which was 82.09 ms longer on average than what was seen in Pre. Most of the trials were between 300-600 ms (Fig. 5C *right panel, blue bars*).

Changes in flexion duration were also observed in the Horizontal condition, and were more pronounced than those that occurred in the Vertical condition. Monkey 1 showed an overall significant increase of the duration of the first flexion from 336.96 ms in Pre to 555.03 ms in Post (p=4.5753e-13, Fig. 5B *white and black bars respectively*), while Monkey 2 showed an overall significant increase from 544.53 ms in Pre to 845.1 ms (p= 2.5335e-10, Fig. 5D *white and black*

bars respectively). Both monkeys showed a greater number of trials with a longer duration than what was seen in Pre. Most of the trials were between 300-600 ms for Monkey 1, and 600-800 ms for Monkey 2 (Fig. 5B and D *right panel, black bars* respectively).

These changes were observed both in the severe and the mild group, and were more pronounced in the former. Indeed, in Monkey 1 for the severe group there was a mean flexion duration of 690.66 ms (p= 3.1283e-16, Fig. 5B *red bar*) after inactivation, which was 353.70 ms longer than what was seen in Pre. Most of the trials were between 400-500 ms with some trials lasting up to 1600 ms (Fig. 5B *right panel, red bars*). In the mild group, there was a mean flexion duration of 515.69 ms (p= 2.2582e-07, Fig. 5B *blue bar*) after inactivation which was 178.73 ms longer than what was seen in Pre. Most of the trials were between 300-600 ms (Fig. 5B *right panel, blue bars*). Furthermore, for Monkey 2 the severe group showed a mean of 890.34 ms in Post, which represents a mean increase of 345.81 ms relative to Pre (p= 6.1962e-10, Fig. 5D *red bar*). This group had a majority of trials between 600-700 and 1100-1200 ms, with some trials lasting up to 2400 ms (Fig. 5D *right panel, red bars*). The mild group had also a significant increase of flexion duration, with a mean of 782.66 ms in Post, an increase of 238.13 ms relative to Pre (p= 2.0695e-07, Fig. 5D *blue bar*). Most of the trials were between 700-900 ms (Fig. 5D *right panel, blue bars*).

In contrast to the previous results section, both groups and monkeys showed an increase of duration of the first index flexion after inactivation. These increases indicated a slower and/or weaker movement. Since these deficits in contralateral hand use lead the monkeys to alter their grasping strategies to grip the pellet, we therefore we assessed the type of grasping they performed after inactivation.

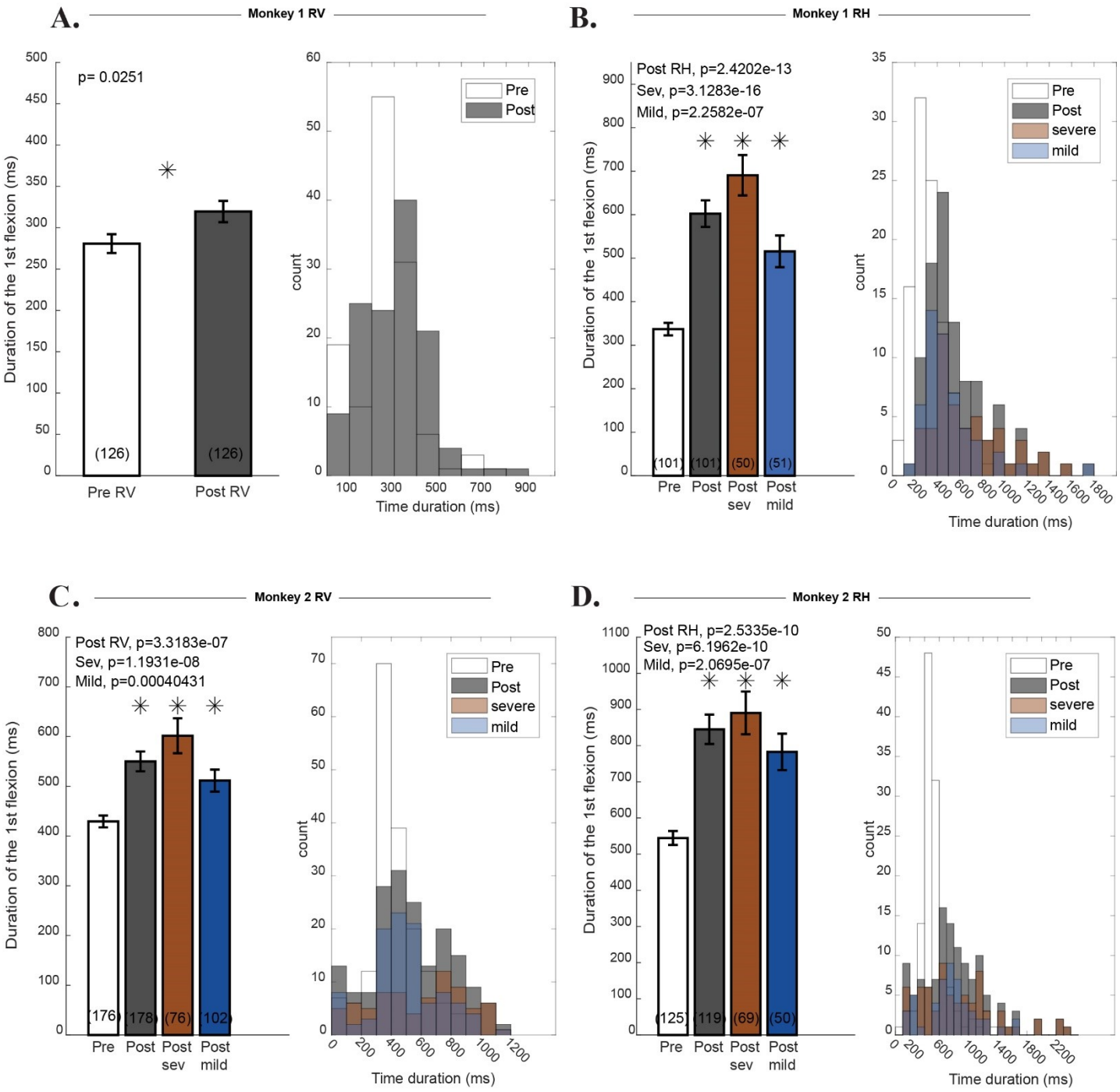


Figure 5: Monkeys duration of the first flexion of the index finger before and after inactivation. A, B, C, D, shown are the durations of the first flexion for Monkey 1 (A,B) and Monkey 2 (C,D) during the vertical condition (A,C) and the horizontal condition (B,D). ***Left panels,*** the duration of the 1st flexion done by the index to retrieve the pellet was calculated based on a frame-by-frame video analysis (1second = 30 frames; see methods). The duration of the 1st flexion is expressed as a mean +/- standard error and comparisons between Pre and Post data were performed using unpaired t-tests. ***Right panels,*** histograms that represent the count of trials depending on the time duration of the first flexion. Each bar represent 100 ms.

4. The effect of inactivation on the grasping configuration of the contralateral arm

The execution of grasping movements depends on the integrity of the primary motor cortex (M1). Prior to M1 inactivation, monkeys performed the task with a normal precision-grip (N-PG, Fig. 6B). Following inactivation the capacity to grasp the pellet was impaired and such that alternate grasping configurations were used by Monkey 1 for the horizontal condition and by Monkey 2 in both conditions. These alternate grasping configurations included the use of the other phalanx of the thumb in abnormal precision-grip (AN-PG); or the use of other digits in abnormal precision-grasp (AN-PGrasp) and abnormal power-grasp (AN-PoG) (Fig. 6B, *see methods for definitions*).

Before inactivation, and despite different hand starting positions (Fig. 6A), monkeys executed a normal precision (N-PG) grip to retrieve the pellet from the well more than 80% of the time. More precisely, Monkey 1 performed this grip 98.40% of the time in the vertical condition, and 81.38% in the horizontal condition. Monkey 2 performed this grip 98.31% of the time in the vertical condition, and 99.20% in the horizontal condition (see Table 1).

Following inactivation both monkeys showed changes in the grasping configuration of the contralateral arm, except for the Monkey 1 in the vertical condition. In the vertical condition Monkey 1 showed a high percentage of N-PG configuration use to grasp and retrieve the pellet from the well in Pre (98.40%) and in Post (96%). There was only a small increase of abnormal precision grip from 0.8% in Pre to 4% in Post (see Table 1). In contrast, in the vertical condition Monkey 2 showed changes in the grasping configuration with an overall decrease of N-PG use from 98.31% in Pre to 49.77% in Post. In parallel the overall results showed there was a small increase in the use of the AN-PG configuration from 1.14% in Pre to 12.82% in Post, and an increase in the use of the AN-PGrasp configuration from 0.56% in Pre to 33.94% in Post (see Table 1).

These changes were more pronounced in the horizontal condition for both monkeys. In this condition Monkey 1 showed an overall decrease in the use of the N-PG configuration from 81.38% in Pre to 45.5% in Post. In parallel there was an increase in the use of abnormal grasping configurations, with an increase in AN-PG (Pre = 8.69% and Post = 32.62%) and AN-PGrasp configuration use (Pre = 7.96% and Post = 20.88%, see Table 1). Similarly, Monkey 2 showed an

overall decrease of N-PG configuration use from 99.20% in Pre to 34.84% in Post. Furthermore the overall results showed an increase of AN-PG (Pre = 0.8% and Post = 20.21%), AN-PGrasp (Pre = 0% and Post = 17.30%), and AN-PoG configuration use (Pre = 0% and Post = 21.24%, see Table 1).

These changes were mostly driven by the severe group that showed the largest changes, but could also be seen in the mild group. The Monkey 1 in the horizontal condition showed a decrease of N-PG for both group with only 40% in Post for the severe group, associated with an increase of AN-PG (Pre = 8.69% and Post = 36%), AN-PGrasp (Pre = 7.96% and Post = 22%), and AN-PoG (Pre = 0.96% and Post = 2%). The mild group showed a decrease of N-PG to 51% in Post, with an increase of AN-PG (Post = 29.23%) and AN-PGrasp (Post = 19.77%) (See Table 1). The Monkey 2 in the vertical condition showed a decrease in the use of N-PG for both groups with only 27.44% for the severe group and 66.52% for the mild group. The severe group showed largest changes with an increase of AN-PG (Pre = 1.14% and Post = 10.31%), AN-PGrasp (Pre = 0.55% and Post = 56.92%), and AN-PoG (Pre = 0% and Post = 5.33%). The mild group showed a decrease of N-PG to 66.52% in Post, with an increase of AN-PG (Post = 14.70%) and AN-PGrasp (Post = 16.70%) (See Table 1). The Monkey 2 in the horizontal condition showed a decrease of N-PG for both groups with only 19.41% in Post for the severe group, associated with an increase of AN-PG (Pre = 0.8% and Post = 12.35%), AN-PGrasp (Pre = 0% and Post = 23.51%), and AN-PoG (Pre = 0% and Post = 34.07%). The mild group showed a decrease of N-PG to 58% in Post, with an increase of AN-PG (Post = 32%), AN-PGrasp (Post = 8%) and AN-PoG (Post = 2%) (See Table 1).

In conclusion the ability to execute a normal precision-pinch was impacted following the inactivation. In the worst cases, there was a loss of independent finger movement as shown by the increase of AN-PGrasp and AN-PoG configurations, where all the digits moved together.

Tableau 1. – Grasping configurations before and after inactivation reported in percentage

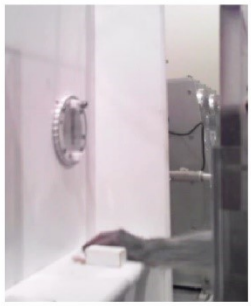
			Monkey 1 - RV	Monkey 1 - RH	Monkey 2 - RV	Monkey 2 - RH
N-PG	Pre		98,40%	81,38%	98,31%	99,20%
	Post	All	96%	45,50%	49,77%	34,84%
		Severe	n/a	40,00%	27,44%	19,41%
		Mild	n/a	51,00%	66,52%	58%
AN-PG	Pre		0,80%	8,69%	1,14%	0,80%
	Post	All	4%	32,62%	12,82%	20,21%
		Severe	n/a	36%	10,31%	12,35%
		Mild	n/a	29,23%	14,70%	32%
AN-PGrasp	Pre		0,80%	7,96%	0,55%	0%
	Post	All	0%	20,88%	33,94%	17,30%
		Severe	n/a	22%	56,92%	23,51%
		Mild	n/a	19,77%	16,70%	8%
AN-PoG	Pre		0%	0,96%	0%	0%
	Post	All	0%	1%	2,29%	21,24%
		Severe	n/a	2%	5,33%	34,07%
		Mild	n/a	0%	0%	2%

The categorization of the grasping was defined by the shape of the grasp used to dislodge pellets from the target based on the frame-by-frame video analysis for each trial. The data were reported in percentage for both monkeys and conditions.

A. Hand start position:

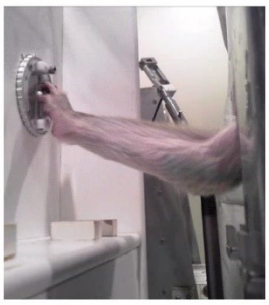
Monkey 1 right hand

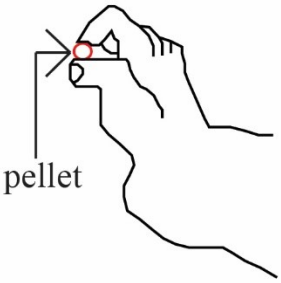
Monkey 2 right hand

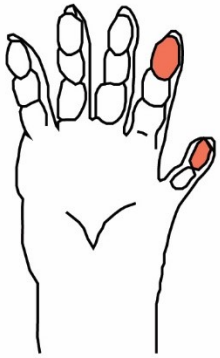


B. Grasping configurations:

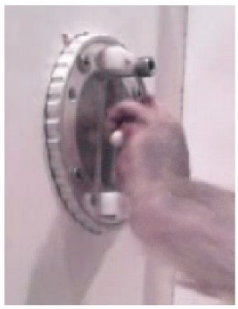
Normal precision-grip
(N-PG)

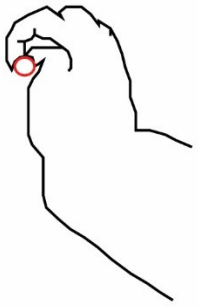


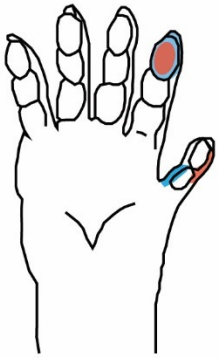




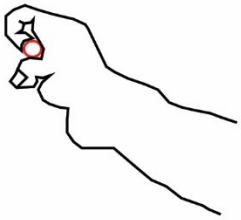
Abnormal precision-grip
(AN-PG)

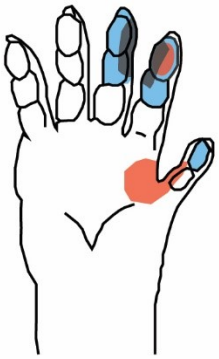




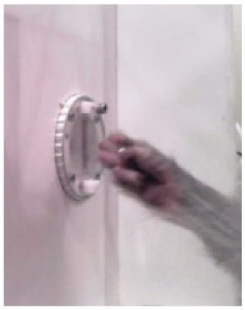


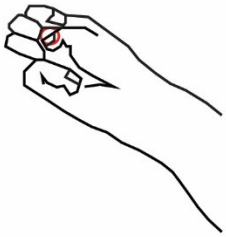
Abnormal precision-grasp
(AN-PGrasp)





Abnormal power-grasp
(AN-PoG)





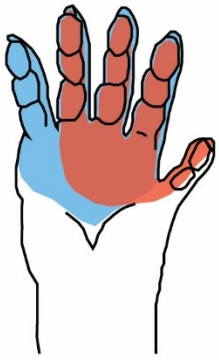


Figure 6: The hand start position and the grasping configurations used during the reach-to-grasp task before and after inactivation. A, photos of the hand start position. Monkey 1 showed a neutral hand start position that required a forearm pronation to retrieve a pellet from a Vertical orientation. **B,** photos and cartoons of the different grasping configurations. For each grasping configuration, the surface area of contact with the pellet represented in the photos are shown in *red* on the hand diagram. The other possible surface area of contacts observed were represented in *blue,* and in *black* (only for AN-PGrasp), on the hand diagram.

5. The effect of inactivation on the reach and grasp phase duration

The duration of the reach and grasp phases were measured using the sensors of the task and compared before and after the inactivation to see if the single Muscimol injection had an effect on the speed of the movement. Following inactivation, the reaching and the grasping components of the movements were impacted. The Contact time was significantly increased in Post for both monkeys, with greater increases for the severe group than the mild group. This can be explained by the injections having targeted the digit area of M1 and thus impacting the grasping ability of monkeys in Post. There was also a more moderate, but significant change, in the reach duration for both conditions in Monkey 2 and in the horizontal condition in Monkey 1.

Prior to inactivation the animals executed the reach-to-grasp task with a high success rate and with a certain speed. The reach and grasp phase duration were similar between monkeys. In the vertical condition, the mean reach duration was 215.59 ms for Monkey 1 and 159.1 ms for Monkey 2. The mean Contact time duration was 738.77 ms for Monkey 1 and 838.33 ms for Monkey 2 (Fig. 7A, B). In the horizontal condition, the mean reach duration was 151.91 ms for Monkey 1 and 151.87 ms for Monkey 2. The mean Contact time duration was 1157.8 ms for Monkey 1 and 1096.4 ms for Monkey 2 (Fig. 7C, D).

In the vertical condition Monkey 1 showed only a significant change during the grasp phase of the movement, with a mean increase of the Contact time of 100.68 ms in Post ($p=0.0188$, Fig. 7A).

In the horizontal condition of Monkey 1 showed changes in both the reach and grasp phases of the movement in Post, with the biggest change occurring during the grasp phase. Following inactivation, the mean reach phase duration significantly increased to 163.06 ms ($p= 2.4240e-05$, Fig. 7C *left panel, black bar*). The severe group showed a mean reach duration of 159.72 ms ($p= 0.0195$) and the mild group a mean of 166.03 ms ($p= 2.8388e-06$, Fig. 7C *left panel, red and blue bars respectively*). The greatest changes occurred during the grasp phase of movement after the inactivation. Indeed the mean Contact time for all data significantly increased up to 1682.3 ms in Post ($p= 3.6166e-08$, Fig. 7C *right panel, black bar*). The severe group showed the biggest significant increase with a mean duration of 2026.4 ms after inactivation ($p= 9.3723e-13$, Fig. 7C *right panel, red bar*), corresponding to an increase of 868.6 ms. The mild group also showed a significant increase with a mean of 1398.5 ms ($p= 0.0053$, Fig. 7C *right panel, blue bar*), corresponding to an increase of 240.7 ms.

The Monkey 2 showed significant changes in both conditions and in both phases of the movement in Post, with the biggest changes occurring for the severe groups.

In the Vertical condition, the mean reach phase duration significantly increased to 175.27 ms following inactivation (p= 2.2462e-04, Fig. 7B *left panel, black bar*). This was due to a significant increase of the reach duration in the severe group with a mean of 183.34 ms (p= 8.1109e-05, Fig. 7B *left panel, red bar*) and in the mild group with a mean of 170.58 ms (p= 0.0185, Fig. 7B *left panel, blue bar*) in Post. In addition, there were also significant changes in the grasp phase duration of the movement. The mean Contact time for all data significantly increased to 1179.3 ms in Post (p= 9.1168e-09, Fig. 7B *right panel, black bar*). The severe group showed the biggest change with a mean Contact time of 1436.3 ms in Post (p= 1.4550e-14, Fig. 7B *right panel, red bar*). The mild group also showed a significant increase of the mean Contact time with a mean of 1024.6 ms in POST (p= 7.6917e-04, Fig. 7B *right panel, blue bar*) in Post.

In the Horizontal condition, the mean reach phase duration for all data significantly increased to 170.31 ms following inactivation (p= 2.2559e-04, Fig. 7D *left panel, black bar*). This was due to a significant increase for the severe group with a mean reach phase duration of 191.49 ms in Post (p= 1.6082e-09, Fig. 7D *left panel, red bar*). There was no significant change for the mild group. The grasp phase of the movement showed important changes in Post. Indeed, the mean Contact time for all data showed a significant increase with a mean of 1732.7 ms in Post (p= 5.9169e-08, Fig. 7D). This was due to significant increases for both the severe group with a mean of 1921.1 ms (p= 3.5130e-08, Fig. 7D) and for the mild group with a mean of 1506.7 ms (p=0.0038, Fig. 7D).

Our results demonstrated that both phases of the movement were impacted following inactivation for both monkeys. The only exception was the reach phase of Monkey 1 in the Vertical condition that remained unchanged. How were these behavioral changes supported by muscle activity? To answer this question we looked at the temporal activation patterns of arm and hand muscles as well as the coordination between them.

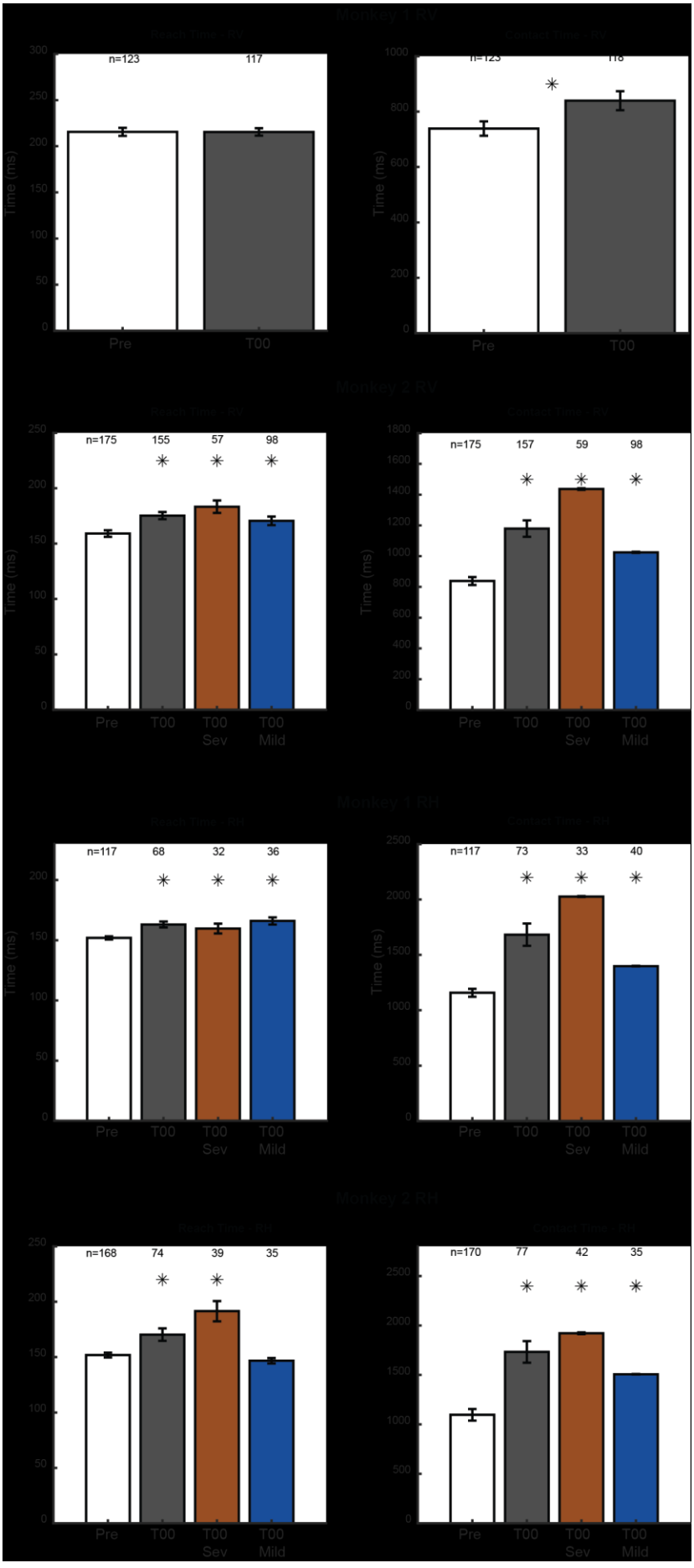


Figure 7: Changes in the reach and grasp phase duration after inactivation. The duration of the two phases were calculated from all trials based on the sensors of the task. The reach duration was defined as the time between the hand leaving the home plate (Home plate sensors Off) and the digits entering the slot (Slot sensors On). The grasp duration (i.e. Contact time) was defined as the time between the digits entering the slot (Slot sensors On) and leaving the slot (Slot sensors Off). The Contact time did not take into account the number of flexions, only the time spent by monkeys' digits inside the slot to retrieve the pellet. The data are expressed in mean +/- standard error and compared with an unpaired t-test. **A, B,** in Post RV both monkeys showed a significant increase in the Contact time. Only Monkey 2 showed a change in the reach phase duration as well. **C, D,** in Post RH both monkeys showed changes in the two phases of the movement. Those changes are more important in the Contact time and for the severe group.

6. Example of EMG signals with Monkey 2 in the Vertical condition

Monkeys had to perform a reach-to-grasp task that required a precise control of the limb trajectory, and of the coordination between digits. The Figure 8 shows examples of filtered and averaged EMG activity in Pre and Post of Monkey 2 during the reach-to-grasp task in the vertical condition (i.e. RV). All EMG signals were synchronized on the start of the grasp (i.e. slots' sensors ON = 0 ms).

Before inactivation, based on the average and smooth EMG signal, FDC had two reach-related bursts (Fig.8, R.1 and R.2) and two grasp-related bursts (Fig. 8, G.1 and G.2). For ED2, one reach-related burst was identified. However, a second grasp-related burst was sometimes visible. However, the offset of this burst was not stable across experiments. Thus, only the first burst was selected for analysis due to its consistence. The three last muscles (FCR, BB and FCU) had three bursts identified, one reach-related burst (Fig. 8, R.1) and two grasp-related bursts (Fig. 8, G.1 and G.2). For the cases of FCR and FCU in Pre, the grasp-related bursts labeled G.1 showed a double peak in the average and smooth EMG signals but was identified as one burst. Indeed, these peaks were not consistent across trials because the burst showed either the double peak, or one of the two peaks in individual trial. Therefore it was treated as one burst, which was consistent across trials. In contrast, FDC consistently exhibited the second reach-related burst (R.2) and G.1 across trials.

While each muscle had at least one burst, some muscles exhibited up to 4 bursts throughout the whole movement. Different behavioral deficits were observed following inactivation that could be due to changes in muscle activity. With regards to analysis we first looked at the muscle temporal activation, to see any modification in the timing at the start of the two phases of the movement. Then, we looked at the coordination between muscles during the whole period.

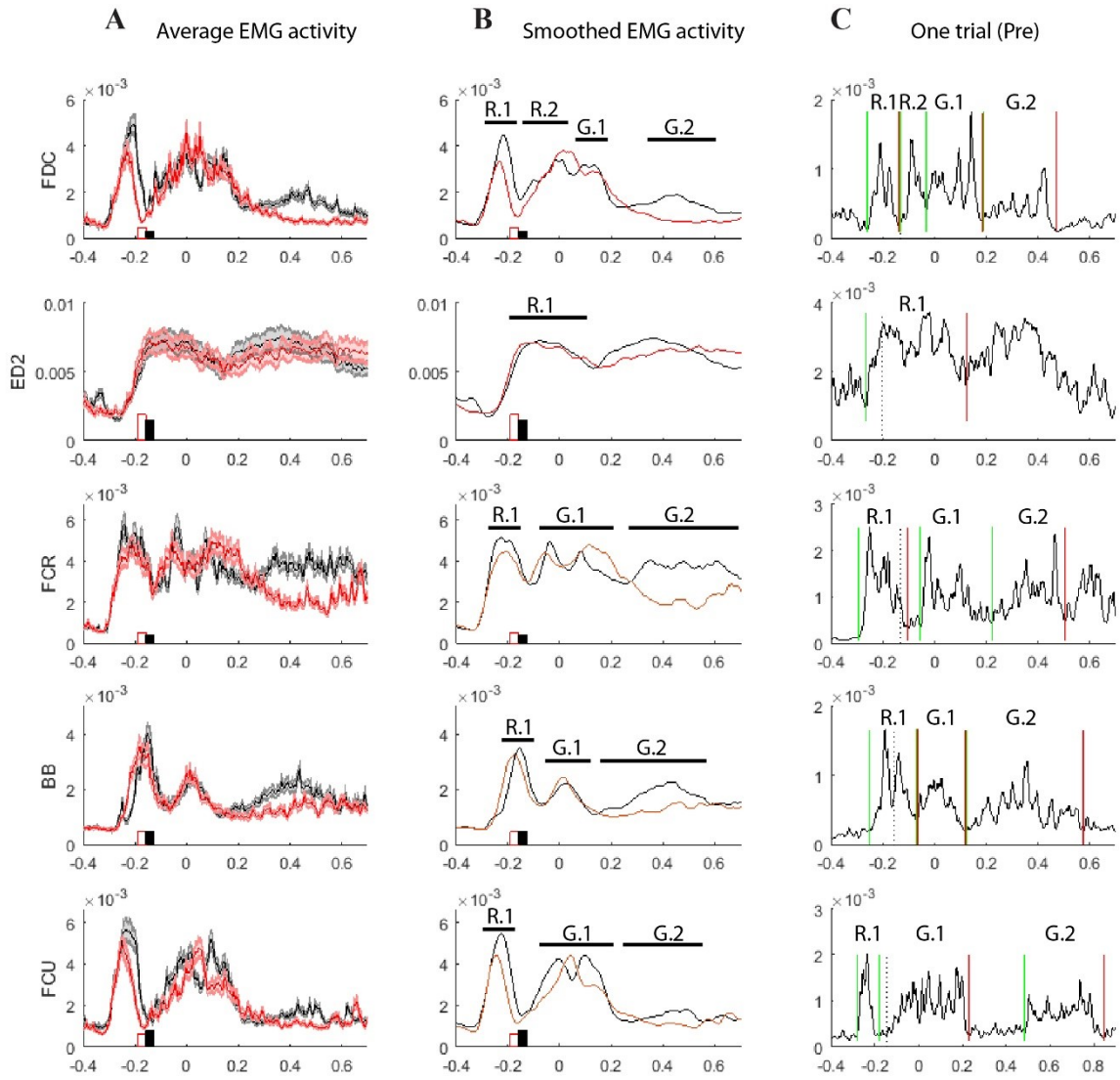


Figure 8: Comparison of the EMG activity in Pre and Post of Monkey 2 during the reach-to-grasp task in Vertical condition. Black traces represented the signal in Pre. Red traces represented the signal in Post. **A,** Average EMG activity with shaded error bar from one block in Pre (n=17 trials) and one block in Post (n=25 trials). These blocks came from the same experiment. On the x-axis was represented the mean start of Reach (+/- 1 standard deviation) in Pre with the black rectangle, and in Post with the red rectangle. **B,** Same as in (A) but smoothed (lowpass filter with a span of 200). **C,** Example of one trial per muscles with the bursts' onsets and offsets marked. Onset was represented by a green line and offset by a red line. A green line was visible if an offset and an onset of a following burst was the same. A red line was predominantly visible if offset and onset partially overlap. The dotted line represented the start of the Reach for this trial.

7. The effects of partial M1 inactivation on the temporal activation of the contralateral arm's muscles

Muscle onset time, expressed in relation to the start of the movement, was used to reflect the temporal activation of the muscle during the reach-to-grasp task. A total of 7 muscles of the right arm were recorded from Monkey 1: Deltoid (*musculus deltoideus*), FCU (*flexor carpi ulnaris*), FCR (*flexor carpi radialis*), FDC (*flexor digit communis)*, ED23 (*extensor digitorum 2 and 3*), ADD1 (*adductor of the thumb)*, and intD1 (*first dorsal interosseus)*. These muscles showed a total of 17 bursts of EMG activity in the RV condition, and 12 bursts in the RH condition. A total of 5 muscles of the right arm were recorded from Monkey 2 with a total of 14 bursts of EMG activity in the RV and RH condition: BB (*biceps brachii*), FCU (*flexor carpi ulnaris*), FCR (*flexor carpi radialis*), FDC (*flexor digit communis)*, and EDC (*extensor digit communis)*.

Reach onset was re-aligned on the start of the reach (see Methods *Muscles Latencies*), and grasp onset was kept aligned on the start of the grasp. Overall, some muscles had one or two bursts during the reach phase, and/or the grasp phase. During the reach, muscles mostly exhibited one burst. Only the FDC of Monkey 2 showed two bursts (FDC#1 and FDC#2 respectively). The first burst's onset occurred before the start of the reach and the second onset occurred around the start of the reach. During the grasp, the first burst of grasp (grasp#1) was associated with the pre-shaping of the hand, and the second burst of grasp (grasp#2) was associated with the first flexion of the grasp phase.

For each muscle the mean latencies of muscle activation were always represented by crosses (+/- 1 standard error). The mean latency observed in Pre was represented by a black cross. After inactivation, the mean latency observed for the mild group was represented by a blue cross, the severe group was represented by a red cross, and both combined by a black cross (mild + severe). The mean latencies observed in Post were represented on the figures in an upper line relative to Pre (see Fig. 9, 10, 11, 12). If there was significant change in the mean latency after inactivation ($p<0.5$, Unpaired t-test), a star was displayed with the same color code. In addition, a blue star was used to represent a significant change between Pre and the mild group, a red star between Pre and Post for the severe group, and a black star between Pre and Post for all the data combined.

The muscle temporal activation was exhibited in a sequential or co-activated manner. The sequential activation was defined as onsets of two muscles that occurred separately but in succession; co-activation was defined as onsets of two muscles that occurred simultaneously.
These muscle sequential activations or co-activations were dependent on the phase of the movement. They were mostly sequential during the Reach, and mostly co-activated during the grasping of the pellet.

a) Reach

The reach was self-initiated and, in some conditions, required a rotation of the forearm. This rotation of the forearm during the reach required more coordination between distal muscles to obtain a proper position of the hand before entering into the slot and retrieve the pellet. A forearm rotation was required for Monkey 1 in RV condition and for Monkey 2 in RH condition. This was due to the different hand start position between monkeys. Indeed, Monkey 1 showed a start hand position that was lateral and required a pronation of the forearm in the RV condition (Fig. 6A). The Monkey 2 showed a start hand position that was flat on the platform, and thus required a supination of the forearm in the RH condition (Fig. 6A). Overall, the mean reach duration was between 150 to 215 ms before inactivation (i.e. time between Home plate sensors off and the slot sensors on).

During the reach, the EMG burst onsets showed a sequential activation with rare co-activation burst onsets. In general the Reach was initiated by wrist/digit flexor muscles, followed by proximal muscles, digit extensor muscles, intrinsic hand muscles, and ended with the digit flexor FDC (Fig. 9A, 10A, 11A, 12A).
After inactivation, the temporal activations of muscles during reach became altered for both monkeys and conditions. The predominant pattern of significant changes was a delay in burst onset times, although in some rare cases we observed earlier burst onsets instead. A later onset was defined as an onset observed significantly later relative to Pre. By contrast, an earlier onset was defined as an onset observed significantly earlier relative to Pre. These significant changes were observed in at least half of the bursts in both monkeys and conditions.
Interestingly, in the case of Monkey 1 RV there was a high success rate, no changes in the reach duration, and no visible deficits after inactivation, but the sequential activation of the muscles

during the reach was altered (Fig. 9A). There were significant changes in 6 of the 7 bursts with later onsets for deltoid, ED23, FCU, FCR and ADD1, and one earlier onset for FDC (Fig. 9A).

The temporal activation was altered and the order of muscle recruitment changed in the severe group of Monkey 1 RH and Monkey 2 RV. For these two cases, there were visible behavioral deficits and no rotation of the forearm required. The Monkey 1 RH showed significant changes in 2 of the 4 bursts depending on the group. The severe group exhibited later onsets for ED23 and FCR (Fig. 10A). The Monkey 2 RV showed significant changes in 3 of the 6 bursts depending on the group. The severe group exhibited later onsets for FCU, FCR, and FDC #1. Moreover, a greater variability of onsets (especially for FCR and ED2) and more co-activation were observed between different muscles (Fig. 11A).

In addition, significant changes in muscle temporal activations were observed in the mild group of Monkey 1 RH and Monkey 2 RV. However, the order of muscle recruitment was preserved. The mild group in Monkey 1 RH exhibited earlier onsets for Delt and ED23. Nonetheless, the order of recruitment was similar to what was seen in Pre, starting with a single activation of FCR, followed by Delt, then ADD1 and at last ED23 (Fig. 10A). The mild group in Monkey 2 RV also exhibited later onsets for ED2, FCR, and FDC #2. However, sequential activation was preserved and remained similar to what was seen in Pre (Fig. 11A).

There were also significant changes in the muscle temporal activations when a rotation of the forearm was required in Monkey 1 RV and Monkey 2 RH. The order of muscle recruitment was altered in Monkey 1 RV and in the severe group of Monkey 2 RH. But the mild group of Monkey 2 RH kept a similar order of recruitment like observed in Pre. This could be due to the type of wrist rotation executed by the monkey. Indeed, because they showed a different hand start position, Monkey 1 needed a pronation of the wrist and Monkey 2 needed a supination of the wrist.

In the case of Monkey 2 RH, where the deficits were the most visible and required a rotation of the forearm, the sequential activation was altered in the severe group. Monkey 2 RH showed significant changes in 3-4 of the 6 bursts depending on the group. The severe group exhibited later onsets for FCU, FCR, FDC #1, and FDC #2 (Fig. 12A). The mild group exhibited significant later onsets for FCU, FCR and FDC #1, but the overall sequential activation was more preserved (Fig. 12A).

The reach phase was supported by burst onsets that were mostly activated in a sequential manner, with one or two bursts co-activated in the normal condition. After inactivation, muscle temporal activations during reach were changed for both monkeys and conditions. The reach phase showed later onsets and more co-activated bursts. These changes were dependent on the severity of the impairment and the difficulty of the task. The order of muscle recruitment was altered in the severe group while it was preserved in the mild group.

b) Grasp

During grasp there was two periods of muscle activation: the pre-shaping of the hand close to the slot (i.e. 0 ms), and the grasping of the pellet inside the well (after 0 ms). Due to the rotation of the wrist the pre-shaping was slightly different between the two conditions in each monkey. In Pre, the muscle temporal activation during the pre-shaping was supported by mostly co-activated bursts, with one or two bursts sequentially activated. The pellet grasping was supported by mostly co-activated bursts.

Prior to Muscimol injection, the pre-shaping of the hand started with digit onsets (intD1 and ADD1), followed by the wrist/digit flexor muscles and the deltoid in both conditions of Monkey 1 (Fig. 9B, 10B). In the case of Monkey 2 the pre-shaping of the hand started with FCU and ended with a different wrist/digit flexor. In between there were activations of proximal and forearm muscles (Fig. 11B, 12B).
The grasping of the pellet started with the activation of proximal muscles followed by mostly co-activated bursts of distal muscles in Vertical condition of both monkeys (Fig. 9B, 11B). In the Horizontal condition, the grasping was realized only by co-activated bursts of distal muscles (Fig. 10B, 12B).

After inactivation there were changes in the temporal activation of muscles during pre-shaping and grasp for both monkeys and both conditions. Usually significant changes were observed in at least half of the bursts. During pre-shaping mostly earlier onsets and more co-activated bursts were observed. The grasping of the pellet always showed later onsets. In addition, the changes to the onsets were not necessarily the same between the severe and the mild group.

Overall, the temporal activation of muscles during the pre-shaping showed significant differences and an altered order of muscle recruitment in the RH condition for both groups and monkeys. This altered recruitment was associated with more co-activation in burst onsets. In the case of Monkey 1 RH, there were significant changes in 3 of the 6 bursts depending on the group. The severe group exhibited earlier onsets for FCR and deltoid, and a later onset for intD1 #1. These changes showed a tendency of the 6 burst onsets to start at a similar time. The mild group exhibited earlier onsets for FCR and FDC, and a later onset for FCU (Fig. 10B).

In the case of Monkey 2 RH, there were significant changes in 2-3 of the 4 bursts depending on the group. The severe group exhibited earlier onsets for BB and FCR, and a later onset for FCU #1. The mild group exhibited earlier onsets for FDC#1 and FCR (Fig. 12B).

The temporal activation during the pre-shaping of the hand showed significant changes but a more preserved order of recruitment in the RV condition of both monkeys. In the case of Monkey 1 RV, there were significant earlier onsets for 2 (FCU #1 and deltoid #1) of the 6 bursts (Fig. 9B). Therefore, the sequential activation during the pre-shaping of the hand was almost preserved. In the case of Monkey 2 RV, there were significant changes in 2-3 of the 4 bursts depending on the group. The severe group exhibited earlier onsets for BB #1, FCR #1, and FDC#1. The mild group exhibited an earlier onset for BB #1 and a later onset for FCU #1 (Fig. 11B). Despite these significant changes, the pre-shaping sequential activation for both groups was similar to what was observed in Pre.

In addition, the bursts involved in grasping were also significantly different, and always exhibited later onsets.

A total of 4 bursts were recorded in the RV condition for both monkeys. The order of recruitment was preserved in the mild group of both monkeys, but altered in the severe group of Monkey 2. In the case of Monkey 1 RV, there was a later onset for deltoid #2, the only significant change in 3 grasp bursts (Fig. 9B). Therefore, except for a later activation of deltoid #2, the sequential activation was similar to what was observed in Pre and the 3 other bursts (intD1#2, FDC#2, and FCU #2) showed slightly more co-activation (Fig. 9B). In the case of Monkey 2 RV, there were significant later onsets for all 4 bursts of the grasp (BB #2, FCU#2, FCR #2 and FDC #2) for the severe group, and in 3 of the 4 grasp bursts (BB #2, FCR #2 and FDC #2) for the mild group (Fig. 11B). The severe group exhibited greater delays than the mild group. Therefore, the results indicate

that the sequential activation was changed in the severe group while it was more preserved and exhibited more co-activation in the mild group (Fig. 11B).

A total of 2 bursts were recorded in the RH condition for both monkeys. There were significant changes in the severe group of both monkeys, and in the mild group of Monkey 2. Unfortunately, due to this small number of bursts the interpretation for RH was more limited. The Monkey 1 RH showed a later onset for intD1 #2 in 2 grasp bursts for the severe group only (Fig. 10B). Thus, the severe group retained some co-activation between intD1 #2 and ADD1 #2, but still less than what was observed in Pre. In contrast, the mild group showed a different temporal activation than in Pre, with pellet grasping initiated by a single activation of intD1 #2 followed by a single activation of ADD1 #2 (Fig. 10B). The Monkey 2 RH showed significant later onsets for both bursts (FCU #2 and FDC #2) in both groups, but the two bursts remained co-activated like in Pre. Surprisingly, these delays were more pronounced for the "mild" than the "severe" group (Fig. 12B).

In conclusion, before inactivation, the temporal activation of muscles was either sequential or involved co-activation, depending on the phase of the movement. The reach phase was mostly supported by bursts that were activated in a sequential manner, with one or two bursts that were co-activated. The pre-shaping phase was mostly supported by bursts activated in a co-activation manner, with one or two bursts being sequentially activated. Finally, the grasp phase was supported by co-activated bursts.

After inactivation, the temporal activation pattern was changed in all phases of the movement in both monkeys and conditions. The bursts of EMG during reach mostly showed later onsets and had more co-activation. During pre-shaping, bursts mostly showed earlier onsets and greater co-activation. Finally, during grasping, EMG bursts always had later onsets. These changes in the timing of onsets of muscle activity impacted the sequential activation of the muscles. The order of muscle recruitment was changed or preserved in some cases depending on the level of impairment and the difficulty of the task. These alterations in muscle activity explained the behavioral deficits observed previously. In some cases the monkeys could successfully execute the task with a sufficiently preserved temporal activation of the muscles, and in other cases they poorly realized the task because of altered muscle recruitment.

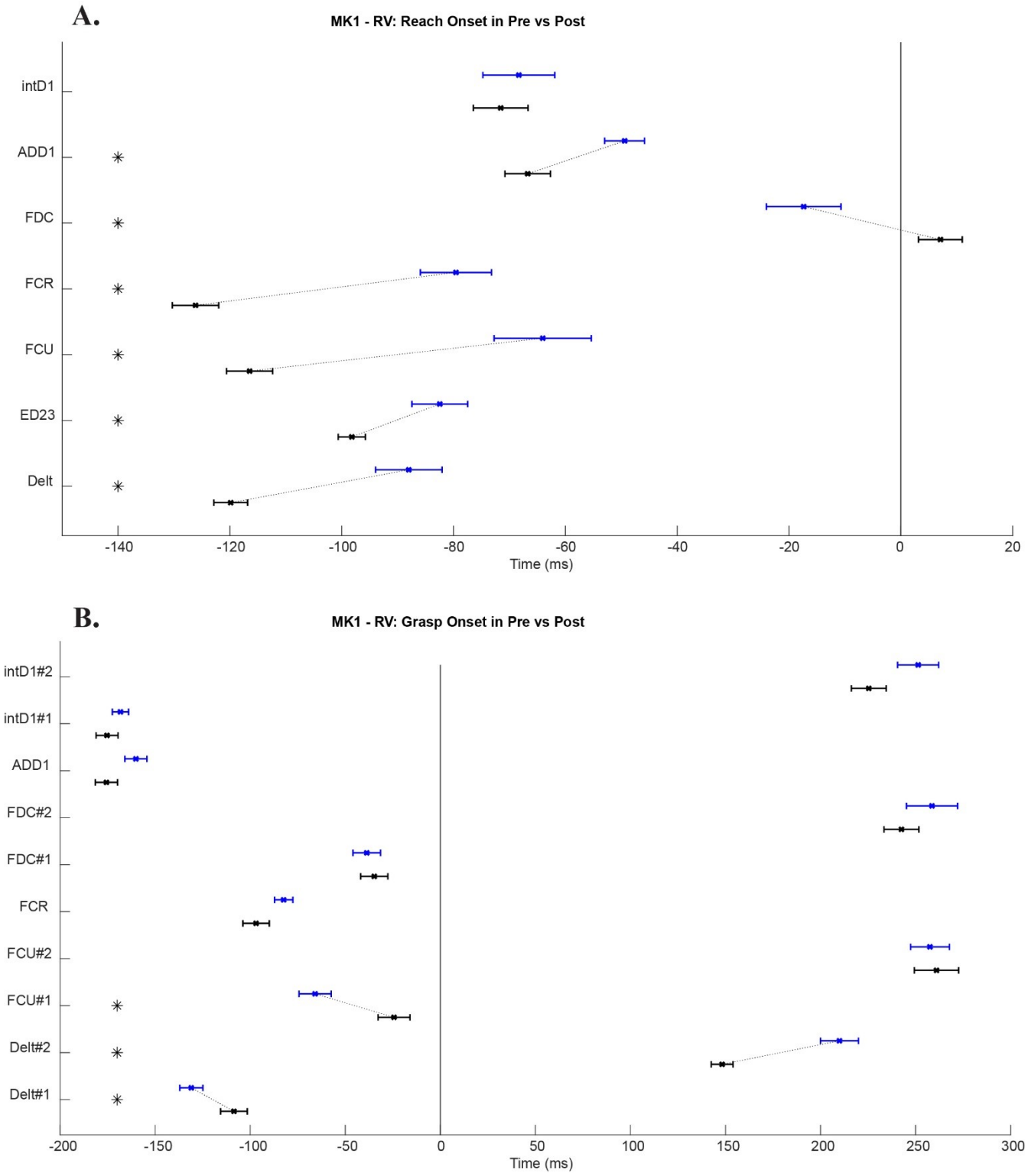


Figure 9: The muscles latencies before (Pre) and after (Post) inactivation for the Monkey 1 in the vertical condition. The mean latency (cross symbol) was expressed in milliseconds with the standard error in black for data in Pre and in blue for data in Post. **A,** Onsets during the reach phase were aligned on the start of the reach (i.e. hand left the home-plate = 0 ms), such that negative values represent EMG activity prior to the start of reach and positive values represent EMG activity after the start of reach. **B,** Onsets during the Grasp phase of the movement were aligned on the start of the grasp (i.e. slots sensors ON = 0 ms) , such that negative values represent EMG activity prior to the start of Grasp and positive values represent EMG activity after the start of Grasp. The first bursts during the Grasp were associated with the pre-shaping of the hand, and the second bursts were associated with the first flexions of the index finger.

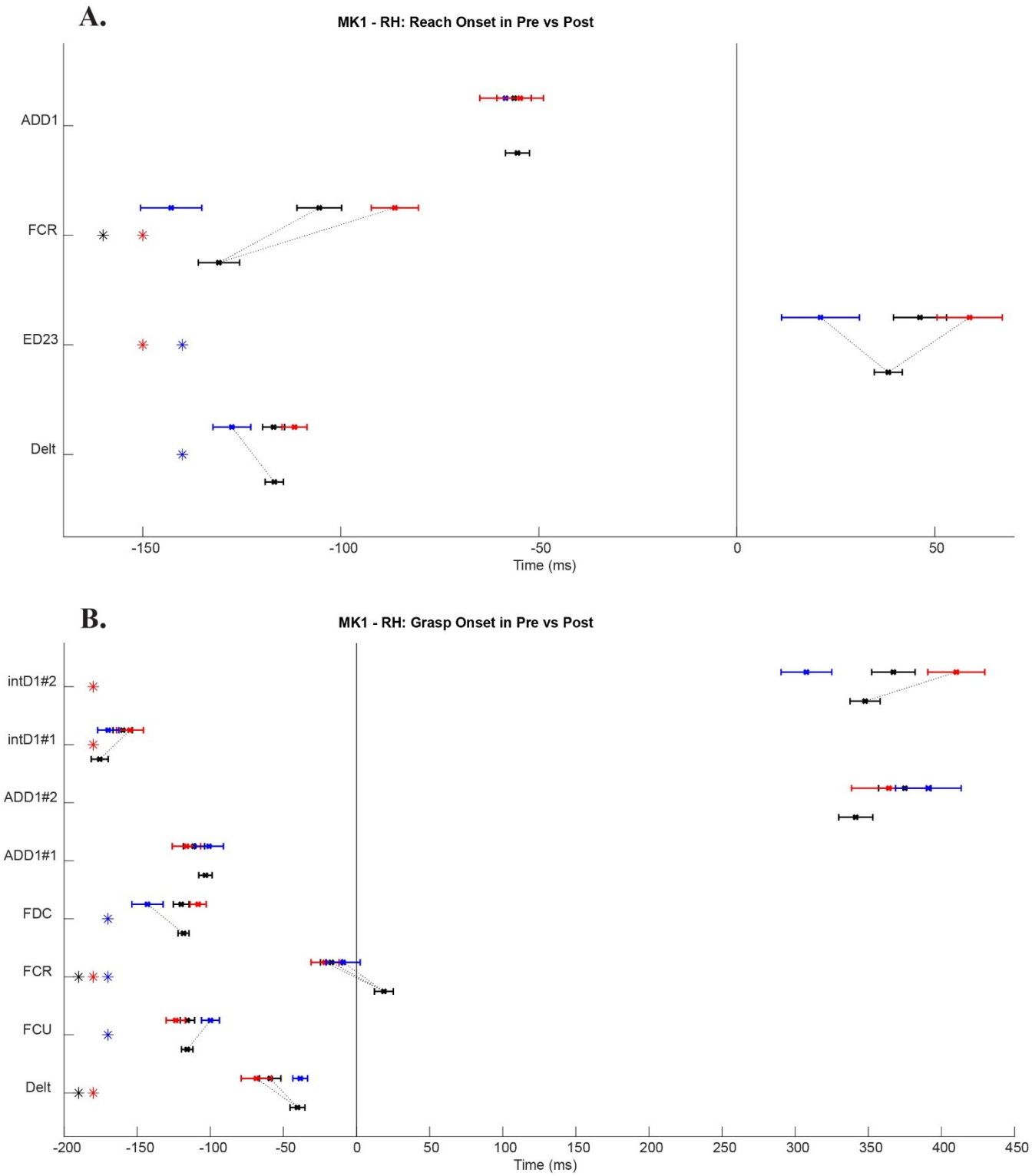


Figure 10: The muscles latencies before (Pre) and after (Post) inactivation for the Monkey 1 in the horizontal condition. The mean latency (cross symbol) was expressed in milliseconds with the standard error in black for data in Pre-inactivation, and in black for all data in Post, in red for the severe group in Post, and in blue for the mild group in Post. **A,** Onsets during the Reach phase were aligned on the start of the Reach (i.e. hand left the home-plate = 0 ms). **B,** Onsets during the Grasp phase of the movement were aligned on the start of the grasp (i.e. slots sensors ON = 0 ms). The first bursts during the Grasp were associated with the pre-shaping of the hand, and the second bursts were associated with the first flexions of the index finger.

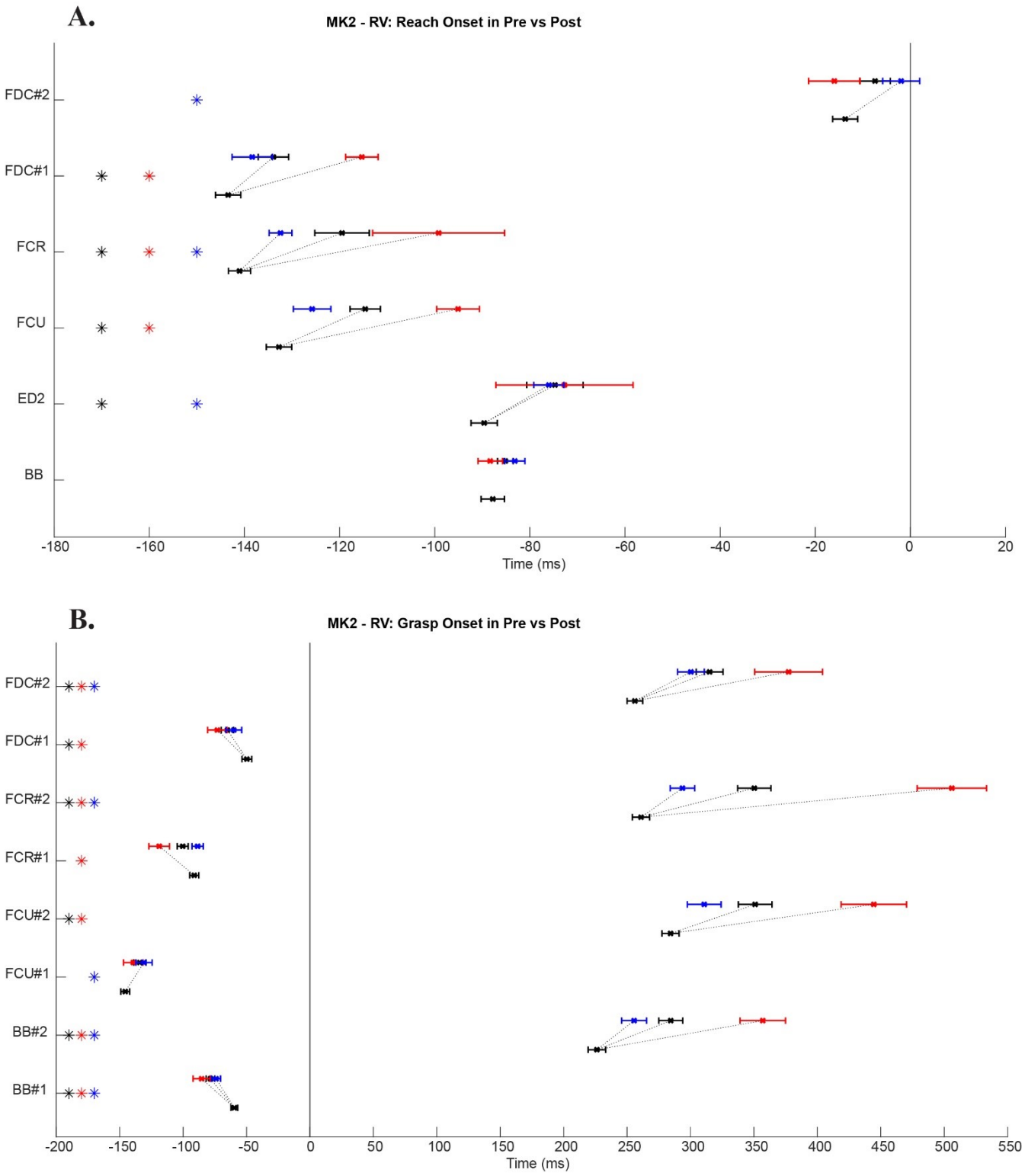


Figure 11: The muscles latencies before (Pre) and after (Post) inactivation for the Monkey 2 in the vertical condition. The mean latency (cross symbol) was expressed in milliseconds with the standard error in black for data in Pre-inactivation, and in black for all data in Post, in red for the severe group in Post, in blue for the mild group in Post. **A,** Onsets during the Reach phase were aligned on the start of the Reach (i.e. hand left the home-plate = 0 ms). **B,** Onsets during the Grasp phase of the movement were aligned on the start of the grasp (i.e. slots sensors ON = 0 ms). The first bursts during the Grasp were associated with the pre-shaping of the hand, and the second bursts were associated with the first flexions of the index finger.

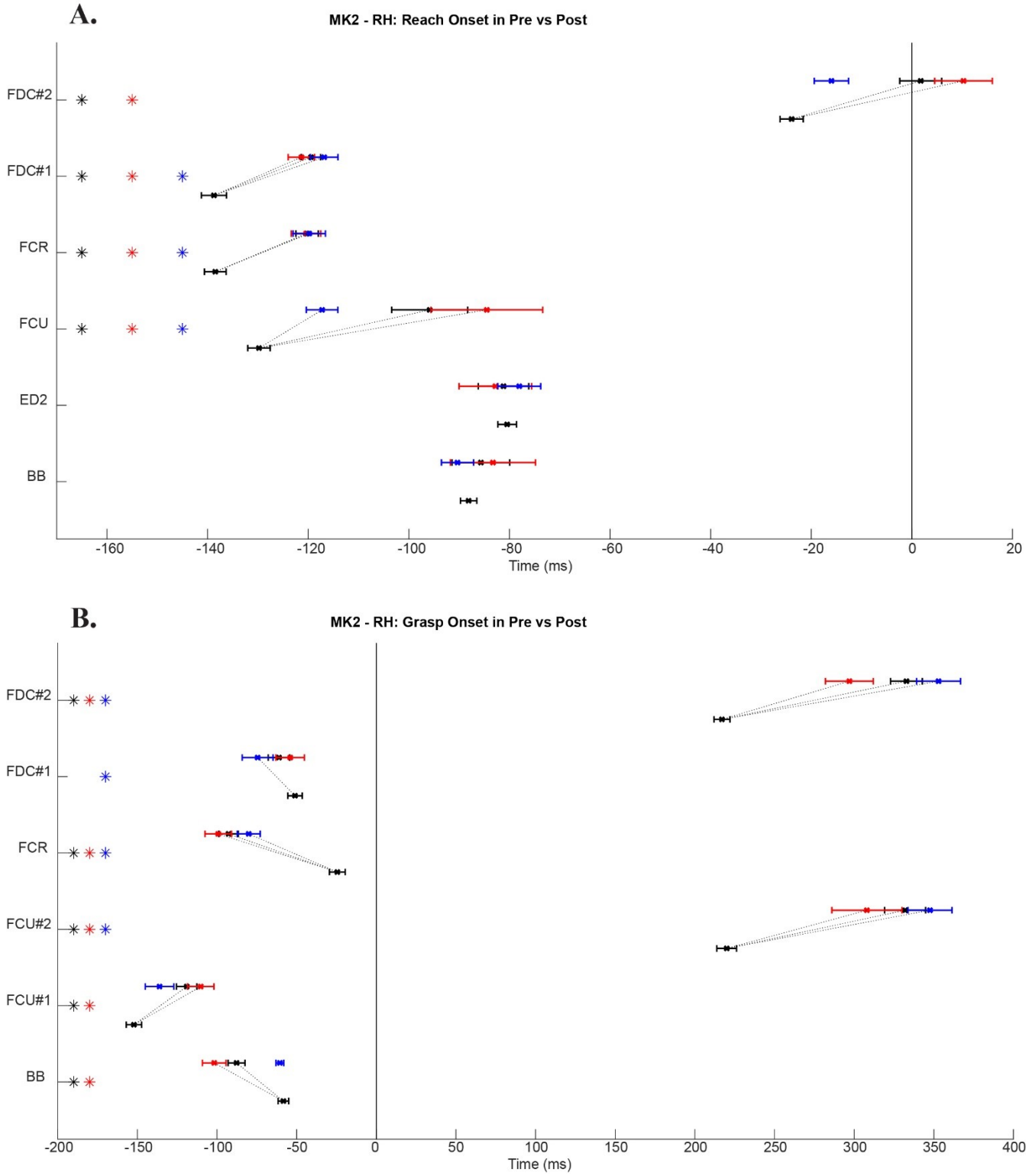


Figure 12: The muscles latencies before (Pre) and after (Post) inactivation for the Monkey 2 in the horizontal condition. The mean latency (cross symbol) was expressed in milliseconds with the standard error in black for data in Pre-inactivation, and in black for all data in Post, in red for the severe group in Post, in blue for the mild group in Post. **A,** Onsets during the Reach phase were aligned on the start of the Reach (i.e. hand left the home-plate = 0 ms). **B,** Onsets during the Grasp phase of the movement were aligned on the start of the grasp (i.e. slots sensors ON = 0 ms). The first bursts during the Grasp were associated with the pre-shaping of the hand, and the second bursts were associated with the first flexions of the index finger.

8. The effect of partial M1 inactivation on muscles coordination of the contralateral arm

Phase-plot analysis was performed to better evaluate the phase relationships between the muscles. The coordination between muscles during a reach-to-grasp task was compared before and after inactivation. The movement was divided into the reach and grasp phase of the movement, and all EMGs were normalized in time. The reach phase of the movement was defined from the home-plate sensors' offsets (i.e. hand left the home-plate; phase 0.0) until the target sensors' onsets (i.e. thumb and index entering the well; phase 1.0). The grasp phase of the movement was defined from the target sensors' onsets (phase 1.0) until the end of the first digit flexion defined by offset bursts (phase 2.0) of a selected muscle (intD1 for Monkey 1; FDC for Monkey 2). Onsets and offsets that occurred during the reach were plotted between phases 0.0 and 1.0, while those that occurred during the grasp were plotted between phases 1.0 and 2.0.

a) Pre-inactivation

Monkeys produced a smooth limb trajectory from the home-plate to the target, with a hand adjustment that occurred when close to the target before the grasping of the pellet. Thus the muscles exhibited a general sequential activation with some muscles co-activated at similar times and different groups of muscles activated at different phases of the movement. The sequential muscle activations were defined by non-overlaping rectangles. Therefore muscle activities showed variation of activation in time and in duration (and thus their offset). Co-activated muscles were defined by overlapped rectangles, and showing similar activation and duration. This was consistent with our previous observations with latencies.

Overall, the initial period of activation was done by wrist/digit flexor muscles (FCR, FCU, and FDC#1 for Monkey 2) to flex the wrist and lift it. Subsequently there was activation of proximal muscles (deltoid for Monkey 1, and BB for Monkey 2) to raise the forearm and the limb from the home-plate. Then the digits were lifted from the home-plate with a burst of activity in digits extensors (ED23 in Monkey 1, ED2 in Monkey 2), and slightly later by intrinsic hand muscles (intD1, ADD1 only in Monkey 1). Flexion of the digits (FDC) was activated (except in Monkey 1 RH) during the transport of the limb. Close to the slot, there was activation of the digits (intD1, ADD1 only in Monkey1) in preparation for the proper aperture of the hand. At this point

the hand position was adjusted by wrist flexor muscle activation (FCR or FCU), and moved toward the slot by proximal muscle activations (deltoid and BB). Once inside the slot, digits were brought into contact with the pellet by wrist flexor muscle activations (usually FDC or FCR). Last, there was simultaneous activation of several muscles during the grasping of the pellet.

i) Reach

During the reach, Monkey 1 showed 7 bursts in the RV condition and 4 bursts in the RH condition. The Monkey 2 showed a total of 6 bursts in the RV and RH condition. Monkeys did not execute the exact same movement due to the different hand start position. However, some similarities were observed in their reach patterns of activations. The one exception was for the Monkey 1 RH, presented later.

Both monkeys showed common activation of wrist flexor and digit extensor muscles (FCU, FCR, ED2, and ED23). While these muscles showed different burst onset times between monkeys and conditions as discussed in the previous results section, they showed a common pattern of activation in the phase-plot analysis. The start of the reach was realized by FCU and FCR with no overlapped rectangles (gray and green dots, Fig. 12A, 15A, 18A). The wrist flexors were followed by activation of digit extensor (ED23 for Monkey1, ED2 for Monkey2, blue dots, Fig. 13A, 16A, 18A). The digit extensor and the last activated bursts (FDC for Monkey 1, FDC#2 for Monkey2, red dots, Fig. 13A, 16A, 18A) showed independent rectangles, with no overlap with other bursts.

Both monkeys showed some differences in their patterns of activations for the proximal muscles and the intrinsic hand muscles. Indeed, these muscle patterns were different between monkeys. For Monkey 1, the proximal muscle recorded was the deltoid, and for the Monkey 2 it was the BB. In addition, only the Monkey 1 had intrinsic hand muscles recorded (intD1 and ADD1).

For Monkey 1, the Deltoid burst was in co-activation with the FCR and showed extensive overlapped rectangles. This was also true for the Monkey 1 RH condition. In contrast, for Monkey 2 the BB rectangle had almost no overlap with the FCR. Overall, proximal muscle activations (deltoid and BB) were realized between the FCR and the extensor muscle activations.

Another difference was the two intrinsic hand muscles recorded only in Monkey 1 (intD1 and ADD1). These muscles showed a co-activation with extensive overlapped rectangles. This brief

co-activation was realized between the activation of the digit extensor and the last wrist flexor muscle activation (FDC).

Finally, there was a difference in the FDC. In comparison to Monkey 1, an additional burst was observed for Monkey 2, in both conditions. A first burst (FDC#1) in co-activation with the FCU was observed, and thus showed extensive overlapped rectangles.

The Monkey 1 RH showed a different pattern of activation with only 4 bursts (Fig. 14A). The reach was initiated by the wrist flexor FCR like what had been was observed in other conditions and for the other monkey. This FCR and the deltoid burst were in co-activation like in the RV condition and showed overlapping rectangles. This co-activation was followed by the ADD1 burst with almost no overlapping rectangles, and finally by an ED23 burst. In this case, most of the transport of the limb was supported by the digit extensor muscle activation.

i.i) Grasp

During grasp, the first burst of grasp (grasp#1, represented by crosses) was associated with the pre-shaping of the hand, and the second burst of grasp (grasp#2, represented by diamonds) was associated with the first flexion of the grasp phase. The pattern of activation during the pre-shaping showed there were more muscles co-activated and few of them were sequentially activated. The grasping of the pellet showed bursts that were extensively overlapped.

Monkey 1 showed 9 bursts in the RV condition (6 bursts in pre-shaping and 3 in the pellet grasping, Fig. 13B) and 8 bursts in the RH condition (6 bursts in pre-shaping and 2 in pellet grasping, Fig. 14B). Monkey 2 showed 8 bursts in the RV condition (4 bursts in the pre-shaping and 4 in pellet grasping, Fig. 16B) and 6 bursts in the RH condition (4 bursts in the pre-shaping and 2 in pellet grasping, Fig. 18B).

During the pre-shaping of the hand, both monkey showed common activation of wrist flexor and proximal muscles. In general the pre-shaping was initiated by the activation of a wrist flexor muscle, the FCU (except for Monkey 1 RV with the FCR). The end of the pre-shaping was also done by the activation of another wrist flexor muscle to bring the digit against the pellet, the FDC in RV condition and the FCR in RH condition. Usually the first and the last burst of activation showed no overlapped rectangles (except in Monkey 1 RV that showed a small overlap). In

between, to move the hand toward the target, there were proximal muscle activations (deltoid or BB) that had overlapping rectangles with the wrist flexor muscle activations.
In addition, and only in Monkey 1, there were intrinsic hand muscle activations. The intD1 and ADD1 showed the earliest activations during the pre-shaping. Therefore, the pre-shaping of Monkey 1 always started with an intrinsic hand burst. In the RV condition, it started with both muscles and no overlapped rectangles (intD1 and ADD1). In the RH condition, it started with the intD1 and the ADD1 showed a later activation that had some overlapping rectangle with wrist flexor activation.

During the grasping of the pellet, for both monkeys and conditions, there were simultaneous activations with extensive overlapped rectangles.

In Pre, the pattern of activation was sequential or included co-activation depending on the phase of the movement. Reach mostly exhibited a sequential activation of muscles. Pre-shaping of the hand included more co-activation of muscles, with few sequential activations. Grasping mostly relied on co-activation of muscles. This was consistent with our previous observations with latencies (see above). Interestingly, common muscles in the two monkeys showed a similar pattern of activation (FDC, FCU, and FCR).

b) Post-inactivation

Following inactivation the pattern of activation showed the same number of bursts. However, the pattern of activation was nonetheless altered. There were more co-activations during the reach and during the pre-shaping of the hand. In addition, changes could also be observed when the task required a forearm rotation.

i) Reach

The pattern of activation of the Reach was changed in both monkeys and conditions following inactivation.

The severe group in both monkeys showed abnormal co-activation and altered patterns of activation (Fig. 15A, 17A, 19A). Some muscles that has been sequentially activated in Pre were now co-activated following inactivation. This is particularly true between the initial wrist flexor

muscle activations (FCR, FCU and FDC#1) with proximal muscle activations (deltoid, BB), and intrinsic hand muscles for Monkey 1 (ADD1).

While there was significant changes in the mean latencies (see above), the mild group of Monkey 1 RH and Monkey 2 in both conditions showed a preserved pattern of activation with the phase-plot analysis (Fig 15C, 17C, and 19C respectively).

The pattern of activation could also be changed because of the difficulty of the task (i.e. required a forearm rotation). This was the case for Monkey 1 RV that required a pronation of the wrist. While there was no decrease in the success rate, this mild group showed slight motor deficit (as measured by the number of flexions and the duration of the first flexion) and significant changes in the mean latencies (see above). Interestingly, the pattern of activations was altered during the reach with extensive overlapped rectangles in all flexors and digit bursts (Fig. 13C)

Only the digit extensor muscles showed a similar pattern of activations following inactivation (ED23, ED2) in both monkeys and conditions.

After inactivation, the overall coordination during the reach phase became changed based on the severity of impairment. The biggest changes were visible in the severe group with altered patterns of activations. In contrast, the mild group presented a more preserved pattern of activations during the reach. Interestingly, these changes were consistent with our observations with the muscle mean latencies. If there was a different order of recruitment, an altered pattern of activation was observed too.

i.i) Grasp

The patterns of activations were also altered during the hand pre-shaping in both monkeys and conditions.

The pre-shaping pattern of activation showed abnormal co-activation in the severe groups for both monkeys. Indeed, all bursts related to the pre-shaping exhibited a striking co-activation with no organization between muscles left.

These changes were visible for Monkey 1 RH, and Monkey 2 RV and RH. In the case of Monkey 1 RH, the 6 bursts involved during pre-shaping showed a greater co-activation as visualized by all rectangles overlapping (Fig. 15B). In the case of Monkey 2 RV and RH, the 4 pre-shaping bursts were all more co-activated and displayed wider rectangles (Fig. 17B and 19B respectively).

The pre-shaping pattern of activation was mostly preserved in the mild group of Monkey 1 RH, and Monkey 2 (Fig. 15D, 17D, and 19D respectively). In the case of Monkey 1 RH, the 6 bursts involved during the pre-shaping of the hand were preserved. However, there was more overlap between muscle activations, but they nonetheless kept a similar pattern of activations to what was observed in Pre (Fig. 15D).

In the case of Monkey 1 RV, the pattern was mostly preserved albeit with more co-activation. The changes were visible mostly in FCU grasp#1 and also in FCR and FDC grasp#1. The FCU grasp#1 showed an earlier activation (*grey crosses*) and thus partially overlapped with intD1 grasp#1 and FCR. The FCR (*green crosses*) showed a decrease in duration (and thus the offsets) and no longer overlapped with ADD1 anymore. FDC grasp#1 showed increased duration (*red* crosses) and was more co-activated with FCR (Fig. 13D).

A similar pattern of activations was observed during grasping. The co-activations observed in Pre were similar following the inactivation. However, the patterns of activations showed more variability with larger rectangles for severe impairments.

The 4 bursts involved during the grasping of the pellet in Monkey 1 RV showed similar co-activation as what was observed in Pre. Only Deltoid (*yellow diamond*) showed a greater variability and greater co-activation with the other bursts (Fig. 13D). In the case of Monkey 1 RH, the severe group showed similar activation of the 2 digit bursts (intD1 and ADD1) with a preserved co-activation (Fig. 15B). In contrast, the mild group had a similar activation but appeared to have less overlap. This could be due to the smaller number of trials obtained in the mild group (n=25, Fig. 15D).

In the case of Monkey 2 RV, the 4 bursts involved in the grasping of the pellet showed a simultaneous activation pattern but with a greater variability of onsets in the severe group (Fig. 17B). The 4 bursts of the mild group were all overlapped like what had been previously observed in Pre (Fig. 17D). In the case of Monkey 2 RH, the 2 bursts in the severe group were still extensively overlapped but in addition showed a greater variability in their onsets and offsets (Fig. 19B). The 2 bursts in the mild group showed extensive overlap with a slightly later activation (Fig. 19D).

After inactivation, the patterns of activations during pre-shaping and grasping was changed in both monkeys and conditions. These changes in muscle coordination were dependent on the severity of

the impairment and the difficulty of the task (i.e. whether a forearm rotation was required or not). During the pre-shaping, the severe group showed an altered pattern of activation with all muscles being co-activated as shown by the extensively overlapped rectangles of digit, wrist, and proximal muscles. However the pattern of activations was more preserved in the case of mild impairment. During the grasping, the pattern of activations showed co-activated bursts with more variability in the severe impairment group.

In conclusion, these changes were consistent with our previous behavioral observations. In the case of mild impairment, the monkeys were able to execute mostly a precision grip to retrieve the pellet. The mild group showed some motor deficits but kept a high success rate thanks to the preserved EMG pattern of activations following inactivation. Thus, changes in the muscle temporal activations and the pattern of activations were sufficient to perform the task with success. In contrast with severe impairment, monkeys showed atypical grasping and used other compensatory hand-shapes to perform the task (abnormal precision grip, abnormal precision grasp, or abnormal power grasp). Indeed, the EMGs' temporal activations and pattern of activations were altered and showed more co-activation. As a consequence the success rate was low, and monkeys were not able to perform the precision grip anymore. The compensatory movements were not sufficient anymore to successfully realize the task.

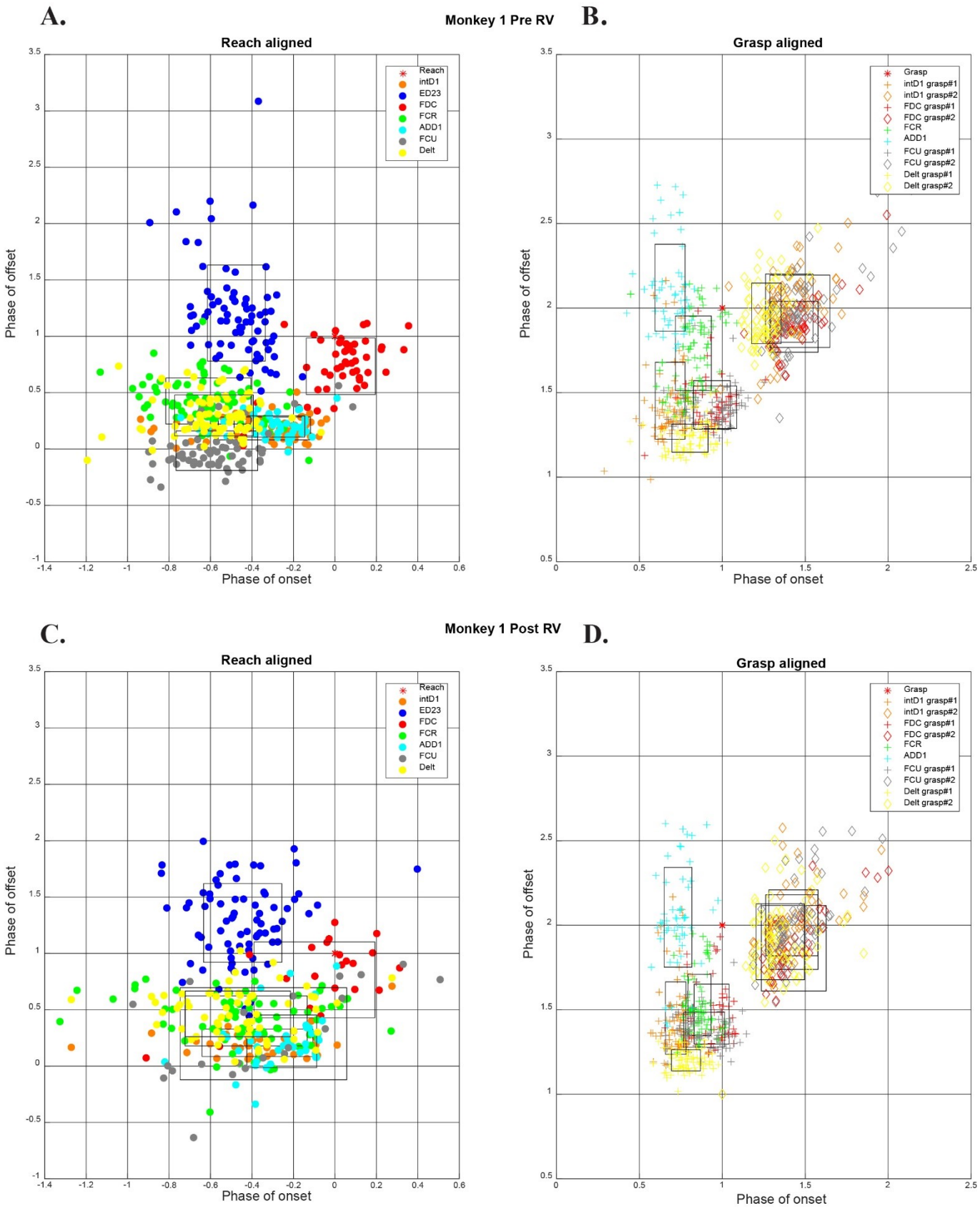


Figure 13: Monkey 1 phase-plots of EMG activity for the Vertical condition. Illustrated is the period of activity for each muscle in phase space for Monkey 1 in Pre and Post RV by plotting the phase offset of activity of burst of EMG activity as a function of the phase of its onset. **A, B,** Scatter plot of muscle activations for the reach and grasp phases before inactivation (Pre). **C, D,** Scatter plot of muscle activations for the reach and the grasp phases after inactivation (Post).

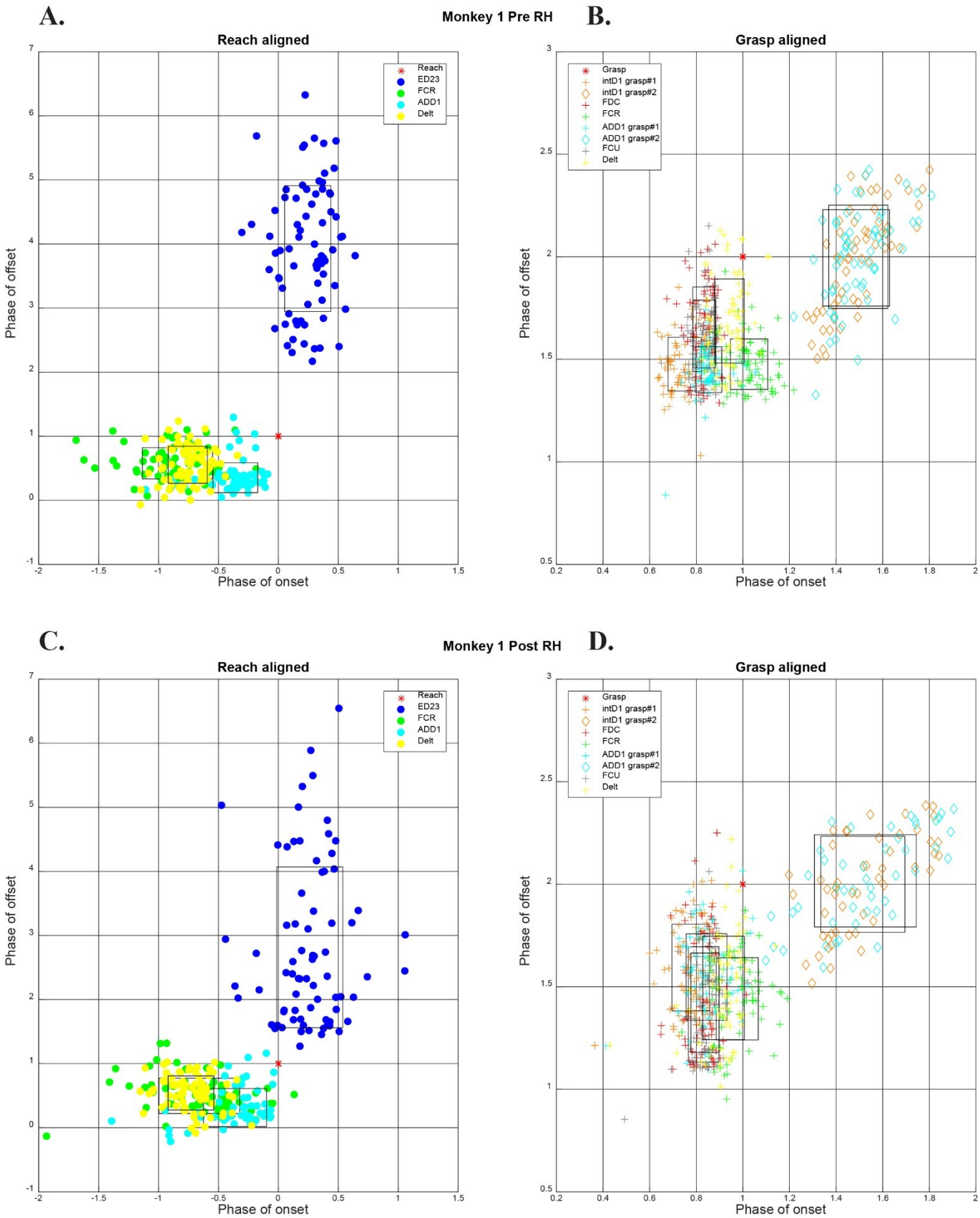


Figure 14: Monkey 1 phase-plots of EMG activity for the Horizontal condition. Illustrated is the period of activity for each muscle in phase space for Monkey 1 in Pre and Post RH by plotting the phase offset of activity of burst of EMG activity as a function of the phase of its onset. **A, B,** Scatter plot of muscle activations for the reach (left) and grasp (right) phases before inactivation (Pre). **C, D,** Same muscle activities after inactivation (Post) all pooled together.

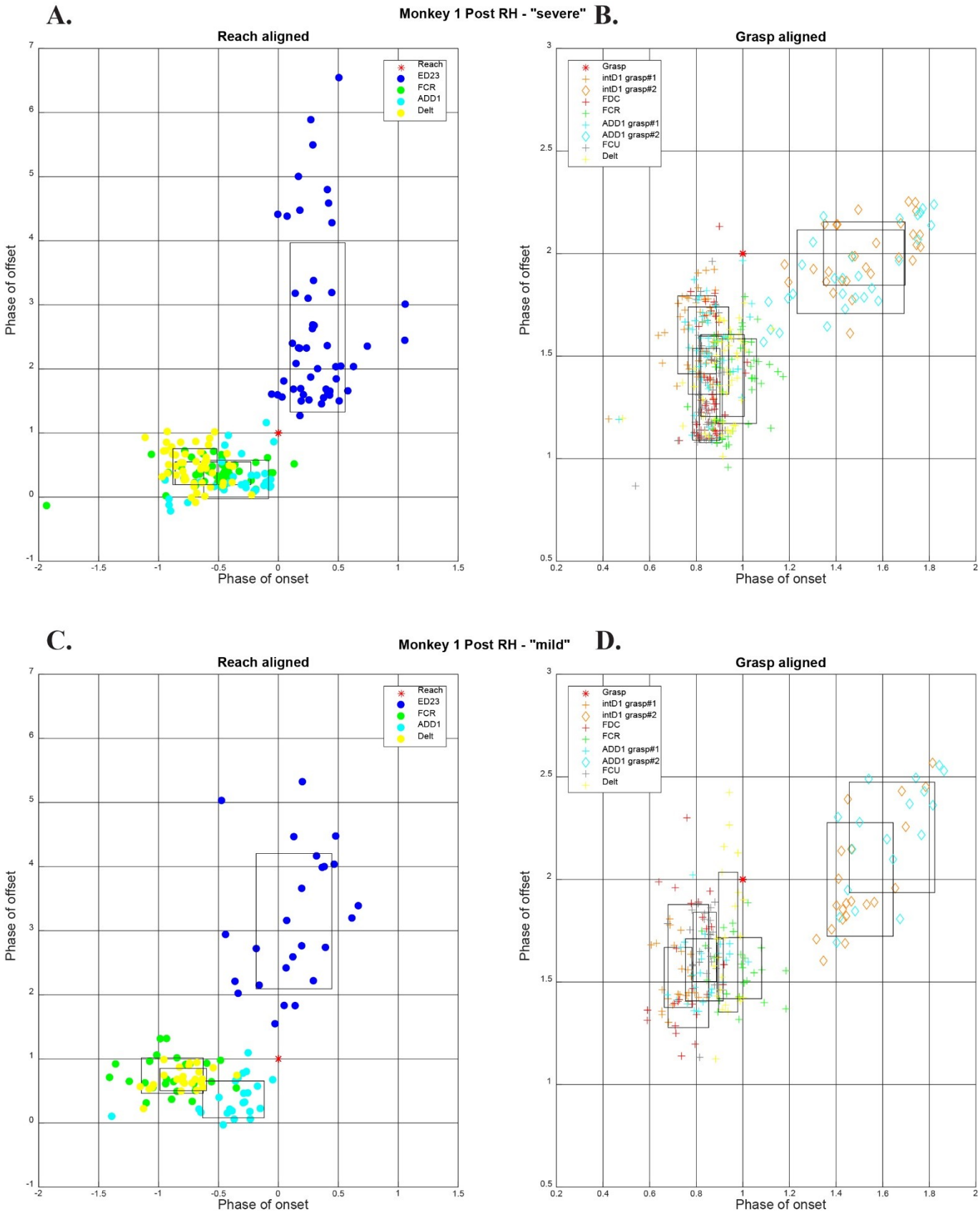


Figure 15: Monkey 1 phase-plots of EMG activity for the Horizontal condition for each post-inactivation group. A, Scatter plot of muscle activations only for the severe group during the reach. **B,** Scatter plot of muscle activations only for the severe group during the grasp. **C,** Scatter plot of muscle activations only during the reach for the mild group after inactivation (Post). **D,** Scatter plot of muscle activations only during the grasp for the mild group after inactivation (Post).

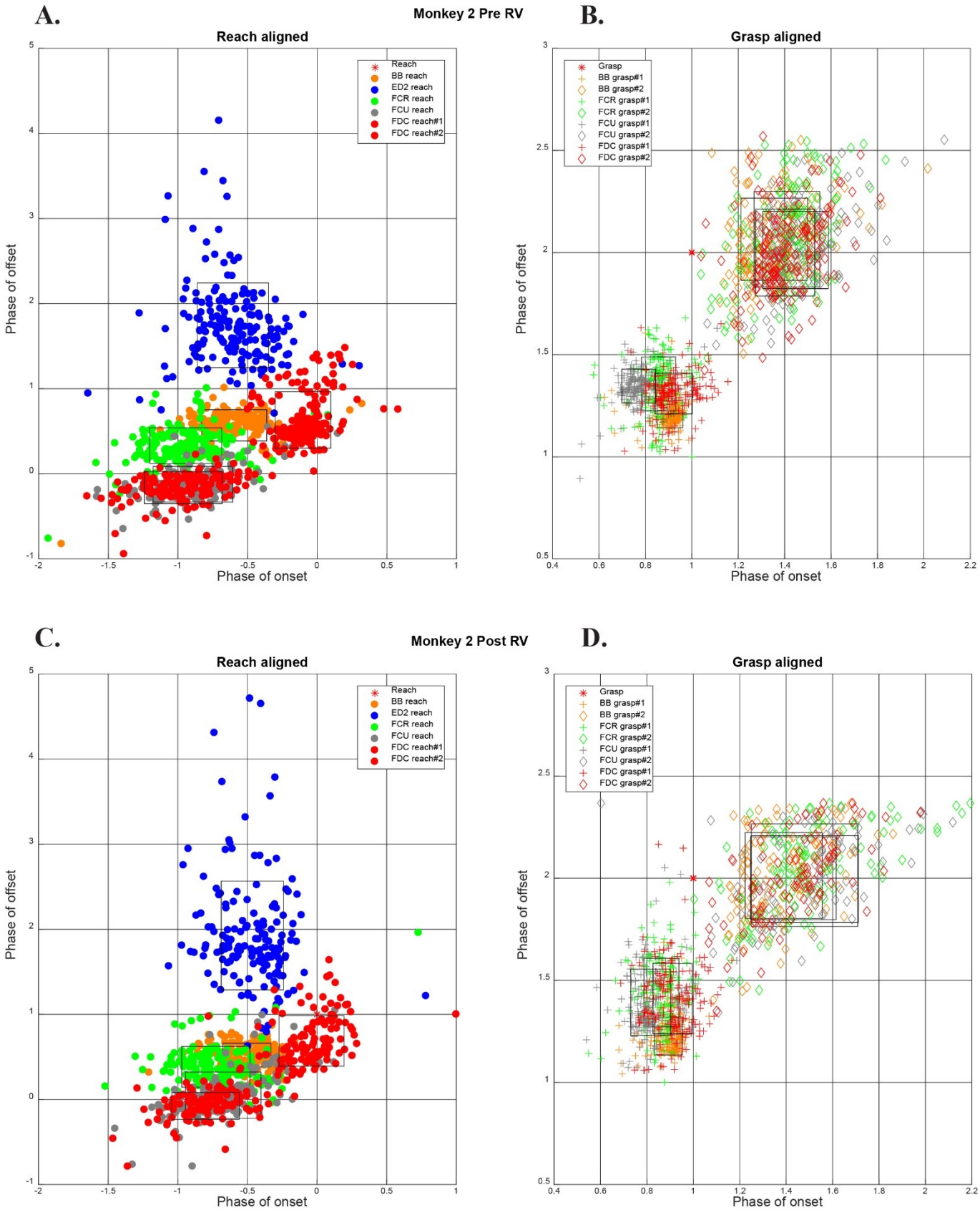


Figure 16: Monkey 2 phase-plots of EMG activity for the Vertical condition. Illustrated is the period of activity of each muscle in phase space for Monkey 2 in Pre and Post RV by plotting the phase offset of EMG burst activity as a function of the phase of its onset. **A, B,** Scatter plot of muscle activations for the reach and grasp phases before inactivation (Pre). **C, D,** Scatter plot of muscle activations for the reach and the grasp phases after inactivation (Post).

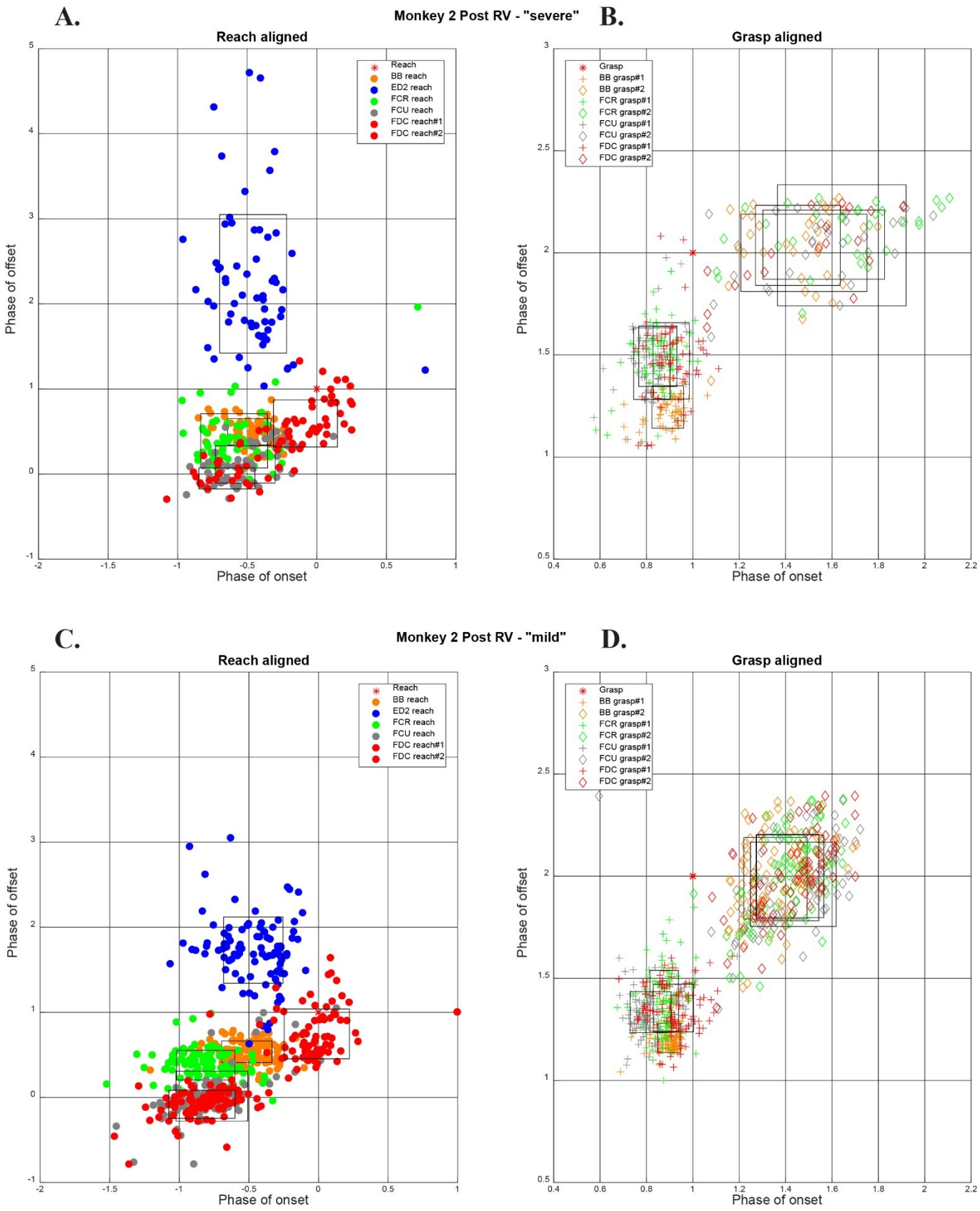


Figure 17: Monkey 2 phase-plots of EMG activity for the Vertical condition for each post-inactivation group. A, Scatter plot of muscle activations only for the severe group during the reach. **B,** Scatter plot of muscle activations only for the severe group during the grasp. **C,** Scatter plot of muscle activations only during the reach for the mild group after inactivation (Post). **D,** Scatter plot of muscle activations only during the grasp for the mild group after inactivation (Post).

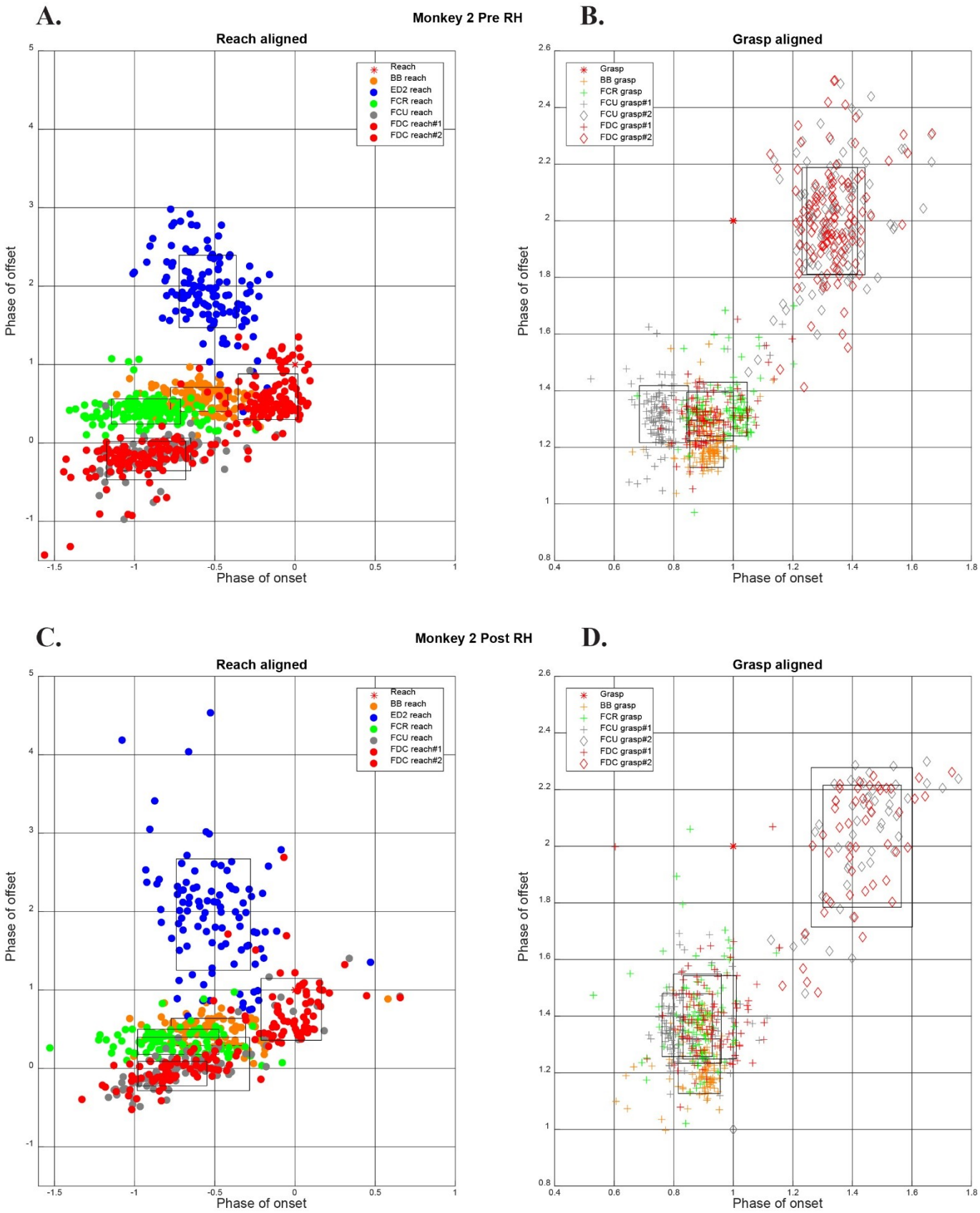


Figure 18: Monkey 2 phase-plots of EMG activity for the Horizontal condition. Illustrated is the period of activity of each muscle in phase space for Monkey 2 in Pre and Post RH by plotting the phase offset of EMG burst activity as a function of the phase of its onset. **A, B,** Scatter plot of muscle activations for the reach and grasp phases before inactivation (Pre). **C, D,** Scatter plot of muscle activations for the reach and the grasp phases after inactivation (Post).

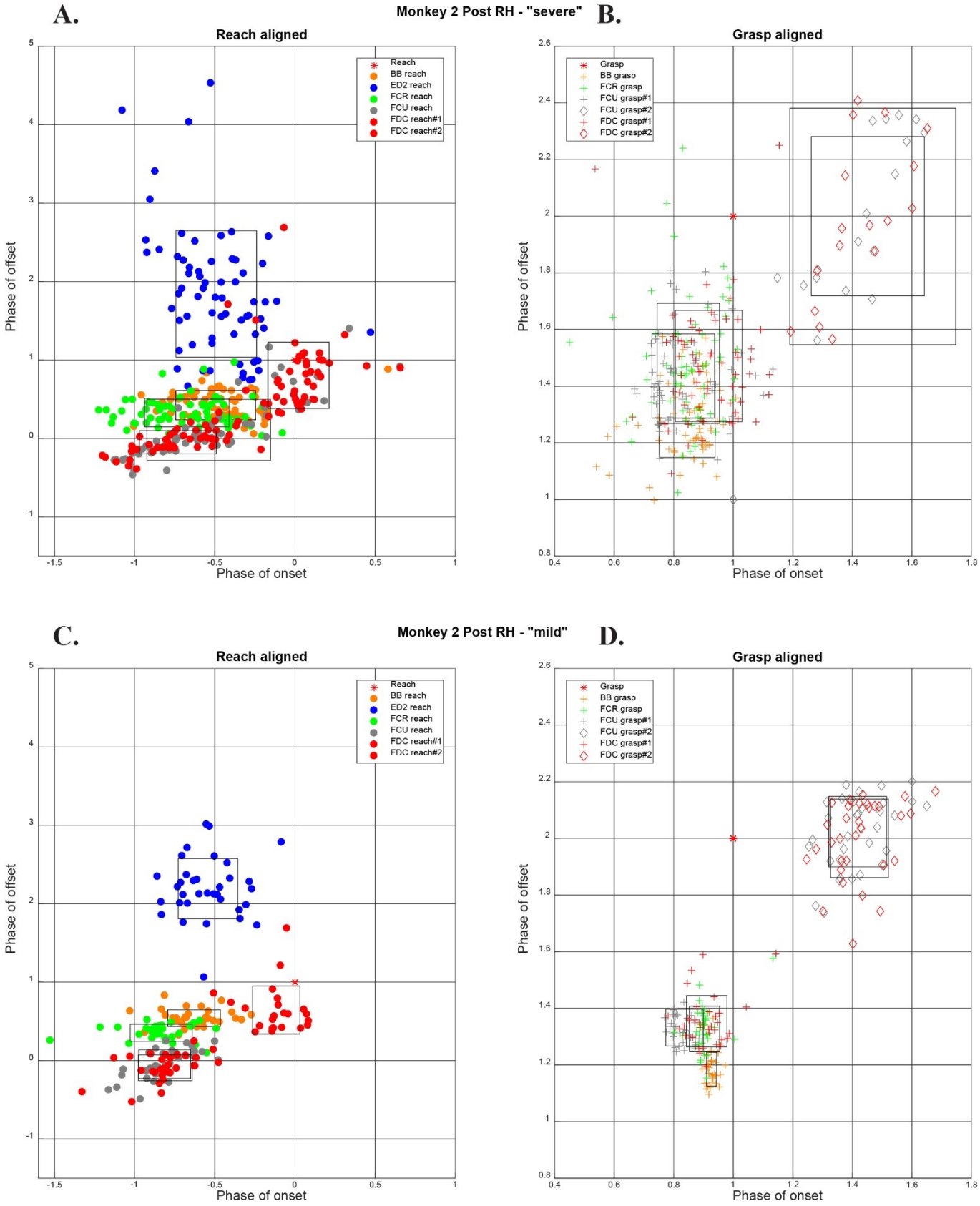


Figure 19: Monkey 2 phase-plots of EMG activity for the Horizontal condition for each post-inactivation group. Illustrated is the period of activity of each muscle in phase space for Monkey 2 in Post RH. **A,** Scatter plot of muscle activations only for the severe group during the reach. **B,** Scatter plot of muscle activations only for the severe group during the grasp. **C,** Scatter plot of muscle activations only during the reach for the mild group after inactivation (Post). **D,** Scatter plot of muscle activations only during the grasp for the mild group after inactivation (Post).

Chapter 4 – General Discussion

1. General summary

In this study we evaluated the behavioral performance and upper limb muscle activities of two monkeys during a reach-to-grasp task cumulating in either a vertical or horizontal precision grip. These data were compared before and after partial inactivation of the digit area of M1 induced by Muscimol injection. In total 12 inactivation experiments were included in the analyses (5 in Monkey 1, 7 in Monkey 2)

The results of this study demonstrated that partial inactivation of the M1 hand area produced behavioral impairments and inappropriate muscle activation patterns of the distal musculature in the contralateral, paretic limb. These latter changes were dependent on the severity of the behavioral impairment and on the difficulty of the task, in particular whether or not the reach and grasp required forearm pronation or supination. Based on the extent of behavioral deficits, we subdivided inactivation experiments into two groups, “mild” and “severe”.

In the case of the mild group, while behavioral deficits were visible the overall muscle activation patterns were similar to prior inactivation in most cases. This preserved pattern of activation allowed animals to perform the task successfully. However, some changes in the muscle activation patterns were observed when the task required a forearm pronation or supination (i.e. when the task was more difficult). The severe group showed both behavioral deficits and detrimental changes of muscle activation patterns, such that monkeys were not able to perform the task with success. This was true regardless of task difficulty.

Our results demonstrated that behavioral and muscular changes can be observed relatively early after the injection of Muscimol (between 13 to 45 min from the offset of the injection). This confirmed that small inactivations of the M1 digit area induces a loss of fine digit control in the paretic limb, consistent with previous studies (Matsumura et al. 1991; Schieber and Poliakov 1998; Brochier et al. 1999). More precisely, in the current study this loss of control took the form of alterations in the order of muscle recruitments and patterns of activation during reach, preshaping and grasping with the paretic limb. In addition, we found that changes in the patterns of activations may be related to the severity of motor impairment; severe behavioral impairments were accompanied by an altered pattern of muscle activation, whereas mild impairment showed a more preserved pattern of muscle activation.

2. Dexterous motor control of the contralateral hand by the primary motor cortex (M1)

The inactivation of the digit representation area of M1 induced deficits in the execution of reach-to-grasp movements with the contralateral hand. Our results confirm that the loss of independent digit movements is one of the main consequences of M1 inactivation, and is accompanied by weakness/slowness of the movement. These symptoms are in agreement with previous studies using large (Kubota 1996) and partial inactivation of M1 in monkeys (Brochier et al. 1999; Matsumura et al. 1991; Schieber and Poliakov 1998; Fogassi et al. 2001).

After training, the monkeys became highly stereotyped in their performance on the pellet retrieval task, using one precision grip in the vast majority of trials. This manual dexterity associated with the precision grip was disturbed by the injection of Muscimol as measured by the number of flexions of the index to retrieve the pellet. The increased number of flexions to retrieve the pellet reflected decreased fine motor control. Similar deficits were observed following small ischemic lesion of the digit representation area in M1 (Friel and Nudo 1998; Friel et al. 2005). These changes were accompanied by a change in movement strategy. Immediately after inactivation, monkeys retrieved the pellets using atypical grasping configurations which ranged from abnormal precision grip to, in worst cases, whole-hand grasping with digits moving all together. These different grasping configurations were observed in a group dependent manner, such that the mild group used mostly N-PG and AN-PG while the severe group used mostly AN-PGrasp and AN-PoG (see results section 4). Our results demonstrated that there was a loss of independent finger movement as shown by the progressive loss of thumb and index digit specific movements, and more precisely their distal phalanx, to grasp the pellet.

These deficits are concordant with the functional segregation of M1 motor outputs. Indeed, electrophysiological and anatomical evidence demonstrates that motor output maps of M1 are organized with a central core of distal (wrist, digit, and intrinsic hand) muscle representations, surrounded by a "horseshoe"-shaped zone of proximal (shoulder and elbow) muscle representations (Park et al., 2001; He et al., 1993). Our Muscimol injections specifically targeted the digit area of M1 and resulted in deficits that mostly impacted the distal segment of the upper

limb. In contrast to previous M1 inactivation in monkeys (Brochier et al. 1999; Fogassi et al. 2001; Kubota 1996; Matsumura et al. 1991; Schieber and Poliakov 1998), our results also demonstrated that more proximal muscles were impacted by our inactivation.

The increased number of flexions needed by the animals to retrieve the pellet raised the question of how the first flexion was being executed after Muscimol injection. Our results demonstrated that there were significant increases in the duration of the first flexion in all groups and conditions, likely due to a slowness or an inability to execute the grasp. This reduction in the speed of the movement was previously reported by inactivation studies, which measured either the premovement reaction time in addition to the movement time itself (Schieber and Poliakov 1998; Matsumura et al. 1991), or the grasp phase only (Kubota 1996), or the reach and the grasp phase duration (Fogassi et al. 2001). Two of these studies used a similar task as ours, Matsumura et al. (1991) with the raisin pick-up task, and Fogassi et al. (2001) with an apparatus that contained a thin plate in its center. They both required a reach-to-grasp movement culminating into an opposition of the thumb and the index finger to grasp the object. Our results demonstrated that the significant increases in movement duration reported in these studies is at least in part due to the longer execution of the first flexion.

Interestingly, our results showed increased duration in the first flexion in both groups and conditions whereas the deficit in manual dexterity was mostly significant for the severe group and less so for the mild group. Therefore, mild groups could present longer first flexion duration but without increasing the number of flexions required to retrieve the pellet as observed in Monkey 1 RH and Monkey 2 RV (Fig. 4B, and C respectively). In the case of the mild impairments, there was weakness/slowness of movements. In contrast, in the case of severe impairment there was weakness/slowness in association with a loss of independent digit movements. This might suggest that the loss of independent digit movement and the slowness/weakness might be dissociable deficits and thus reflect dissociable aspects of M1 dysfunction.

This is consistent with the observations of Schieber and Poliakov (1998). After partial inactivation of the M1 hand area, some monkeys showed prolonged response time (i.e. reaction time and the movement time) without a loss of independent finger movement and vice-versa. The authors suggest that the prolonged response time could result of a slowness/weakness of the instructed digit

movement, whereas the loss of independent digit movement could result in a loss of stabilization of non-instructed digits involved during the task (i.e. stabilization of middle, ring and little digit during the precision grip). Indeed, Humphrey and Reed (1983) showed evidence in the primate brain of two independent motor control systems during wrist flexion or extension against torque perturbations: one for the reciprocal activation of antagonist muscles during voluntary control of the wrist, and another for active muscle co-contraction to stabilize the posture against a perturbation (Humphrey and Reed 1983). More recently, cortical neurons were also identified that showed specific firing in relation to arm resting opposed to reach movement (Velliste et al. 2014). Thus, M1 could utilize different control systems during the active control of intended movement versus the active stabilization of an intended movement. The weakness/slowness of the first flexion reflects the loss of the active control of the digit movement, whereas the increased number of flexions in association with the progressive recruitment of the other fingers (AN-PGrasp, AN-PoG) could be the result of progressive loss of the active stabilization of the non-instructed digits (i.e. middle, ring and little).

In the cases of mild impairment, monkeys were still able to oppose the thumb and the index. In the worst cases, our results showed that AN-PoG was associated with the inability to perform individual digit movements due to a loss of active stabilization of other digits. In addition, monkeys struggled to preshape the wrist and digits (extension of thumb, index, and wrist in contrast to flexion of middle, ring and little digit). To retrieve the pellet, monkeys placed the hand against the board of the task and managed to enter the index into the well, then executed the flexion in association with wrist up and down movements. In addition, the pellet was grasped between the digits and the palm because they were unable to oppose the thumb and the index.

These observations are consistent with descriptions of previous inactivation studies, which have observed monkeys' digits all moving together when their hand failed to preshape in a variety of functional tasks. This was seen during the execution of a force task (Brochier et al. 1999), during retrieval of a food morsel from large and small wells (Schieber and Poliakov 1998), and during attempts to grasp a small object placed on a flat surface (Fogassi et al. 2001). This inability to preshape the hand was described as a 'flat' hand posture by Fogassi et al. (2001) after small (5 µg) and large (90 µg) Muscimol injections of the hand field area of M1. Interestingly, following small inactivations, one monkey showed a wrist impairment in addition of the 'flat' hand posture (Fogassi

et al. 2001). This suggests that in the current study the wrist muscles could have been impacted by the Muscimol injection, which would explain, at least in part, the struggle to preshape the hand that we observed in the severe group.

The motor deficits that we observed were similar to deficits caused by lesions of the pyramidal tract (Lawrence and Kuypers 1968a), ischemic infarct in M1 (Nudo et al. 1996; Nudo and Milliken 1996), and larger doses of Muscimol in M1 hand area (Kubota 1996; Fogassi et al. 2001) (30 and 90 μg respectively) that have been performed in primates. Our results add further support to the notion that the corticomotoneuronal system plays a critical role in the performance of individual digit movements. Indeed, there is a general agreement that the CM projection originating from M1 has direct and potent connection onto motoneurons of hand muscles contralaterally, and play an important role in precision handling (Fetz and Cheney 1980; Porter 1985; Lawrence and Hopkins 1976; Muir and Lemon 1983).

3. Reach versus Grasp

The M1 inactivation resulted in motor deficits that affected the most distal segments of the hand during grasping including a reduction in the speed of movement. In contrast, the reach phase was relatively preserved and monkeys could still accurately move the hand from the starting position to the target. Nonetheless, our results showed that both phases of the movement were impacted to some degree, and were executed slower than what had been observed prior to inactivation as measured by the task sensors (see Fig.7).

In the case of Monkey 1 in the RV condition, we observed no changes in the reach duration after inactivation (see Fig. 7A *left graph*). However, there were significant changes in onsets times of shoulder, wrist and hand muscles relative to Pre (see Fig. 9A). This could be explained by the hand start position that required this monkey to execute a rotation of the forearm (i.e. pronation) during reach in the RV condition. This pronation during the transport phase required coordination between more forearm muscles than the RH condition which required no rotation of the forearm with this starting hand position. Despite a normal reach duration, theses muscle changes reflected an improper coordination.

In the other cases, while deficits were less pronounced during reach as compared to grasp, we observed significant increases of the reach duration. The reach was self-initiated such that the increased reach duration could be due to difficulty in preshaping the hand. During reach, most of the transport phase of the movement was supported by the proximal muscles of the shoulder and elbow. We observed that the deltoid was affected by the inactivation, but not the biceps. This could explain in part the prolonged reach duration. In addition, the end of the reach normally involves preshaping of the hand, which involves mostly the distal musculature including both the digits and wrist. The motor control of the former, and possibly the latter, were affected by the inactivation; and could have led to a prolonged reach duration, as the monkeys had more difficulty to preshape the hand. These longer durations of movement are similar to what was reported in previous studies (Fogassi et al. 2001; Kubota 1996; Schieber and Poliakov 1998) which have shown slower limb movements in parallel with behavioral deficits. These studies measured the duration of the movement in different ways, such as the whole movement (Fogassi et al. 2001), or the different phases of the movement separately (Kubota 1996), or included the reaction time as well (Schieber and Poliakov 1998). Despite these differences, all these studies showed a reduction in the speed of the movement.

We also observed no significant increase in aiming errors, in contrast to a previous lesion study of Friel et al. (2005) (*data not shown*). That study showed a significant increase of aiming errors following small lesions restricted in the rostral portion of M1 where proximal movement representations are located (Friel et al. 2005). Thus, our results showed that this type of deficit was not due to inactivation of distal representation in M1.

Our results showed a loss of independent digit movements in association with weakness/slowness of the hand muscles during reach and grasp. Although weakness and slowness could be dissociable deficits, our paradigm could not distinguish between them. These behavioral deficits could be due to a partial loss of descending commands, for example due to a loss of corticomotoneuronal signals. In the following section we discuss the changes in the muscular patterns of activations induced by the inactivation 1in parallel to these behavioral deficits.

4. Impact of the inactivation on muscle activation patterns

This study is the first to examine the temporal activation of muscles in a reach-to-grasp movement before and after partial inactivation of M1 in the awake monkey. Immediately after inactivation, our results demonstrated that both the mild and severe groups showed significant changes in the latencies of muscle activations. More precisely, we observed later muscle onsets during reaching and grasping, and earlier muscle onsets during hand preshaping. These changes in the latencies resulted in more co-activation between muscles.

Interestingly, our results showed that Muscimol injection induce significant changes in latencies of muscles across different segments of the paretic limb, from proximal musculature like the deltoid, to distal intrinsic hand muscles like the intD1. Therefore, even a small inactivation focused on the digit representation area of M1 impacted the temporal activation of all segments of the upper limb, and not just the hand. These results were consistent with anatomical and stimulation studies of the corticospinal tract (CST). It has been demonstrated that a single M1 neuron can facilitate a number of different muscles (Fetz and Cheney 1980). In addition, direct CM cells could produce postspike effects in both proximal and distal muscles (McKiernan et al. 1998). This is also supported by the anatomical existence of the corticospinal divergent projections, where a previous study demonstrated that individual corticospinal axons made connections with motoneurons of different muscles (Shinoda, Yokota, and Futami 1981). At a cortical level, the proximity between regions representing hand muscles with different regions representing elbow and shoulder muscles (Park et al. 2001) could explain the combined impact on distal and proximal muscles that was observed in the present study.

Our results regarding the abnormal muscle latencies during the reach phase are comparable with lesion studies in monkeys and humans (Hoffman and Strick 1995; Wagner et al. 2007), albeit less pronounced in severity. Our results are also similar to those obtained with an unilateral lesion of the M1 arm area in monkeys (Hoffman and Strick 1995). This study showed an alteration in the temporal recruitment of the contralateral musculature with abnormal timing in the onset of agonist muscle activity, and failure to deactivate antagonist muscles. Furthermore, there was a delayed muscle onset of the wrist flexor accompanied by earlier onsets of agonist muscles. Our results are also similar to the observations of Wagner et al. (2007) which studied hemiparetic patients at an acute time point (mean = 9 days post-stroke). In this study, they recorded six muscles of the upper

limb (deltoid, biceps, triceps, wrist extensors and flexors) which showed altered muscle activations such as later and more variable onset latencies compared to the control group (Wagner et al. 2007). However, our results showed quantitatively less profound changes in the reach latencies due to our small size of our Muscimol injections. These suggest that the use of a larger size of inactivation using multiple Muscimol injections would likely have produced the same inappropriate temporal activation as lesion studies. While both of these lesion studies observed the abnormal temporal activation of muscles during the reach, our results go one step further by showing that there were significant temporal changes during the prehaping and grasping phases as well.

In our paradigm, the orientation of the slot led monkeys to execute a rotation of the forearm during the reach. However, the rotation of the forearm was different and executed in different conditions between monkeys due to the different hand start position of each monkey. Indeed, Monkey 1 executed a pronation of the forearm whereas Monkey 2 executed a supination of the forearm. In addition, the rotation of the forearm was required in the Vertical condition for Monkey 1 whereas it was required in the Horizontal condition for Monkey 2. Our results showed abnormal temporal muscle activation during reach if a rotation of the forearm was required, even during the case with the least deficits (i.e. Monkey 1 in the RV condition).

In addition, we looked at how partial inactivation of M1 impacted the coordination between muscles as represented by our phase-plot figures (see Fig. 13, 14, 15, 16, 17, 18, and 19). The changes were not restricted to the muscle burst onsets as we saw previously with muscle latencies, but seemed to also occur through the whole movement.

Following inactivation, we observed that muscle coordination was preserved in the mild group, with most of the bursts showing a similar pattern of activation as what had been observed prior to Muscimol injection. In contrast, we observed an altered pattern of activation in the severe group with mostly co-activated bursts throughout the different phases of the movement involving both proximal and distal musculature, which likely contributed to motor impairment. These results showed that the significant changes in the latencies previously observed in both groups could be due to changes that either reflect muscular compensation to preserve the coordination of the whole movement, or reflect the loss of motor control associated with the inability to preserve proper coordination between muscles.

This implies that spared premotor areas and remaining descending pathways cannot fully compensate for the fine motor control provided by M1 even in the case of partial inactivation. Even other premotor areas of the ipsilesionnal hemisphere, that also have direct corticomotoneuronal connections, could not compensate for the behavioral and muscular loss. This is consistent with the observations of Schmidlin and collaborators (2008), which showed that motor responses evoked in hand muscles by repetitive ICMS in the PMv were reduced or abolished by small inactivation of the M1 hand area (Schmidlin et al. 2008). This demonstrated that the F5 subdivision of the PMv could be dependent on the integrity of M1 due to the connections between the F5 and M1. Indeed, there are reciprocal connections through distinct populations of cortico-cortical neurons between F5 and M1 (Dum and Strick 2005). Therefore, M1 does not act simply as a relay to motor outputs; the integrity of M1 and reciprocal connections are essential for the motor control of the hand.

5. Future direction

The next step in the analysis of our data will be to apply the associative clustering analysis developed by Drew and collaborators (Krouchev, Kalaska, and Drew 2006). The first step of this analysis will be to determine whether each burst, represented by rectangles in our phase plot figures, belong to one or another cluster. If the distance between the centers of each burst are separated by <1SD, it will be considered to be part of the same cluster. Therefore, sufficiently overlapped bursts form clusters, as also knows as synergies (Krouchev, Kalaska, and Drew 2006). The next step would be to compare the synergies that exist across recorded muscles before and after inactivation by superimposing the phase-analysis of the EMG activity (Yakovenko and Drew 2015). The Euclidean distance between clusters centroids will be calculated to evaluate the changes in the muscle coordination relative to the phase of the movement. For example, with the mild group that showed a preserved pattern of muscle activations, we would expect similar cluster compositions (i.e. the same EMG bursts form the same clusters seen in Pre) with similar Euclidean distance. In contrast, with the severe group that showed altered patterns of activations, we would expect different cluster compositions and changes in Euclidean distance between centroids. Indeed, with the increases in observed co-activation we would expect smaller Euclidean distance between

clusters, or even a merging of clusters. Therefore, the differences in the number of muscle synergies would reflect disruptions in descending neural pathways and would be correlated to motor deficits. It is worth noting that a previous study with mild or severe motor impaired patients have used the non-negative matrix factorization (NMF) technique to quantify synergies and demonstrated 3 different patterns of muscle synergies after stroke. More precisely, in cases of mild-to-moderate impairment, the paretic arm showed preserved synergies. In contrast, in cases of severe impairment, multiple synergies became merged after stroke. A third pattern emerged years after stroke injury, where some of the synergies in the affected arm appeared to be fractionations of the synergies observed in the unaffected arm. Even though the lesions were cortical, the merging and fractionation of these synergies might be due to either neural changes at the cortical level, or other processes at the brainstem or spinal cord levels unmasked by the cortical lesions (Cheung et al. 2012). Although additional research is needed to have a better understanding of the production of these motor patterns observed in patients, these results suggest that muscle synergy patterns could be used as physiological markers. These results could be compared with the results obtained by the associative clustering analysis to determine if the detected changes in muscle synergies are similar across both techniques. If so, these findings would show that the use of Muscimol in monkeys represent a great model to evaluate the contribution of motor cortical areas to control of upper limb movements. Muscle synergy analysis will offer a better view of how neural structure and motor behaviors change after a trauma. Such information could help clinicians to design and choose the most effective therapies for their patients.

Further insights into the contribution of M1 to the control of muscle synergies of the hand could be achieved by repeating the same analysis in the paretic hand at different post-injection times. Indeed, we could extend our analyses to recordings performed at later time points and with larger inactivation sizes in our monkeys. In addition to the data presented here, we recorded behavioral and EMG data 3, 10 and 24 hours after the Muscimol injection, and also performed experiments with different sizes of inactivation (up to 5 injections of Muscimol). At the 3h and 10h time points, we would expect to see greater behavioral deficits with disruptions of grasping for the worst cases. In parallel, we could expect to see changes in the patterns of muscle activities with possibly no more EMG signal for some of the most distal muscles, and more variable and abnormal temporal activations for extensor and proximal muscles. Therefore, we would expect to see the

evolution of impairment in the muscle activation patterns over time. This would be important information about the differential changes that occur with time.

Conclusion

Skilled hand function is critical to many different aspects of our daily life, from interaction with our environment, to technology, communication, and social interaction. The hand is one of the most complex structures of the human body and can be used to perform a considerable number of combinations of movements due to the large number of muscles that control it and the number of degrees of freedom it has available. A precise temporal activation of multiple muscles are required to move a single or several digits together (Schieber 1995; Maier and Hepp-Reymond 1995). The motor cortex and the corticospinal tract are crucial for the control of such fine finger movements (Porter and Lemon 1993). To date, only one study in monkeys investigated the changes in muscle activation patterns of the hand and forearm after large lesions in M1 (Hoffman and Strick 1995), but no study performed the same analysis following small M1 lesions. We wanted to investigate the changes in behavioral performance and in muscle activation patterns during a reach-to-grasp task following a partial, localized inactivation of the M1 hand area. We used small Muscimol injections to partially inactivate the digit representation area of M1 unilaterally in the awake monkey. In addition to the deficits in behavioral performance as represented by the loss of independent finger movement with weakness/slowness of the reach and grasp, our results demonstrated changes in the temporal muscle activations and muscle coordination during movement. We observed that these muscle activation patterns were preserved for mild impairment and were altered for severe impairment, with more co-activations of muscles occurring. In future studies, the associative cluster analysis would be a powerful tool to quantify these changes in terms of muscle-synergy patterns. These results could also be compared to the changes in muscle-synergy patterns following larger inactivations of M1, which were not analyzed here. Furthermore, future studies could aim to inactivate other cortical areas in order to identify their contributions to the fine motor control of the hand, such as PMv (Fogassi et al. 2001; Kurata and Hoffman 1994; Moll and Kuypers 1977). Due to the commonality in deficits observed between Muscimol inactivations, lesions or stroke damage to M1, the results could also be compared to clinical studies with stroke

patients. Such comparisons will help us develop a better understanding of how muscle-synergy patterns reflect cortical damage.

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