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DEPARTMENT OF BIOENGINEERING

HUMAN MOTOR AUGMENTATION - SPINAL
MOTOR NEURONS CONTROL OF
REDUNDANT DEGREES-OF-FREEDOM

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DECLARATION OF ORIGINALITY

I hereby declare that the work presented in this thesis is my own, except otherwise acknowledged. Some parts have been conducted in collaboration with other researchers, and this is indicated where relevant.

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ABSTRACT

Human motor augmentation - spinal motor neurons control of redundant degrees-of-freedom

Mario Bräcklein

In 1963, Stan Lee introduced a new villain to the Spiderman Universe: Dr Octopus – a human equipped with multiple robotic arms that can be controlled seamlessly in coordination with his natural limbs. Throughout the last decades, turning such fiction into real-life applications gave rise to the research field of human motor augmentation, ultimately aiming to enable humans to perform motor tasks that are sheer impossible with our natural limbs alone. While a significant process was made in designing artificial supernumerary limbs, a central problem remains: identifying adequate bodily signals that allow moving supernumerary degrees-of-freedom together with our natural ones. So far, neural activity in the brain seems to hold the greatest potential for providing all the flexibility needed to ensure such coordination between natural and supernumerary degrees-of-freedom. However, accessing neural populations in the cortical regions is accompanied by an unacceptable risk for most users. A different group of neural cells can be found in the outmost layer of the motor pathway, driving the contraction of muscles and generation of force – spinal motor neurons. The development of novel neural interfaces has made it possible to study single motor neuron activity with minimal harm to the user. This allows a direct and non-invasive window into the neural activity orchestrating human movement. In this dissertation, I investigate whether these neurons innervating our muscles could provide supernumerary control signals. The results indicate, in essence, that features extracted non-invasively from motor neuron activity have the potential to overcome current limitations in supernumerary control and thus could significantly advance human motor augmentation.

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¹ This is a list of all peer-reviewed publication that contributed to this dissertation. All publications are arranged in chronical order.

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LIST OF ABBREVIATIONS¹

AI	-	artificial intelligence	-	Degree of intelligence performed by a computer
BCI	-	brain-computer interface	-	Command interface using brain activity
CMC	-	corticomuscular coherence	-	Method to estimate the spectral coupling between cortical and muscular activity
CNS	-	central nervous system	-	Unity of brain and spinal cord orchestrating body functions
CoV	-	coefficient-of-variation	-	Ratio of standard deviation and mean of a value
CST	-	cumulative spike train	-	Sum of all motor neuron activity
ECoG	-	electrocorticography/-gram	-	Procedure to measure electrical potentials on the surface of the cortex
EEG	-	electroencephalography/-gram	-	procedure to measure electric potential due to brain activity at skin-level
EMG	-	electromyography/-gram	-	procedure to measure electric potential due to muscle contraction inside the muscle or at skin-level
FCST	-	filtered cumulative spike train	-	Filtered sum of all motor neuron activity
HDsEMG	-	high-density surface EMG	-	EMG recorded on the surface of the skin via multiple electrodes arranged in a grid
IMC	-	intramuscular coherence	-	Method to estimate the strength of common input to a motor neuron

¹ The short definitions only serve as an exemplification and do not aim to provide a sophisticated explanation of the respective terms.

In addition, abbreviations are reintroduced in each chapter.

			pool in the spectral domain
MEG	-	magnetoencephalography/-gram	- Procedure to measure magnetic fields due to brain activity
MU	-	motor unit	- Conglomerate of motor neuron and all its innervated muscle fibres
MUAP	-	motor unit action potential	- Action potential along the muscle fibres innervated by a motor neuron
MVC	-	maximum voluntary contraction level	- Maximum force during a specific movement that can be generated voluntarily
PC	-	principal component	- Direction along which data explains the most variance
PCA	-	principal component analysis	- Statistical method to reduce dimensionality of datasets
pps	-	pulses-per-second	- Unit describing the occurrence of action potentials
sDoF	-	supernumerary degrees-of-freedom	- Additional directions on which movement can occur that cannot be utilized with the natural limbs

CHAPTER 1 INTRODUCTION

Equipping humans with artificial robotic limbs that can be controlled as effortlessly as the natural ones is a popular theme in science fiction and art (Kac 1997). In the last decades, the field of human motor augmentation has emerged to turn this phantasm into reality: extending human motor capacities beyond anatomical boundaries. Since then, significant progress has been made, especially in the development of artificial supernumerary limbs. One major bottleneck, however, is the coordination of natural limbs and such artificial actuators. A harmonized orchestration of, for example, a robotic third arm or cursor in augmented and virtual realities, demands the central nervous system (CNS) to provide control commands to and receive sensorimotor information from such additional effectors. While compromises for coordinating natural and supernumerary degrees-of-freedom (sDoF) were found for specialised and general-purpose applications (Prattichizzo *et al.* 2021), they come at the expense of limiting natural motor function. Hence, current control paradigms do not allow for an effective extension of *all* natural DoF and thus do not leverage the full potential of human motor augmentation. Assuming it exists, a fully sovereign navigation paradigm based on bodily signals that can be accessed easily and modulated volitionally without impeding existing motor functions is yet to be found.

1.1 Outline of dissertation

In this dissertation, I present work solely focusing on finding new ways to produce supernumerary control signals. I reveal and assess the potential that lies in the activity of motor neurons, the final link in the motor pathway that bridges the CNS and muscles, to act as an adequate resource for extending human motor function.

In the remainder of Chapter 1, I review the current state-of-the-art in human motor augmentation research, characterise current attempts to coordinate natural limbs and sDoF, identify limitations and describe why motor neurons could comprise all the necessary features to overcome these roadblocks (section 1.2). In section 1.3, I provide additional context on how motor neuron activity can be tracked and monitored non-invasively and review how the CNS orchestrates motor neurons in movement generation and what potential resources this opens up to extract features for additional control in section 1.4.

In Chapter 2, I use a novel neural interface to investigate whether spectral components within the beta range (13-30 Hz) of spinal motor neurons, can be modulated partially uncoupled from the resulting force of the innervated muscle. The work in this chapter suggests that high-frequency components to motor neuron pools may allow an expansion of DoF.

In Chapter 3, I aim to establish a better understanding of beta oscillations at the level of motor neurons, observed in Chapter 2, and their relationship to cortical beta components by simultaneously recording motor neuron and cortical activity. Furthermore, by using the experimental biofeedback paradigm from Chapter 2, I test how volitional modulations of beta activity in the periphery relate to cortical beta power.

In Chapter 4, I focus on single motor neurons as an alternative potential resource for controlling sDoF in contrast to the beta frequency feature from Chapters 2 and 3 which was extracted from an entire motor neuron pool. I introduce a biofeedback task that encouraged subjects to gain flexible control over motor neuron pairs. I provide evidence for potential strategies that could allow subjects to succeed in the biofeedback task for various combinations of motor neuron pairs.

In Chapter 5, I conclude all findings and discuss potential future directions that could help to translate the findings made in this dissertation towards novel human-machine applications. Finally, I will provide my personal view on the role motor neurons could play in human motor augmentation.

1.2 Human motor augmentation

Whether moving a chair, walking up a flight of stairs, preparing food, or typing a text message – the only way for humans to interact with their environment is via their motor output. From the earliest beginnings of human civilisations to the present day, humans have used technology to extend such motor skills to accomplish novel interactions that are impossible with the natural motor functions alone (Ambrose 2001). This includes simple tools such as stones to open nuts that cannot be opened with the bare hands alone, a bicycle to cover further distances, or even more sophisticated technologies such as exoskeletons (Dollar & Herr 2008) and suits (Lee *et al.* 2017) to reduce the physical load on the human body during heavy lifting, and carrying of objects (de Looze *et al.* 2016) or robotic devices to extend the spatial reach of the natural limbs during surgery (Sung & Gill 2001) or outer space manipulations (Gu & Xu 1995). The use of technology to enable humans to perform motor

tasks impossible with the natural motor function alone is understood as *human motor augmentation* (Eden *et al.* 2022).

While bicycles or exoskeletons improve an existing motor function, a new category of human motor augmentation has recently emerged, aiming to extend the overall motor capacities by allowing humans to orchestrate sDoF, such as controlling artificial supernumerary limbs (Dominijanni *et al.* 2021; Eden *et al.* 2022). This type of augmentation, recently coined as “*DoF augmentation*” (Eden *et al.* 2022), has the potential to tremendously reshape the way humans interact with their environment beyond what is possible with our natural limbs alone.¹

Recent advances in wearable robotics and human-machine interfaces have promoted the rapid development of a multiplicity of artificial supernumerary limbs (Makin *et al.* 2017), for instance, artificial fingers (Clode 2018; Cunningham *et al.* 2018; Hussain *et al.* 2015; Malvezzi *et al.* 2019; Prattichizzo *et al.* 2014a; Wu & Asada 2014a), arms (Bonilla & Asada 2014; Guggenheim *et al.* 2020; Khodambashi *et al.* 2016; Nguyen *et al.* 2019; Parietti *et al.* 2014; Parietti & Asada 2017; Penaloza *et al.* 2019; Sanchez *et al.* 2019; Sasaki *et al.* 2017; Veronneau *et al.* 2020), and legs (Hao *et al.* 2020; Khazoom *et al.* 2020; Parietti *et al.* 2015; Treers *et al.* 2017). The potential application spectrum of these artificial actuators is vast. It reaches from, for example, overhead assembly work (Parietti *et al.* 2014), load reduction (Bonilla & Asada 2014; Hao *et al.* 2020; Parietti *et al.* 2015), stabilisation aid (Kurek & Asada 2017), surgical robotics (Huang *et al.* 2020), ‘third hand’ applications to overcome bimanual constraints (Guggenheim *et al.* 2020; Nguyen *et al.* 2019; Veronneau *et al.* 2020) by enhancing dexterity of single hands (Prattichizzo *et al.* 2014a; Wu & Asada 2014a), to applications in

¹ For this reason, the remainder of this dissertation focuses on *DoF augmentation* when referring to human motor augmentation in general.

entertainment such as playing the piano with an extra thumb (Cunningham *et al.* 2018) or drumming with an extra arm (Khodambashi *et al.* 2016).

For a supernumerary limb to provide an effective augmentation of motor capabilities, seamless integration of the additional sDoF with the existing effectors in the human body needs to be given (Dominijanni *et al.* 2021; Eden *et al.* 2022). For example, this can be the concurrent manipulation of the human hand equipped with an artificial third thumb (Clode 2018). Hence, in an ideal case, the control of sDoF should be as volitional as but independent from natural limb movement. Throughout the years, several concepts were developed to allow for effective coordination between natural and sDoF. These attempts can be grouped into three different categories as we recently introduced in Eden *et al.*, 2022:

“Autonomous DoF augmentation extends the number of DoFs involved in one or more tasks using autonomously controlled devices. For instance, a robot may help carry an object with its human user.

DoF augmentation by transfer, in contrast, lets the user control the sDoFs. However, it only extends the number of movement DoFs involved in a task by re-purposing other existing body DoFs that are task-irrelevant. An example would be a third arm controlled by foot movements for three-tool surgery.

*Augmentation by DoF extension lets the user control the sDoFs by extending the body’s total number of movement DoFs. An example would be a third arm driven by neural activity that can be controlled independently from and concurrently with the natural limbs while preserving the full” (Eden *et al.* 2022)*

1.2.1 Autonomous augmentation

One possible approach to allow for coordination between natural and sDoF is to employ an autonomous agent. In such cases, the user is not in direct control of the sDoF. Instead, an artificial intelligence (AI) (Bonilla & Asada 2014) or remote human operator (Veronneau *et al.* 2020) coordinates movement along sDoF, for example, an artificial third arm, to support the human user during specific tasks. Such autonomous agents are mainly utilised in specialised purpose applications such as overhead assembly work (Bonilla & Asada 2014), aircraft manufacturing (Parietti & Asada 2014), or grasping support (Wu & Asada 2014b), to just name a few. In Veronneau *et al.*, for instance, the supernumerary limb was navigated by an additional human operator via a remote controller that mimics the kinematics of the supernumerary arm (Veronneau *et al.* 2020). Therefore, in such teleoperated scenarios, an additional human supervises the coordination between both natural and sDoF.

A different approach is to replace the human operator with an AI agent to control the sDoF subserviently to the natural limb movement inside the task environment. To reduce the complexity in coordination between natural and sDoF, the tasks in which sDoF are utilised are well-defined, i.e. all steps within a task are known a priori and follow a specific order. Moreover, the natural limbs perform the complex aspect of a task. At the same time, the sDoF are used on simple executions such as holding an object in place (Bonilla & Asada 2014), reducing the load on the user while performing manufacturing tasks in a static body position (Parietti *et al.* 2014) or during near-ground work (Kurek & Asada 2017). In applications with a more dynamic task process, the AI agent observes the natural limb movement to identify control commands that serve the coordination between natural and artificial limbs. Such

motion coupling was shown, for example, using an artificial finger activated in synergy with the natural ones by analysing fingertip movement and gesture of the augmented hand (Prattichizzo *et al.* 2014b, 2014a; Wu & Asada 2016). Other prototypes mimic the lower extremities, such as artificial supernumerary legs (Hao *et al.* 2020; Khazoom *et al.* 2020; Parietti *et al.* 2015), exploiting information about the user's gait cycle to provide support and load reduction during standing and walking.

In conclusion, using autonomous agents to drive sDoF shows great potential for various use cases in domestic and industrial environments. Since an external operator is fully dedicated to orchestrating the augmentation device, it allows for the exploitation of complex robotic systems which may provide several sDoF at once. Moreover, as little to no input is required by the user during the usage of such sDoF, simple human-machine interfaces, e.g. inertial measurement units (Kurek & Asada 2017), provide enough information to operate these systems. This limited communication between the operator and the sDoF provides evidence for short learning and adaption periods by the user when leveraging these sDoF. Nevertheless, utilising such autonomous agents is limited to well-structured and simple tasks. The lack of voluntary control over the sDoF does not allow great flexibility during the application process.

1.2.2 Augmentation by transfer

In order to overcome this functional gap autonomous agents leave, the user itself needs to be in charge of coordinating both natural and sDoF. One concept exploits bodily signals to trigger predefined activations of the sDoF, for example, by using voice commands (Vatsal & Hoffman 2018), facial expressions (Fukuoka *et al.* 2019) or eye movement (Maimon-Dror *et al.* 2017). Leigh and Maes identified specific hand gestures from muscle activity in the

forearm to trigger movements of the artificial actuator attached to the user's wrist (Leigh & Maes 2016a). Such concepts, however, are limited to activating pre-defined motions of the sDoF. To ultimately utilise sDoF for general purpose applications, users require volitional *and* continuous control over sDoF. A common approach to enable users for such coordination is to employ body or myoelectric interfaces, traditionally found in computer (Simeone *et al.* 2014; Velloso *et al.* 2015), robotics (Hannaford *et al.* 2013) and prosthetic control (Farina *et al.* 2014a; Ison *et al.* 2016), to record movement (Abdi *et al.* 2015; Clode 2018; Huang *et al.* 2020; Sasaki *et al.* 2017) or muscle activation (Hussain *et al.* 2016; Parietti & Asada 2017) of certain body parts that do not directly interfere with the task-specific motions in which sDoF are used. For instance, Dani Clode designed a neat artificial third thumb to enhance the augmented hand's dexterity (Clode 2018), which was used to investigate the learning of supernumerary control (Kieliba *et al.* 2021). Figure 1-1 illustrates the third thumb design. The two DoF of the robotic limb, in this example, are controlled via pressure sensors mounted to the big toes of the user (Clode 2018).



Figure 1-1: Artificial supernumerary limb model 'Third Thumb' by Dani Clode Design for enhanced dexterity. The artificial thumb is mounted to the wrist and equips the user with two sDoF. The Third Thumb is driven via

two motors worn on the forearm. The user controls these motors via a wireless body interface comprising two pressure sensors placed underneath the user's big toes—courtesy of Dani Clode Design/daniclude.com.

Similarly, the movement of one foot (Huang *et al.* 2020), both feet (Sanchez *et al.* 2019), hand and foot (Cunningham *et al.* 2018) or the elbow (Wu & Asada 2015) may be used to drive sDoF. In contrast to these body interfaces that are worn by or assembled around the user's limbs, camera systems may be used to remap natural limb movement into sDoF control signals (Abdi *et al.* 2015). Instead of recording the movement of entire limbs, single muscle activation recorded via surface electromyography (EMG) can also be mapped into control commands for sDoF. Previous prototypes explored, for example, the EMG of several torsi (Parietti & Asada 2017), facial (Hussain *et al.* 2017; Meraz *et al.* 2018) or shoulder and arm muscles (Gurgone *et al.* 2022) to generate control commands. Moreover, hybrid systems combine various approaches discussed above to optimise the coordination of sDoF. For example, Hussain *et al.*, supplemented the activation of pre-defined movements of an artificial sixth finger triggered by the forearm EMG via continuous control of the grasping tightness via the biceps' EMG (Hussain *et al.* 2016). A hybrid system comprising a motion capture camera system and bending sensors worn on the user's foot (body interface), introduced by Sasaki *et al.*, showed the potential of driving several sDoF concurrently (Sasaki *et al.* 2017).

Taken together, such continuous control is achieved by remapping bodily signals not primarily used in the task-specific operation to manipulate sDoF. Hence, the movement of sDoF is not constrained to follow or imitate the user's body. Instead, the users themselves have direct control, which enables the use of sDoF in complex application scenarios such as surgery (Huang *et al.* 2020, 2021), enhanced dexterity (Clode 2018; Leigh & Maes 2016b) or advanced industrial settings (Srinivas *et al.* 2015), to name a few. However, in such scenarios,

motor augmentation is at the expense of remapping the activation of natural limbs. Therefore, supernumerary control signals extracted from natural limb movement or muscle activation demand that the corresponding effectors are redundant at the time of supernumerary control. For example, in the work of Dani Clode, enhanced dexterity is achieved by controlling an artificial thumb in coordination with wrist and finger movements. The sDoF is operated by re-mapping bodily signals, i.e. toe movements. The ability to use the toes or even the feet for other motor tasks, such as walking, while operating the sDoF is lost due to the feet's occupation for controlling the supernumerary thumb. This type of augmentation is context-dependent and may not always generalise well. To fully leverage the potential of human motor augmentation, the control of sDoF should be able to adapt to different functions and tasks without impeding natural limb functionality.

1.2.3 Augmentation by extension

Neither *autonomous augmentation* nor *augmentation by transfer* provide a supernumerary control scheme that can be volitionally used without impeding natural limb movement. The fundamental question is, thus, whether the CNS has at all the resources to provide a control channel that allows for human motor augmentation by coordinating between natural and sDoF (Dominijanni *et al.* 2021).

Mehring *et al.*, addressed this question by studying the movement abilities of subjects born with natural supernumerary limbs in the shape of an additional finger per hand (Mehring *et al.* 2019). These polydactyl subjects could control and navigate their supernumerary limbs, mostly independent of the fingers. This six-finger control was shown to be superior to five finger movements. Hence, it allowed the subjects to perform one-handed manipulation tasks impossible for normal-bodied humans. Moreover, the study revealed that polydactyl subjects

had not only independent muscles and nerves but also dedicated cortical structures to orchestrate the sDoF (Mehring *et al.* 2019). These findings indicate that the human brain has the resources needed to drive sDoF. However, how can normal-bodied humans leverage these resources?

The human motor system decreases in complexity from the cortical level with millions of neurons, over hundreds to thousands of spinal motor neurons to the activation of single muscles and, finally, the movement of limbs. This motor pathway holds great redundancy or abundance (Latash 2012). Especially these early stages of the motor pathway may include a *motor null space*, i.e. change in underlying neural activation pattern while the motor output remains unaffected. Therefore, augmenting human motor function in normal-bodied individuals may rely on spare biological signals inside this null space of motor control, i.e. neural activity that can be modulated voluntarily without impeding or directly changing natural motor function (Kaufman *et al.* 2014). In fact, previous research suggested that the underlying high dimensional neural activity during specific motor tasks can be projected on low-dimensional manifolds (Gallego *et al.* 2017) not at all utilising the full dimensionality potentially available on the neuron level. Moreover, although only shown in the absence of movement, single neurons inside the primary motor cortex may behave dissociated from their correlated limb movement (Schieber 2011). This may mean that certain neural modulation appears inside a null space that does not directly alter the human motor output (Kaufman *et al.* 2014). However, the underlying mechanism behind these results is yet to be fully understood (Lebedev 2017).

Several brain-computer interfaces (BCIs) studies explored adequate sources for supernumerary control signals inside such a neural null space. Pioneering non-human primate

studies demonstrated that supernumerary control features extracted from intracortical single-unit recordings could be modulated during natural limb movement (Milovanovic *et al.* 2015; Orsborn *et al.* 2014). In a recent study performed by Penaloza and Nishio, able-bodied human subjects were instructed to perform a balancing task involving both of their arms (natural DoF) while the movement of a robotic arm (sDoF) was triggered via BCI control (Penaloza & Nishio 2018). The trigger signal, extracted non-invasively from the electroencephalogram (EEG), was used to activate predefined movements of the artificial limb. The results suggest that most subjects successfully performed the BCI task concurrently with the balancing task within a single experimental session. However, it remains unclear which underlying cortical mechanism provoked the movement of the artificial actuator as the trigger signal was extracted from a single EEG electrode determined by the highest power output during an imagination task (grasping of a bottle) rather than an underlying functional or anatomical cortical structure. On the contrary, efforts were made by different research groups also using BCI-based sDoF control, but from focal cortical areas. For example, in Cheung *et al.*, human subjects were trained to perform a motor imagination task (movement of the right hand) while controlling a joystick (with the left hand)(Cheung *et al.* 2012). Cortical signals associated with motor imagination were extracted from the EEG. Subjects were then asked to navigate a cursor inside a 2D space in which one axis was controlled via joystick movement (natural DoF) and the other by cortical signals obtained from the EEG (sDoF). It was shown that, to some extent, the three subjects studied here were able to coordinate both motor imagination and hand movement. Similarly, Leeb *et al.*, designed a virtual reality experiment in which subjects navigated an object along the horizontal axis via a joystick (natural DoF) and triggered a movement along the vertical axis (sDoF) via EEG (foot movement imagination task) (Leeb *et al.* 2013). Even though there was no continuous control along the

sDoF, it was shown that subjects were able to perform natural movement overt to EEG control. Both of these studies have used cortical signals arising from different cortical structures, i.e. different hemisphere (Cheung *et al.* 2012) and different cortical sites associated with different body parts (Leeb *et al.* 2013), compared to those responsible for the overt natural movement. Therefore, it remains unknown whether these control schemes only work in the absence of any movement of the limb associated with the motor imagination and thus relies on compensation or substitution as seen in the concepts utilising body or muscle interfaces discussed above (see section 1.2.2).

Bashford *et al.*, have recently shown that it is also possible to extract two control channels (one for natural and one for sDoF control) originating from the same cortical structure (Bashford *et al.* 2018). Three subjects performed a 2D target tracking task similar to the study conducted by Cheung *et al.*, 2012. One axis was controlled by natural limb movement (keypress via finger movement) and the other by BCI activation. The sDoF was controlled continuously via a feature extracted from gamma-band activity via the electrocorticogram (ECoG) emerging from the same cortical structure associated with the finger movement. It was shown that subjects learnt to dissociate their motor ECoG signals from the originally correlated movements. Despite the pre-experimental association between both, gamma-band activity was modulated partially independently from the ongoing finger movement.

The human brain is flexible and can learn and adjust to changing sensorimotor settings (Di Pino *et al.* 2014; Krakauer & Mazzoni 2011). The results of Bashford *et al.*, similar to those obtained in non-human primates (Lansdell *et al.* 2020), provide evidence that such operant conditioning experimental settings may provoke the development of a BCI (sDoF) control map in addition to and independent from the natural control scheme. Such mapping may even

stay present after finishing the BCI task, as observed for other BCI control experiments (Jackson *et al.* 2006) and may be quickly re-employed via advanced decoder adaption (Silversmith *et al.* 2020). More research is necessary, but these findings provide evidence that humans may learn via operant conditioning to employ such sDoF control maps.

These findings suggest that single neurons or entire neural populations yield the potential for controlling sDoF. However, human motor augmentation aims to provide sDoF control for general purpose applications. Hence, one of the biggest challenges in utilising such neural signals is their accessibility. Non-invasive BCIs such as EEG provide a wearable and cost-efficient approach to recording cortical signals with a moderate temporal and spatial resolution. Similarly, magnetoencephalography (MEG), a different non-invasive method, overcomes some shortcomings in resolution observed in EEG systems. However, current MEG systems based on superconducting quantum interference devices require extensive cooling and magnetically shielded rooms, making their operation expensive and non-wearable. Moreover, both EEG and MEG are prone to artefacts and their inverse solution is ill-posed which restricts the confidence in which a neural population that may generate a supernumerary control signal can be tracked across and within sessions. Hence, EEG and MEG might not provide the reliability needed for general-purpose sDoF applications. Recordings of single neurons or field potentials via intracortical or ECoG systems, respectively, provide superior signal quality with a high temporal and spatial resolution. Thus, such systems allow us to acquire neural signals from focal cortical sites. Moreover, due to the better signal-to-noise ratio, such systems allow for neural recordings with a high spectral resolution, as observed, for example, in Bashford *et al.*, 2018. However, such procedures are highly invasive and, therefore, come with a great risk for the user and are unacceptable for human motor augmentation applications by current standards.

So far, none of the techniques discussed here provide a window into human motor control that bridges the access to direct neuronal recordings on one side and non-invasive and wearable design on the other. However, this gap could be closed with recent advances in EMG technology that made it possible to study and record non-cortical neural activity non-invasively (Farina *et al.* 2016). Motor neurons are the final neural cells in the motor pathway, ultimately bridging the CNS and muscles to form movement. Their activity is present in the electrical field generated by muscle contractions and thus can be detected with minimal or even non-invasive EMG recording setups. Hence, decoding single motor neurons non-invasively could be a viable alternative to current invasive approaches to track and monitor human neural populations (Farina & Holobar 2015; Holobar & Farina 2021; Sartori & Sawicki 2021) and potentially extracting a control signal suitable for extending human motor function.

1.3 EMG decomposition – non-invasive access to neural activity

The CNS orchestrates movement generation by commanding the activation of skeletal muscles. Such motor commands emerging from the CNS are propagated and tuned via alpha motor neurons emerging from the ventral horn in the spinal cord on one side and innervating skeletal muscles on the other (Hounsgaard 2017; Merletti & Parker 2004). Therefore, motor neurons build a direct link between the output of the CNS and muscle activation (Hounsgaard 2017). Descending synaptic input, as well as the outputs of afferent loops and spinal interneurons, trigger action potentials at the initial segment of the motor neuron's axon. These action potentials are propagated along the motor neuron axon and arrive at the neuromuscular junction, a synapse between the motor neuron and its innervated muscle fibres. The transmission of motor neuron action potentials at this neuromuscular junction is considered to be reliable (Ruff Robert 2006; Wood & R. Slater 2001), always resulting in action

potentials along the innervated muscle fibres. For this reason, a motor neuron and its innervated muscle fibres are understood as a single functional unit, the motor unit (MU). Due to the secure transmission of action potentials via the neuromuscular junction, MU action potentials (MUAPs), i.e. action potentials propagated along the innervated muscle fibres, represent an amplified version of the initial action potential triggered at the dendrites of the corresponding motor neuron. These MUAPs generate electrical fields, which can be measured either inside the muscle or at skin level via EMG (see Figure 1-2).

Regardless of the measurement technique, i.e. using intramuscular or surface electrodes, the EMG is the convolutive mixture of all MU activity (e.g. spike timings of individual MUAPs) with their respective MUAP shapes detected at the recording site. In order to access the central properties of MU activity, such as their discharge behaviour, for example, to analyse neural strategies that underpin force generation, the convolutive mixture captured in the EMG needs to be decomposed into the underlying neural activity. In the case of intramuscular EMG, where the observation site (EMG electrodes) is near the sources (MUs), the decomposition may be solved by spike sorting techniques known from intracortical recordings, such as template matching (Kleine *et al.* 2007). However, the decomposition problem becomes more challenging when the distance between the source and the observation site increases. During surface EMG recordings, when the electrodes are usually placed above the muscle belly at skin level, the electrical fields of the MUAPs need to traverse through various layers of tissue, for example, muscle, bone, adipose, and skin tissue, depending on the subject and the muscle before reaching the electrodes. These anatomical volume conductors act as a spatial low-pass filter with an unknown finite and non-linear impulse response, ultimately affecting the representation of original MUAP at the skin level (Farina *et al.* 2002).

Moreover, each EMG channel records an average of this underlying convolutive mixture of *all* MU activity at its respective locations. Hence, effects such as amplitude cancellations due to the biphasic nature of the signal itself further distort the MUAP representation in the EMG (Farina *et al.* 2008; Keenan *et al.* 2006). These degradations are compounded by the properties of the EMG electrodes (Roy *et al.* 1986) and insufficient knowledge about the exact MUAP location, and thus the spatial propagation of the generated electrical fields through the underlying tissue (Farina *et al.* 2014b), all resulting in not less than a blurred representation of MU activity in the EMG.

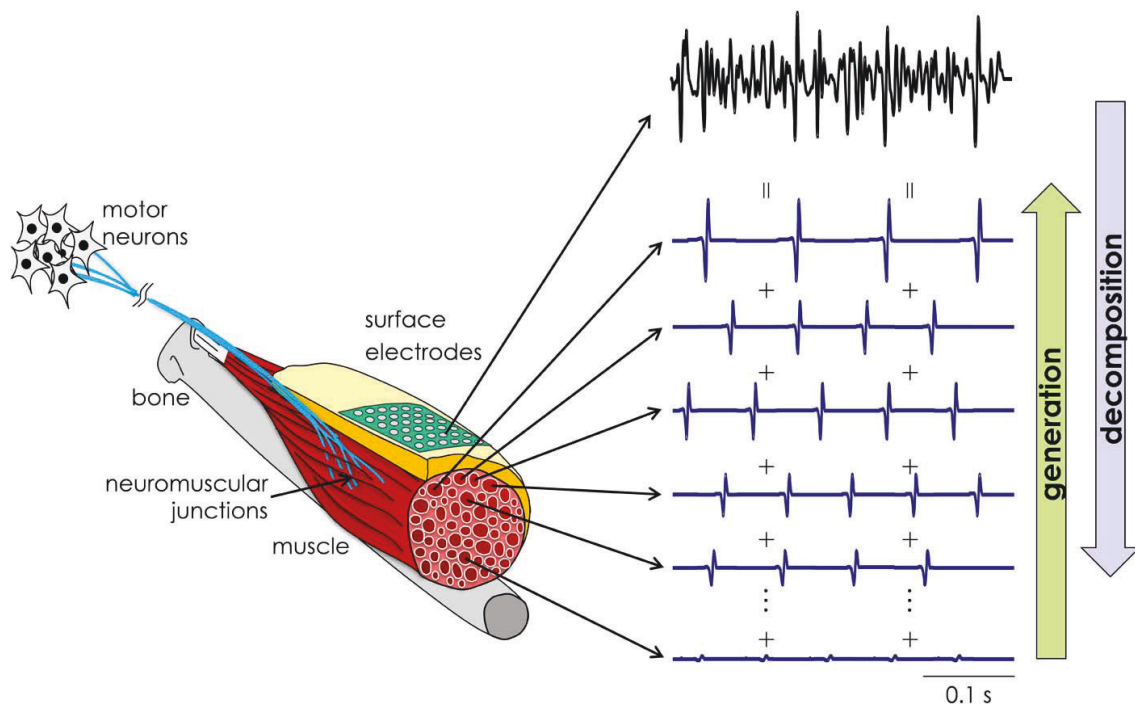


Figure 1-2: Schematic overview of EMG generation. Motor neurons arise from the spinal cord on one side and innervate muscle fibres on the other. The secure transmission of motor neuron action potentials via the neuromuscular junction, i.e. a synapse between the motor neuron and the innervated muscle fibres, always results (in healthy individuals) in an action potential propagated along the muscle fibres. For this reason, the functional conglomerate of the motor neuron and its innervated muscle fibres is understood as a motor unit and their action potentials as motor unit action potentials. The electromagnetic fields due to motor unit action potentials traverse through various layers of tissue, affecting their representation before reaching electrodes on the skin's surface. The resulting electromyogram is thus the sum of all filtered motor unit action potentials. Reproduced from Farina & Holobar, 2016

Recent advances in EMG electrode design (Stegeman *et al.* 2012), computational modelling and advanced source separation algorithms (Holobar & Zazula 2007; Negro *et al.* 2016a), however, made it possible to deconvolute the spatial-temporal mixture of motor neuron activity observed in the EMG into the underlying MUAP spike trains. Recordings from multiple EMG electrode channels arranged in a grid, i.e. high-density sEMG (HDsEMG), allow the tracking of the convoluted MU activity in time and space (Blok *et al.* 2002; Stegeman *et al.* 2012). By increasing the number of observations of the underlying MU activity, i.e. moving from traditional bi-polar EMG recordings towards HDsEMG, the spatial variability of the MUAP propagation inside the innervated muscle can be tracked. Thus, more recording sites in HDsEMG improve the discrimination between different MUs in the EMG (Merletti *et al.* 2008). Over the last decades, several approaches have been developed to identify individual motor neurons from the EMG (Chen & Zhou 2016; Clarke *et al.* 2021; Gazzoni *et al.* 2004; Holobar & Zazula 2007; Negro *et al.* 2016b). One extensively validated and popular approach is to utilise blind source separation techniques based on convolutive kernel compensation (Holobar & Zazula 2007; Negro *et al.* 2016b).

In general, the relationship between MUAP spike trains and the measured EMG can be described as a convolutive mixture in which the MUAP spike trains are modelled as δ -impulses. Moreover, these MU spike trains are sparse due to the refractory period, which prevents MUs from uninterrupted firing. Therefore, the MU spike trains can be modelled as binary codes predominantly comprised of 0, with a few 1 indicating the timing of a MUAP discharge. Following, the measured EMG can be described as:

$$EMG(k) = \sum_{l=0}^{L-1} H(l)s(k-l) + \omega(k) \quad \text{Eq. 1-1}$$

where the observation vector $EMG(k)$ comprises the measured EMG signals of the m recording channels at the k -th sample. The central properties of the n detected MUs, i.e. the timings of MUAP discharges, are noted in the source vector $s(k)$. The additive noise term $\omega(k)$, assumed to be uncorrelated in time and space, comprises all electrical noise due to the recording setup itself and low-power MUAPs undistinguishable by the decomposition model (Bankman *et al.* 1993). The size of the additive noise term $\omega(k)$ is determined by the number of the EMG recording channels m . All MU firing comprised in the source vector are assumed to be sparse and independent. The mixing matrix H , of size $m \times n$, contains all MUAP shapes and their respective spatial variability due to the subject-specific low-pass filter at each observation site for each sample l , whereas l ranges from 0 to $L - 1$ (Negro *et al.* 2016a). L is proportional to the average MUAP length.

The goal of all decomposition techniques is to find the inverse solution, $s(k)$, to the convolutive mixture in Eq. 1-1. This separation problem can be solved by transforming the convolutive mixture into an instantaneous one. For this reason, the source vector $s(k)$ is extended by delayed version of the original sources themselves. The extension factor is limited by the refractory period and usually set to L , i.e. the average MUAP length. Therefore, the extended source vector $\tilde{s}(k)$ incorporates all original sources from Eq. 1-1 and $L - 1$ delayed versions resulting in total length of $nL \times 1$ at every sample k . The original sources and their delayed versions in $\tilde{s}(k)$ remain uncorrelated since L is limited by the refractory period and the MU spike trains are generally sparse. To avoid an ill posed equation, i.e. having more sources than observations, the observation matrix $EMG(k)$ is extended by its delayed

repetitions, too. Hence, its extension factor R is suggested to be $R \geq \frac{n}{m}L$ (Negro *et al.* 2016b), leading to an extended observation vector $\widetilde{EMG}(k)$ of size $mR \times 1$ at every sample k . The extended version of Eq. 1-1 can be written as:

$$\widetilde{EMG}(k) = \tilde{H}\tilde{s}(k) + \tilde{\omega}(k) \quad \text{Eq. 1-2}$$

with the mixing matrix $\tilde{H}$ defined as

$$\tilde{H} = \begin{bmatrix} \tilde{h}_{11} & \cdots & \tilde{h}_{1n} \\ \vdots & \ddots & \vdots \\ \tilde{h}_{m1} & \cdots & \tilde{h}_{mn} \end{bmatrix} \quad \text{Eq. 1-3}$$

$$\tilde{h} = \begin{bmatrix} \tilde{h}_{ij}[0] & \cdots & \tilde{h}_{ij}[L-1] & 0 & \cdots & 0 \\ 0 & \ddots & \ddots & \ddots & \ddots & \vdots \\ \vdots & \ddots & \ddots & \ddots & \ddots & 0 \\ 0 & \cdots & 0 & \tilde{h}_{ij}[0] & \cdots & \tilde{h}_{ij}[L-1] \end{bmatrix} \quad \text{Eq. 1-4}$$

The MUAP of the j -th MU detected at the i -th electrode channel is captured in $\tilde{h}_{ij}$. All MUAPs in $\tilde{H}$ are assumed to be constant throughout the recording, i.e. for all samples k (Negro *et al.* 2016a). After the extension, all EMG channels are decorrelated by applying a spatial whitening to the observation matrix $\widetilde{EMG}$. The over-determined system in Eq. 1-2, i.e. more observations than sources, may then be solved using, for example, a fixed-point algorithm (Hyvarinen 1999; Negro *et al.* 2016a). At its core, the solution to this separation problem mimics an independent component analysis (Hyvärinen & Oja 2000), with the termination

criteria being sparseness rather than independence. Therefore, two sources, i.e. MU spike trains, are separated when they reach a maximum sparseness.

Combined with HDsEMG, these decomposition techniques allow a direct and non-invasive window into the underlying neural structure that orchestrates movement. Therefore, motor neurons are neural cells that can be tracked and monitored without surgery, thus with minimal to no harm to the human user. Over recent years, such novel neural interfaces comprised of surface EMG and decomposition algorithms were advanced to track multiple MUs in real-time during voluntary contractions (Barsakcioglu *et al.* 2021; Bräcklein *et al.* 2021, 2022b, 2022a; Formento *et al.* 2021). The major challenge in translating traditional decomposition methods into real-time ready versions are the computational heavy and thus time-consuming whitening of the data, i.e. decorrelating all the HDsEMG channels, and the calculation of the inverse of the mixing matrix. One extensively validated approach to overcome these challenges is to train the whitening and the inverse of the mixing matrices on a training data set (Barsakcioglu *et al.* 2021). This data set is usually recorded before the actual real-time decomposition is employed. This method assumes that the MUAP shapes decoded in the training data set remain constant throughout the entire experimental session. This pre-trained separation matrix can then be applied online to the recorded data stream, allowing decomposition of MU activity with neglectable delays.¹

¹ Further details on the real-time decomposition technique developed by Barsakcioglu *et al.* can be found in section 2.2.4.

1.4 Motor neurons – a resource for augmentation?

The recent developments in neural interfaces and advanced decomposition techniques allow access to neural activity in the periphery of the human motor system in the same way as intra-cortical recordings allow for the tracking of neural populations in the brain. Given their accessibility, motor neurons may be a promising candidate for augmentation. Yet, it needs to be shown if motor neurons can transmit multiple channels of information, i.e. one regarding movement of natural limb and one unrelated to motor behaviour.

To achieve coordination of sDoF and natural limbs, two, ideally independent, control channels are needed. One that ensures the volitional control of all natural limbs and one that serves the control of sDoF. In the case of motor neurons, a potential supernumerary control signal would need to lie in a null space to motor behaviour, i.e. motor neuron activity that does not necessarily interfere with force generation. Such a null space could potentially be identified in the compound activity of many motor neurons, similar to what was recently shown in a BCI study, where signal components in the population activity of cortical neurons could be controlled volitionally without interfering with the movement triggered by the same neural population (Bashford *et al.* 2018). A different null space approach would be to volitionally control single motor neurons without impeding the motor behaviour of the innervated muscle.

In the following section, I will refer to previous research related to the question of whether the activity of populations of motor neurons or single motor neurons comprises such a null space and suppose it exists, the possibility of being utilised for human motor augmentation. I will comment on two possible pathways for augmentation exploring high frequency

components in the compound activity of an entire motor neuron pool that are outside the muscular-skeletal bandwidth, and on the flexible control of individual motor neurons.

1.4.1 Motor neuron populations – bandwidths of the common input

The nervous system orchestrates movement by sending neural information through a diversity of synaptic inputs converging onto alpha motor neurons, including descending inputs from cortical regions and the brainstem (Lemon 2008) as well as proprioceptive and spinal inputs, for example, from sensory afferents (Windhorst 2007). If the net synaptic input to a single motor neuron, i.e. the integration of all excitable and inhibitory input signals, crosses an excitation threshold, the motor neuron starts to discharge action potentials leading to a twitch of the innervated muscle fibres. Hence, the compound activity of many active motor neurons innervating the same muscle leads to a contraction. The input signals received by such a pool of motor neurons, i.e. a group of motor neurons contributing towards a particular muscle contraction or movement, can be modelled into a common and independent input signal (Farina *et al.* 2014c; Negro *et al.* 2016c) as illustrated in Figure 1-3.

The common input signal is received by all motor neurons in the pool, while an independent input differs across individual motor neurons. Regardless of common or independent input, the transformation of synaptic input to MUAP spike trains by the motor neurons is non-linear. This is mainly because each motor neuron has an excitation threshold determined by its size and intrinsic state (see section 1.4.2). Hence, motor neurons will not discharge action potentials for any net-synaptic input below this threshold. Although, once this critical threshold is surpassed, the firing rate of action potentials partially relates to the strength of the net synaptic input (however, it should be noted that the discharge rate of motor neurons is limited by the refractory period and saturation effects (Fuglevand *et al.*

2015)). The resulting MUAP train represents a sampling of the net synaptic input comprised of a common and independent component. Hence, the output of every active motor neuron within a pool reflects this common input distorted by independent components. Muscle activation and, thus, force production results from the cumulative activity of the entire motor neuron pool, i.e. the sum of all MUAP trains. Such a summation of the individual motor neuron contributions enhances the common activity while the influence of individual independent components is attenuated when populations are considered, as compared to activity in individual motor neurons (Farina *et al.* 2014c; Farina & Negro 2015).

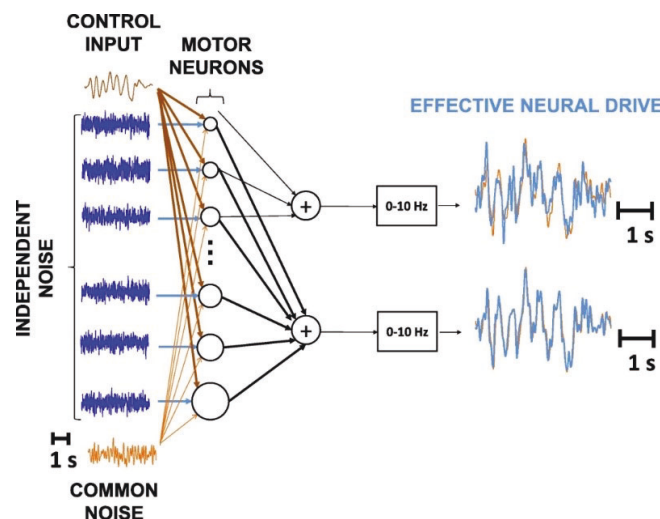


Figure 1-3: Schematic representation of the synaptic input to a motor neuron pool and the resulting force profile. The synaptic input to a motor neuron pool can be modelled into a common component that is shared across the population (here, the low-frequency component of this common input is termed control input and the high-frequency component common noise) and private independent input to each motor neuron (here termed as independent noise). Both common and independent components are present at the motor neuron output, i.e. action potential spike trains. However, the cumulative activity of the entire motor neuron pool mainly highlights the common input, whereas independent components cancel each other out. The average twitch response of the muscle fibres acts as a biomechanical low-pass filter with a cut-off frequency of approximately 10 Hz. Thus, only the low-frequency common input component (control input) is translated into force. This common low-frequency component is the effective neural drive, which ultimately determines the force output of the innervated muscle. This effective neural drive is superimposed on the right with the resulting force profile for considering a sub-pool of only three motor neurons (top) and the entire pool (bottom). As shown for only three motor neurons, if the number of neurons is small, the estimation of the effective drive can be noisy due to the non-linear nature of motor neurons. Reproduced from Farina & Negro, 2015

The strength of this common input signal to a motor neuron pool at a given frequency can be estimated using intramuscular coherence (IMC) (Castronovo *et al.* 2015; Dideriksen *et al.* 2018). Coherence generally measures the linear correlation in the spectral domain between two signals. In the case of intramuscular coherence, the spectral coupling within a motor neuron pool due to a common input drive is estimated, meaning the stronger the coupling, the larger the common component is at a given frequency. This common input signal has usually pronounced activity in the low frequencies (<10Hz) and a prominent peak at around 20Hz (see Figure 1-4). However, these high-frequency components do not get directly translated into the force of the innervated muscle. In fact, the resulting force profile only follows the low-frequency component (<10Hz) of the common input signal. The average twitch of the innervated muscle fibres limits the power spectrum of the resulting force (Dideriksen *et al.* 2012). These biomechanical constraints act as a low-pass filter allowing only a linear transmission of the low-frequency component of the common input to the resulting force profile of the innervated muscle(s) (Farina *et al.* 2014c). This low-frequency component is termed *effective drive* and is the ultimate code that determines movement (De Luca & Erim 1994; Gandevia 2001; Thompson *et al.* 2018).

In contrast, no obvious link to function can be assigned to higher frequency components of the common input drive, which is why they were previously coined as *common noise* (Dideriksen *et al.* 2012; Farina & Negro 2015; Negro *et al.* 2009). This high-frequency common noise component seems to be outside of the motor bandwidth due to the biomechanical constraints of muscles. Hence, activity in this spectral band could lie in a null space and not directly influence motor behaviour. However, if such a null space exists and whether or not activity inside this null space can be volitionally controlled without impeding force control of the innervated muscle is yet to be demonstrated.

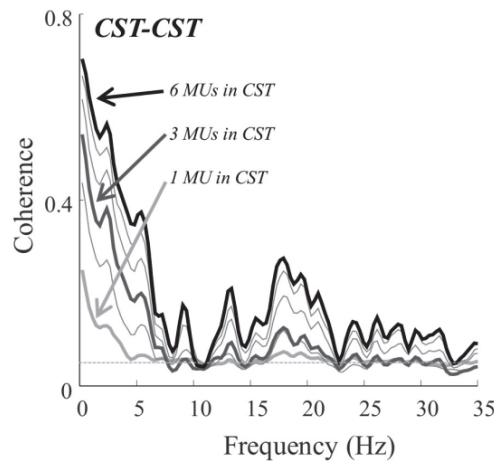


Figure 1-4: Intramuscular coherence of a single motor neuron pool. Intramuscular coherence (IMC) is a method to estimate the strength of the common input signal received by a motor neuron pool. Different IMC estimates are shown for different sizes of motor neuron pools, indicated by the shades of grey. The dashed horizontal line indicates the significance threshold of the estimated coherence values. Similar to the detection of a common input signal in the cumulative spike train, i.e. the sum of all motor neuron action potentials within a pool, the strength of the IMC largely depends on the number of active motor neurons. The strength of common input in the lower frequencies drastically diverges towards a maximum when the number of analysed motor neurons increases. Higher frequency components become “visible”, i.e. reaching significant coherence values for such larger groups of active motor neurons. The discharge rate of individual motor neurons is insufficient to reproduce the high-frequency components of the input drive. The refractory period and saturation effects limit the maximum discharge rate of motor neurons to often less than 40 spikes-per-second, which is too slow to react to the fast oscillation. This effect is comparable to aliasing in classical signal processing (Nyquist 1928). Hence, the cumulative activity of many motor neurons is necessary to accurately estimate the common input drive's high-frequency components to the entire pool. Even though only the low-frequency components (up to 10 Hz) are linearly translated into force production (effective neural drive), the IMC indicates prominent high-frequency components present in the common drive to the motor neuron pool. Yet, their functional role in force production is unknown. Reproduced from Dideriksen et al., 2018.

1.4.2 Single motor neurons – rigid control and the role of biofeedback

A single pool of motor neurons can consist of hundreds of individual neurons (Duchateau & Enoka 2022; Heckman & Enoka 2012). If just a handful of these motor neurons could be controlled flexibly, the information transfer via neural interfaces would increase tremendously. A few motor neurons controlled independently by the remaining tens to hundred motor neurons inside the same pool during force production would result in a multidimensional neural activation without a major impact on the force, in the same way as

the activity in single motor neurons determined by independent synaptic input is filtered in force generation (Farina *et al.* 2014c). Hence, the selective activation of a few motor neurons could provide the basis for driving sDoF without impeding the original functionality of the innervated muscle.

While such a mechanism could revolutionise motor augmentation, independent control of all motor neurons would imply a vast increase in computational load in the CNS, with no known *natural* functional benefit for the human motor system. In fact, most observations suggest that, in contrast to independent control, individual motor neurons are activated in a highly stereotyped manner determined by the common input received and the neuron's biophysical properties.

In 1957, Henneman discovered a dependency between the anatomy of motor neurons and their sensitivity to discharge action potentials (Henneman 1957). The membrane resistance of a motor neuron is anti-correlated to its soma size, meaning that for the same synaptic input, smaller motor neurons start to discharge action potentials earlier and at higher rates than larger ones. This relationship between the size of a motor neuron and its discharge behaviour is known as *size-principle*. Henneman's postulations state that a motor neuron's response to a given input signal is solely determined by its properties. In combination with the concept of a common input signal that determines movement, motor neuron control is based on a simple, one-dimensional activation. This size-principle could be confirmed throughout the years in various muscles (Desmedt & Godaux 1977b; Milner-Brown *et al.* 1973; Monster & Chan 1977; Oya *et al.* 2009; Riek & Bawa 1992; Thomas *et al.* 1986, 1987; van Zuylen *et al.* 1988) and seems to remain robust under various physiological conditions (Adam & De Luca 2003; Desmedt & Godaux 1977a; Fling *et al.* 2009; Jones *et al.* 1994; Thomas *et al.* 1986, 1987, 1978).

Despite this overall consensus that the activity of motor neuron pool underlies a rigid one-dimensional control scheme, humans seem to be able to gain control over individual neurons when provided with biofeedback on their spiking activity, using, for example, auditory and/or visual clues. In the 1960s, various research groups pioneered such investigations in volitional control of single motor neurons showing that subjects could quickly learn to modulate single neuron discharge rates (Basmajian 1963; Harrison & Mortensen 1962; Wagman *et al.* 1965). These results were later confirmed in various finger (Kato & Tanji 1972; Thomas *et al.* 1978) and arm muscles (Illyés 1977). While these findings seem to conflict with the size principle directly, i.e. smaller motor neurons get recruited before larger ones for the same net synaptic input, they share one caveat – the experiments were conducted in multifunctional muscles or changes in posture were allowed during the recording session.

Recent studies argue that the nervous system accommodates such multifunctionality using multiple common inputs to the same muscle (Bawa *et al.* 2014). In line with this view, one can distinguish between an *anatomical* motor neuron pool, i.e. all motor neurons innervating muscle fibres of one muscle, and a *functional* motor neuron pool which comprises all motor neurons that contribute towards one specific motor task. Such functional motor neuron pools are not limited by anatomical boundaries. For example, they can span across multiple muscles, as observed in extrinsic and intrinsic hand muscles during grasping (Tanzarella *et al.* 2021). On the other hand, a single muscle can be comprised of several functional units (Hoffer *et al.* 1987; ter Haar Romeny *et al.* 1984; Wakeling 2009), i.e. motor neuron sub-pools that receive different common inputs depending on the motion in which the innervated muscle is involved. These findings suggest that the distribution of common input is not limited to anatomy, and thus task-specific activation of certain motor neurons in multifunctional muscles is likely due to task-specific common inputs. It is for this reason that

volitional control of individual motor neurons and thus violations of the size principle should be assessed using this updated definition of motor neuron pools (i.e. grouping motor neurons based on their contribution to function rather than their anatomical innervations):

“We suggest that when discussing recruitment order, the motoneuron pool should be operationally defined as the group of motoneurons that receive excitatory synaptic input to drive the functional movement, not the pool of motoneurons defined by anatomy. The validity of the Size Principle should then be evaluated within this operationally defined motoneuron pool to determine if recruitment proceeds from small to large.” (Bawa et al. 2014).

Taking the functional grouping of motor neuron pools and the size principle into account, flexible control of individual neurons would rely on intentional modulation of either a neuron-specific input signal or its intrinsic excitability¹. While the design of the original biofeedback studies from the last century does not allow to pin the underlying mechanism that led to flexible motor neuron control, they provide evidence for biofeedback providing subjects with crucial information to alter motor neuron firing patterns intentionally. Similarly, more recently, it was suggested that subjects exposed to closed-loop biofeedback could learn to break up pairs of muscles that are usually driven by the same task-dependent neural input (Laine et al. 2015), and establish new, arbitrary synergies of muscles (Nazarpour et al. 2012; Radhakrishnan et al. 2008). Nevertheless, if similar flexible activation, as previously suggested for muscle synergies, of motor neurons from the same functional pool could be achieved in a closed-loop biofeedback task is yet to be shown.

¹ Changes in the excitability of a neuron can be seen as a virtual change in its size. For example, if the intrinsic excitability of a neuron is decreased, it would start firing action potentials at higher net synaptic input compared to the base line value.

CHAPTER 2 MODULATING MOTOR NEURON BETA INSIDE A FORCE NULL SPACE¹

Effective human motor augmentation should rely on biological signals that can be volitionally modulated without compromising natural motor control. We provided human subjects with real-time information on the power of two separate spectral bands of the spiking activity of motor neurons innervating the tibialis anterior muscle: the low-frequency band (<7Hz), which is directly translated into natural force control, and the beta band (13-30Hz), which is outside the dynamics of the neuromuscular system. Subjects could gain control over the powers in these two bands to navigate a cursor towards specific targets in a 2-D space (experiment 1) and to up- and down-modulate beta activity while keeping steady force contractions (experiment 2). Results indicate that beta projections to the spinal motor neuron pool can be voluntarily controlled partially decoupled from natural muscle contractions and, therefore, they could be valid control signals for implementing effective human motor augmentation platforms

¹ This chapter was published as “Towards human motor augmentation by voluntary decoupling beta activity in the neural drive to muscle and force production” in *Journal of Neural Engineering* (2021) with the co-authors Jaime Ibañez^{2,3}, Deren Barsakcioglu², and Dario Farina²; see (Bräcklein *et al.* 2021). The chapter has been slightly altered to accommodate the format of this thesis.

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2.1 Introduction

One of the biggest challenges in human-machine interfacing is the expansion of human capabilities by providing novel ways to interact with the environment (Nicoletti 2001). Real motor augmentation through supernumerary artificial limbs may provide users with new means of interaction with the environment through artificial actuators controlled using spare biological signals (Mehring *et al.* 2019). However, in most cases, human motor augmentation is achieved at the expense of limiting already existing motor functions, e.g., by making users learn to control endogenous bio-signals that would otherwise be used for the movement of existing parts of the body (Parietti & Asada 2017; Salviati *et al.* 2017). Identifying biological signals that can be reliably decoded and volitionally controlled independently from natural motor function is critical to achieve real human motor augmentation.

Neural or muscular signals are commonly used to implement human-machine interfaces (Donoghue 2002; Ferreira *et al.* 2008). Electromyography (EMG) represents a non-invasive, direct and simple source of information tightly linked to the intent to move natural limbs (Farina *et al.* 2014b, 2017; Farina & Holobar 2016; Merletti *et al.* 2001; Merletti & Farina 2016). Due to biomechanical constraints, the effective frequency band within which common inputs to motor neurons translate into movements is rather narrow and concentrated at frequencies $< 7\text{Hz}$ (Farina *et al.* 2014d). EMG information contained at higher frequencies, such as the cortical beta band (13-30Hz), is known to be caused, at least in part, by projections from the motor cortex (Baker 2007; Gwin & Ferris 2012; Kilavik *et al.* 2013; Mima & Hallett 1999; Negro & Farina 2011b). Yet, the functional role of these higher frequency components in the EMG is unclear (Baker 2007; Engel & Fries 2010; Jenkinson & Brown 2011; Little *et al.*

2019). If cortical beta signals observed in the muscles could be uncorrelated from motor function, they could be used to effectively expand the degrees-of-freedom of humans to control artificial limbs during muscle contractions by enabling a new control channel independent from the existing natural ones. In principle, this approach could indeed lead to duplicating the degrees-of-freedom of muscles from which independent beta sources are extracted.

Here we hypothesize that spectral components within the beta range in the common inputs to a motor neuron pool can be partially decoupled from the low-frequency components and, therefore, they might provide a resource for controlling additional degrees-of-freedom concurrently with the motor control of natural limbs. To test this hypothesis, we used a recently validated real-time EMG decomposition technique to extract the firing patterns of pools of motor neurons projecting to a single muscle to directly decode the neural input the muscle receives (Barsakcioglu *et al.* 2021; Barsakcioglu & Farina 2018). These firing patterns allowed us to separate information about the effective drive to the muscle (components at low frequencies causing muscle contraction and movements) from common projections to the motor neuron pool in the beta band (~20Hz) that are not directly translated into force because of their high frequency relative to the musculoskeletal bandwidth (Farina *et al.* 2014d). With this, we tested to what extent our subjects could independently control components in the neural projections to muscles that do not directly determine force and that are therefore not directly associated with the natural control of movements (Farina & Negro 2015). Figure 2-1 summarizes the concept of our framework where common contributions to a motor neuron pool in two separate frequency bandwidths are extracted from real-time decomposed motor neurons to allow subjects to volitionally control them assisted by visual feedback. Specifically, we run two experiments to test our hypothesis. In

the first experiment (which is the one represented in Figure 2-1), subjects had to learn to perform a 2-D target-tracking task by means of which they were encouraged to independently control the low (translated into force) and high (outside the force production bandwidth) spectral components in the activity of the motor neuron pool. With this, we aimed to assess the suitability of common high-frequency components in the motor neuron pool for controlling additional degrees-of-freedom while natural motor function was unaffected. In a second experiment, we studied if volitional modulation of beta activity in the muscles could occur in the context of steady force contractions. Overall, the results of this study show for the first time a potential way to extract endogenous bodily signals for human motor augmentation that could be controlled without distorting the natural motor repertoire and, by doing this, we provide new insights into the possible links of beta cortical oscillations with motor function.

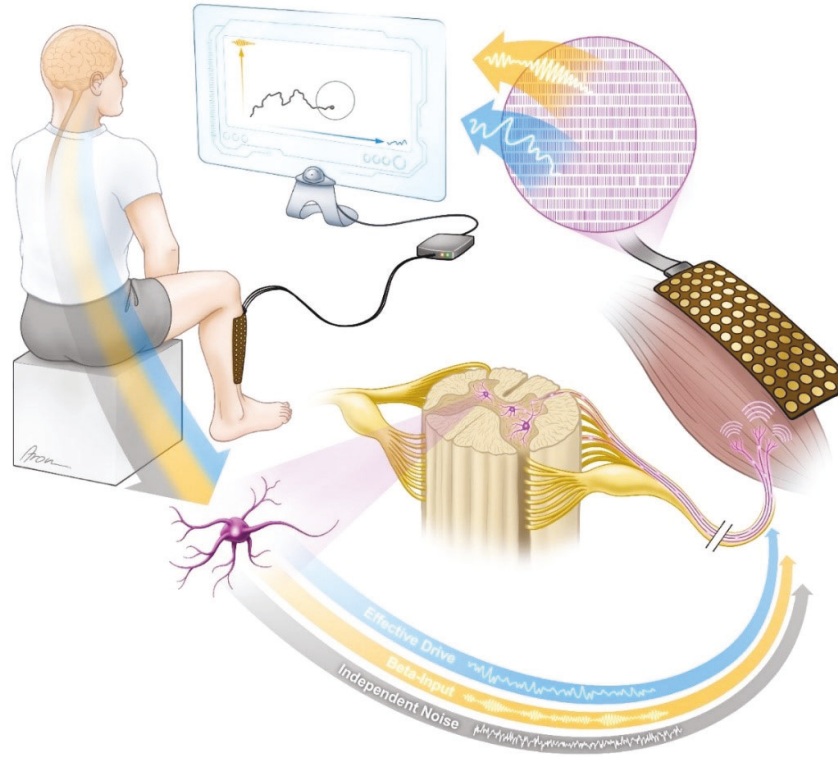


Figure 2-1: Real-time spectral separation of the neural output of a motor neuron pool to augment the degrees-of-freedom of muscles. In the model used here, the spinal motor neuron pool projecting to the tibialis anterior muscle of the right leg receives common neural projections in a broad frequency range. These projections are demodulated by the motor neuron pool to produce an output neural signal that includes low frequency components driving movements (effective drive to the muscle; <7Hz; blue arrow projecting to the muscle). In addition, motor neurons send common projections to the muscle in the beta band (~20Hz; yellow arrow) and each neuron receives an additional input, referred to as independent noise (grey arrow). Spiking activity of motor neurons is decomposed in real-time from high-density surface EMG. Decomposed spiking activity is used to retrieve information about the effective drive and beta projections. In experiment 1, this information is fed-back continuously through visual feedback to subjects who are asked to volitionally modulate the activity projected to motor neurons to navigate a cursor in a 2-D space.

2.2 Materials and Methods

2.2.1 Subjects

Nine male, healthy subjects (age: 28.44 ± 5.10 yrs [mean $\pm$ standard deviation]) were recruited and participated in the study. None of the subjects were familiar with the experimental paradigm nor experienced any neuromuscular diseases prior to the

experimental sessions. The ethics committee at Imperial College London approved the experiments (reference number: 18IC4685).

2.2.2 Acquisition

High-density surface EMG (HDsEMG) was acquired from tibialis anterior muscle of the dominant leg with a 64-electrode grid (5 columns and 13 rows; gold-coated; 1-mm diameter; 8-mm interelectrode distance; OT Bioelettronica, Torino, Italy). The adhesive electrode grid was placed over the muscle belly aligned to the fiber direction. The signals were monopolar recorded, amplified via a Quattrocento Amplifier (OT Bioelettronica, Torino, Italy), sampled at 2048Hz, A/D converted to 16 bits, and digitally band passed filtered (10 – 500Hz). The communication between the amplifier and the computer was conducted via data packages of 256 samples (one buffer corresponds to a signal length of 125ms). The foot of the dominant leg was locked into position to allow dorsiflexion of the ankle only. The force due to ankle dorsiflexion was recorded via a CCT TF-022 force transducer, amplified (OT Bioelettronica, Torino, Italy), and low-pass filtered at 33Hz.

2.2.3 Biofeedback

A MATLAB (The MathWorks, Inc., MA, USA) based version of the online decomposition algorithm described in the next subsection together with a custom-made biofeedback suite were used in all experiments. The biofeedback suite provided visual feedback on discharge timings of single motor neurons, sub-pools or the entire motor neuron pool, the force level exerted by the ankle in the longitudinal plane, the rectified EMG, and included a target-tracking environment.

2.2.4 Online decomposition of motor neuron activity from HDsEMG

Online decomposition of motor neuron activity was achieved using convolutive blind source separation techniques (Negro *et al.* 2016b; Zazula & Holobar 2007) for identifying motor neurons. The real-time decomposition methods used in this study is extensively described and validated in Barsakcioglu, *et al.*, 2021. The real-time decomposition algorithm is calibrated through an initial training phase during which the separation matrix, to extract motor neuron activity from HDsEMG recordings, is computed along with other real-time decomposition parameters. This step involves convolutive sphering (extension of the observations followed by whitening) of the recorded HDsEMG signals followed by a fixed-point iteration algorithm to extract individual separation vectors (for each source) forming the separation matrix, as well as a secondary iterative procedure during which the separation vectors are further refined. During this calibration phase, the final separation matrix is designed, using the whitening transformation matrix, such that the whitening, which involves singular value decomposition, is not required during the real-time decomposition, saving computational resources during real-time operation.

The real-time decomposition phase comprises several processing steps until the final discharge timings of extracted motor neurons are determined. First is the extension of observations (i.e. recorded HDsEMG signals) followed by extraction of spike trains by multiplying the extended observations with the separation matrix. The extracted spike trains are squared, and the peaks are detected. The detected peaks are compared to noise and signal centroids (computed during the training stage) using Euclidean distance. The timestamps of each peak, classified as a valid discharge, is then output together with the information about which motor neuron it belongs to (i.e. spike label).

2.2.5 Intramuscular Coherence

The intramuscular coherence (IMC) quantifies common inputs to the motor neuron pool in the different frequency bands (Castronovo *et al.* 2015; Dideriksen *et al.* 2018). The IMC was estimated by first randomly dividing the pool of motor neurons into two sub-pools of equal size, followed by calculating the maximum squared coherence between the spiking activity of the cumulative spike train (CST) obtained from the two pools by summing the spiking activity of the motor neurons in the two pools. This way the IMC was estimated as:

$$IMC(f) = \frac{|P_{Pool1,2}(f)|^2}{P_{Pool1}(f)P_{Pool2}(f)} \quad Eq. 2-1$$

where $P_{Pool1}(f)$ and $P_{Pool2}(f)$ are the power spectral densities of both CSTs (Welch's, Hanning windows of 4s, 50% overlap), and $P_{Pool1,2}(f)$ is the cross power spectral density. This procedure was repeated 100 times while the two motor neuron pools were randomly selected in each permutation. The average of all permutations was used to determine the peak of the common beta activity present in the motor neuron pool.

Although the detected motor neuron pool is only a small sample of all motor neuron innervating the TA, this subgroup is sufficient to estimate the common neural input present to the entire anatomical pool of motor neurons (Barsakcioglu *et al.* 2021).

2.2.6 Experimental Paradigm

Pre-experimental Processing

Subjects were asked to perform maximum isometric dorsiflexion of the right ankle to estimate the maximum voluntary contraction force (MVC) that the tibialis anterior could produce. The estimated MVC was then set as a reference for the subsequent parts of the

experiment. In a sub-MVC task, subjects were instructed to follow a 4s ramp trajectory (2.5% MVC per second) followed by a constant phase at 10% MVC of 40s. In both experiments, visually-guided force feedback produced by isometric tibialis anterior contractions was provided. Based on the EMG recorded during this sub-MVC task, the separation matrix used by the online decomposition algorithm was generated. The real-time estimates of motor neuron firing were visually presented to the subjects to make them recruit motor neurons one after another by gradually increasing the contraction level to 10% MVC. This pre-experimental task was performed to visually inspect the decomposition results before proceeding with the main experiment as previously done in (Barsakcioglu *et al.* 2021). Notably, all subjects quickly succeeded (i.e. less than 10min) in recruiting single motor neurons progressively by producing mild contractions.

After initializing the online decomposition algorithm, subjects were asked to perform an isometric contraction at 10% of the MVC for 40s while receiving visual feedback about the force exerted by the ankle. The motor neuron activity extracted from this period was used to generate the feature space for the following experimental sessions. The feature space was composed of two spectral components based on the neural input to the identified motor neuron pool. The estimation of this neural input corresponds with the CST, which is the sum of all discharge time series of all detected motor neurons. Both spectral features were obtained by bandpass filtering (3rd-order Butterworth bandpass filter) the CST and calculating the power of the filtered CST (FCST) over 256 samples to mimic the data stream present during the experiments. The cut-off frequencies for the bandpass of the low-frequency feature were set to 1-6Hz representing information within the effective bandwidth of motor control. The bandpass characteristics for the high-frequency feature were obtained by centering a 5Hz band around the most dominant frequency peak in the common beta activity

projected to the motor neuron pool. This peak was estimated by calculating the IMC. The standard deviations and the maximums of the obtained values for both features were used to standardize and maximum-norm the feature values during the following experiments.

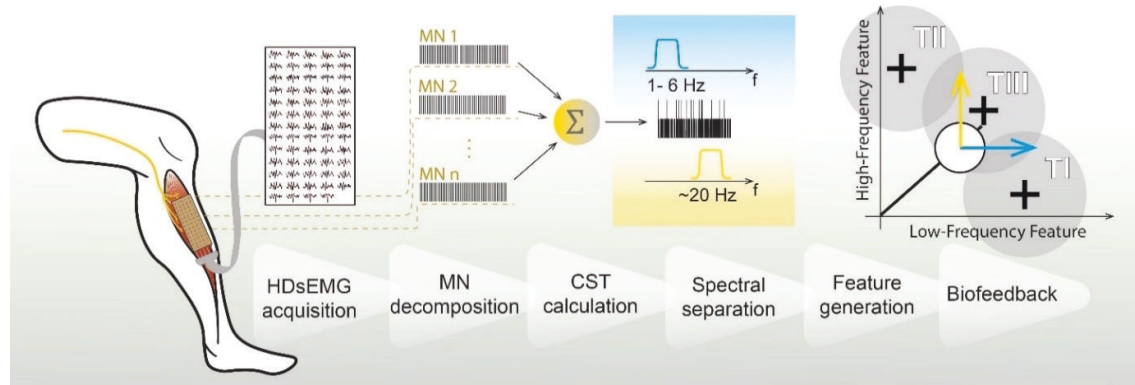


Figure 2-2: Schematic overview of the pre-processing and feedback chain during the target-tracking task. HDsEMG acquisition from the tibialis anterior and real-time decomposition of active motor neuron discharge timings. The sum of all detected motor neuron, i.e. the CST, is filtered in the respective feature bandwidths (green: low-frequency feature inside the effective bandwidth of muscle control; blue: high-frequency feature inside the beta band). Finally, the features are estimated by calculating the power of each individual FCST, mapped into the cursor coordinates, and backpropagated to the subjects via visual feedback by cursor movement inside the 2-D space.

Experiment 1.1 – Target Tracking Task

In the first experiment, subjects had to navigate a cursor in a 2-D space by modulating the common motor neuron activity within two spectral bands: i) inside the effective bandwidth of muscle control (1-6Hz); and ii) in a higher frequency band (beta band) known to receive an important amount of cortical common inputs (5Hz band centered around the peak frequency of the IMC within the beta spectrum). The standardized power of the signals in these two bands was maximum-normed and used to create a 2-D manifold (feature space) with units between 0 and 1 along both axes. The feature space included three targets of equal size placed along the low (TI: 0.7 F1, 0.1 F2 [distance from the origin scaled to the amplitude of the low- and high-frequency features F1 and F2]), high-frequency axis (TII: 0.1 F1, 0.7 F2),

and their diagonal (TIII: 0.5 F1, 0.5 F2), with their centers having all the same distance to the origin (see Figure 2-2). The target radius was set to 30% of the scaled feature amplitude. This target design was determined in pilot studies preceding the experiments. The final selection of target size and placement aimed to encourage naïve subjects to explore the feature space while ensuring that target hits could be achieved.

The acquired HD-EMG signals were online decomposed and filtered in the respective bands as described above. Newly acquired data led to new values for F1 and F2 which determined the cursor's position inside the target space (see Figure 2-2). The cursor movement was smoothed over six buffers (correspondence to 750ms) using a moving average to smooth the estimates of the cursor's position for the visual presentation. In an initial familiarization phase, subjects were instructed to explore the target space while T1 and TII were displayed. The gain to reach each target was set manually to ensure subjects could reach the targets without overexerting themselves to prevent symptoms of muscle fatigue. On average, the gain was increased to 1.98 ± 0.89 F1 and 2.22 ± 0.95 F2 for the low- and high-frequency features, respectively.

The first part of the experiment was a target-tracking task, where the subjects were asked to move the cursor within 40s from the coordinate origin (0 F1, 0 F2) inside one of the three targets (randomly selected) and hold it there for at least seven buffers (one buffer size longer than the moving average; this corresponds to 875ms). In total, each target was presented three times, while the same target never appeared in two consecutive trials. At the end of each trial, subjects needed to move the cursor back into the resting position (origin of the coordinate system) and stay there for at least 10s before the new target was presented. A target hit was indicated via a color change of the target-of-interest from blue to green, while the color switched black when the subject failed to reach it within the 40s-time window.

Experiment 1.2 – Force-Tracking Task

In a force feedback task, performed afterwards, subjects were instructed to follow the mean forces they had used to keep the cursor inside the target-of-interest area. For this purpose, the force exerted during the 875ms preceding a target hit was averaged across all successful trials. Subjects were provided with visually-guided force feedback only. Each block consisted of three constant force trajectories, mirroring the forces used in the previous target-tracking task. Each force trajectory was 20s long and separated by a 20s rest period. The order of the force trajectories was picked randomly from trial-to-trial. In total, this task was repeated three times and subjects were instructed to rest for 60s between trials to avoid muscle fatigue.

Experiment 2 – Beta Modulation Task

The second experiment assessed whether activity in the beta bandwidth present in the tibialis anterior could be modulated volitionally while holding a constant force level with the ankle. Subjects were asked to perform a force-tracking task while they were provided with feedback on both the exerted force level and the beta feature amplitude from experiment 1 (high-frequency feature: the power of the CST filtered in the beta feature bandwidth). Beta power was calculated in the same way as in the target-tracking task. Information on both the force and the beta feature amplitudes were fed-back visually to the subjects by two plots in different colours that were drawn along a time axis as each trial was run. One trial consisted of two phases where the subject was asked to keep the force constant at 10% MVC level while the beta feature amplitude should be either increased (beta up-modulation) or decreased

(beta down-modulation). The order of the beta up- and down-modulation phases was randomized on a trial-by-trial basis. Both phases were separated by 20s rest intervals during which subjects were asked to relax. Ten seconds before the modulation started, subjects were instructed to gradually start exerting the required force of 10% of the MVC. In total, this task was repeated over three trials while subjects were instructed to rest for 60s between trials to avoid muscle fatigue.

2.2.7 Analysis

Experiment 1.1 – Target-tracking Task

All subjects participated in this experiment. If a subject failed to navigate the cursor towards the target-of-interest and to hold it inside for the 875ms period, the nearest miss position was estimated. A nearest miss is the average cursor position during the 875ms interval during which the cursor has the shortest distance to the centre of the selected target-of-interest.

In addition, unintended hits were used as an approach to quantify the probability of cursors hitting non-selected targets before the end of both successful and unsuccessful trials. An unintended hit described the case when the cursor was held inside a non-selected target for at least 875ms. As these did not terminate the trial, unintended hits of the same target could happen multiple times as long as the subject reentered the non-selected target recurrently.

TI and *TII* have the longest centre-to-centre distance inside the cursor space as they required the activation of one feature while suppressing the other. A chance analysis was performed to quantify the probability that the cursor was navigated and held inside either *TI* or *TII* without hitting the other target before, i.e. the probability of hitting the target-of-interest *TI* without a preceding hit of *TII*, and vice versa. All hits that happened without

preceding unintended hits of the other target area were marked as *True*, while no hits or preceding unintended hits as *False*. The chance level was estimated by repeating this analysis on the data gained during the force-tracking task (see section 2.2.7) where no feedback on the cursor movement was given.

Experiment 1.2 – Force-tracking Task

All subjects participated in this experiment. The recorded EMG during the force tracking task was decomposed and filtered in low- and high-frequency feature bandwidths, as described above, to simulate cursor movement. The analysis of hits and unintended hits was repeated for the cursor movement based on the neural activity recorded during the force-tracking task, whereas the target-of-interest corresponded to the associated force trajectory. Moreover, the chance analysis, as described above (see section 2.2.7), was repeated. Particularly, these results of the force-tracking task were used to estimate the chance level.

Experiment 2 – Beta Modulation Task

The mean beta feature amplitude during both beta up- and down-modulation was normalized with the mean beta feature amplitude during the control condition (10% MVC visually-guided force feedback only). Other functional parameters recorded during the modulation phases were also normalized with the respective value gained during the control condition including the mean force, mean rectified EMG, mean low-frequency feature amplitude (standardized and maximum-normed power of the CST filtered between 1-6Hz), coefficient of variation (CoV) of the force, CoV of the rectified EMG, and CoV of the beta feature amplitude. Moreover, this analysis included parameters as the number of active

motor neuron as well as their discharge rates. A motor neuron was classified as active when its discharge rate during the modulation phases (20s time window, 10% MVC force level) was between 4-30 spikes-per-second.

Motor neurons that turned inactive during one of the beta modulation phases were excluded from the analysis. Thus, the beta feature amplitude was recalculated posterior without considering these motor neurons. The power spectral density (PSD) of the CST was estimated using Welch's method (Hanning window, window length: 4s, 50% overlap). One subject was excluded from the analysis as it failed to keep behavioural indicators as force and rectified EMG power at the corresponding amplitudes while modulating beta activity.

2.2.8 Statistics

Statistical analysis was performed to examine the dependency between two or more variables. Results were reported as mean $\pm$ standard deviation. To compare two variables during different conditions, a two-sided pairwise t-test was performed. The normality was tested by using a Shapiro-Wilk test. If the normality assumption was violated, a two-sided Wilcoxon signed rank test was performed instead. However, if the difference between the two variables was not symmetrically distributed, a sign test was used. If more than two variables were the objective, a repeated measures ANOVA was used. If the normality assumptions were not met, Friedman test was performed instead. Post hoc tests were Bonferroni corrected. The threshold for the statistical significance was set to $p < 0.05$.

2.3 Results

2.3.1 Online decomposition and single motor neuron control

In total, 95 motor neurons, 11.9 ± 2.3 motor neurons on average per subject, were detected. Prior to the start of the experiments, subjects were given time to become familiar with single motor neuron control by providing them with visual feedback on the motor neuron spiking activity (see 2.2.6).

2.3.2 Experiment 1: Target Tracking & Force

In experiment 1 we assessed whether low- and high-frequency components of the neural signal that projects to a motor neuron pool and ultimately determine muscle activation can be used to control the movements of a cursor in a 2-D space.

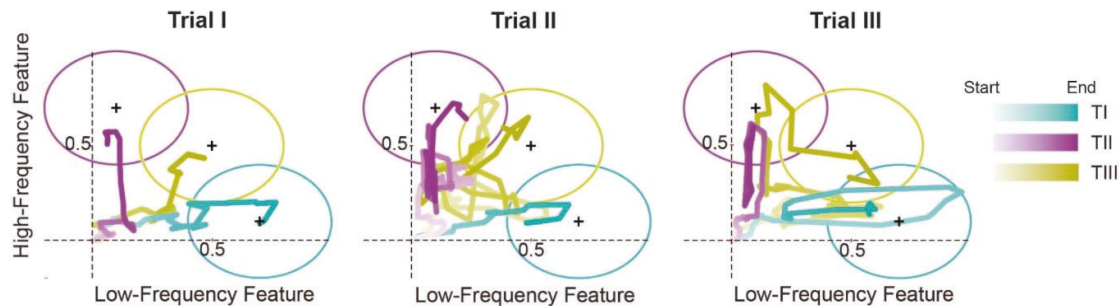


Figure 2-3: Cursor trajectory of one subject during the target-tracking task (experiment 1). Interpolated cursor trajectories during all trials of the target-tracking experiment of one representative subject, all targets, and their centres (black cross). Trial I, II, and III represent the first, second, and third appearance of each target, respectively. The colour intensity of the cursor trajectory increases over time.

The cursor trajectory of one subject during all trials is visualized in Figure 2-3. According to the criteria defining a target hit explained in the methods section (see section 2.2.6), 92.6% of all trials were successful (96.3% for *T1*, 81.5% for *TII*, and 100% for *TIII*). Moreover, all subjects managed to hit all three targets at least once. Figure 2-4A illustrates the cursor movement during the successful trials. When zooming into Figure 2-4A (left), a

trend of each of the cursor movements towards the corresponding target was observed. The average position during the hold period before a hit as well as the nearest misses (the closest cursor position towards the target centre within an 875ms time interval) during unsuccessful trials are shown in Figure 2-4B. In line with the observations in the cursor movements, the cursor end positions are distributed within the corresponding targets. Only three cursor end positions of *TIII* shared a subspace with endpoints of target *TII*. Therefore, both the cursor movement and the end-point trajectories indicated that all subjects were able to navigate within this 2-D manifold to reach the presented targets. This indicates an effective augmentation of human motor output from one dimensional natural control of the pool of motor neurons to a two-dimensional control that includes a second dimension from neural features not directly translated into natural movement.

Despite the spreading of coordinates almost over the entire space, the previous analysis did not reveal whether subjects preferred certain subspaces or directions within the manifold in which they moved the cursor primarily. To study if favoured directions were seen in the movements of the cursor made by the subjects, we computed the two principal components (PCs) of all cursor coordinates in the 2-D space for each subject (Figure 2-4C). On average the first PC explained $64.72 \pm 6.59\%$ and the second one $35.28 \pm 6.59\%$ of the total variability of the cursor coordinate arrangement, which indicates that the first dimension (under natural control) was favoured by the subjects but that the second dimension (spectrally separated from force generation) was also strongly represented.

Taken together, the above findings point to the fact that subjects managed to explore major parts of the feature space while separately controlling the two features used for navigation in the 2-D plane.

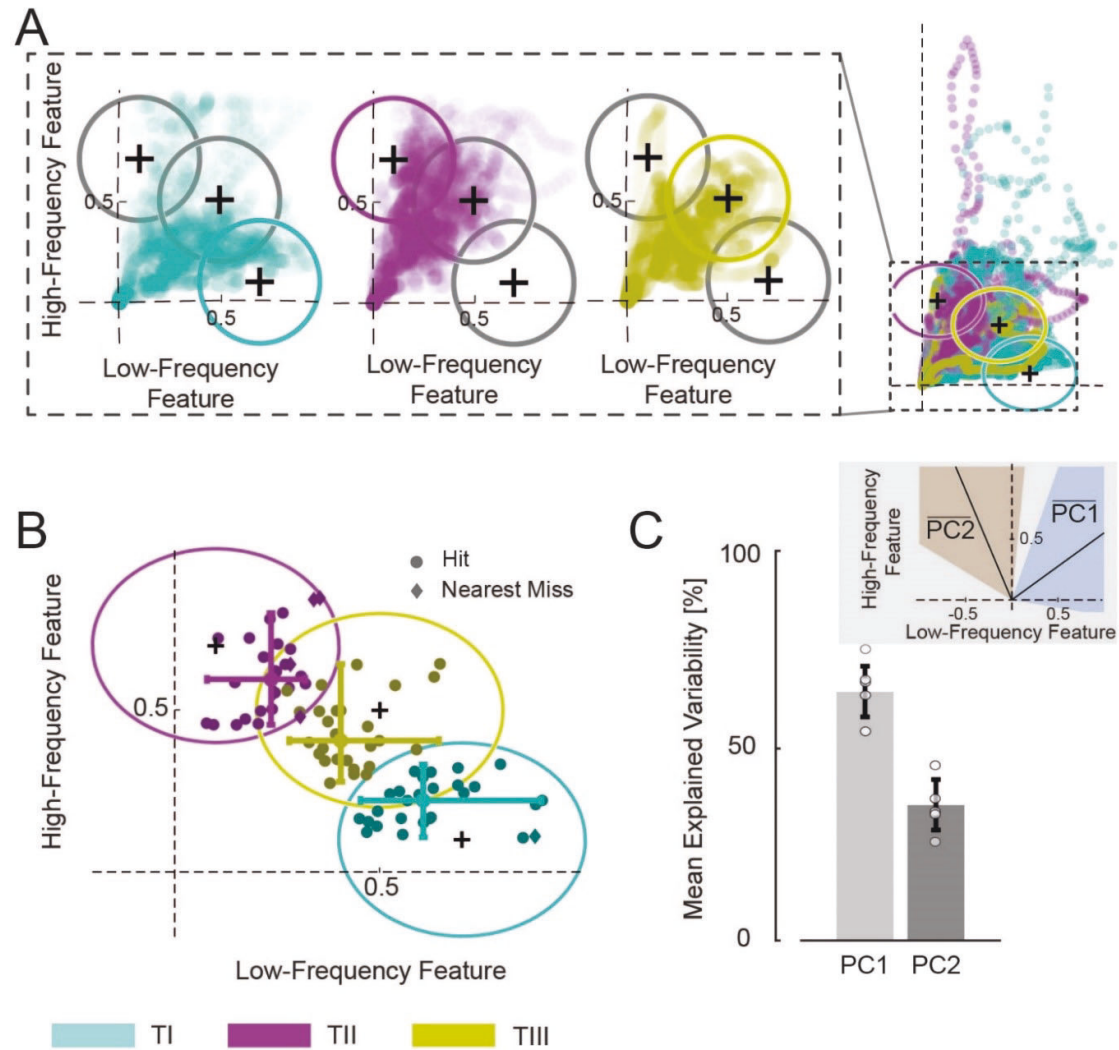


Figure 2-4: Cursor movement during the target-tracking task (experiment 1). A: Cursor movement during all successful trials for all three targets, and all three targets and their centers (black cross). Each dot represents one cursor position. Left inset shows zoomed-in cursor space that was shown to the subjects for each target and the respective cursor movement. B: Average position during hit and nearest miss intervals (875 ms) for each trial and all targets, overlaid by all three targets and their centers (black cross). C: The first two principal components and their explained variability of all demeaned cursor coordinates averaged across all subjects. Black bars indicate the standard deviation. Each circle represents one subject ($n=9$). Mean position of the first and second principal component (black line) and their standard deviation (blue and orange shaded areas, respectively) in relation to the high- and low-frequency feature axes.

The mean forces exerted during the hit and nearest miss periods across all subjects were $8.89 \pm 5.59\%$ of the MVC, $13.01 \pm 2.70\%$ MVC, and $11.72 \pm 4.02\%$ MVC, for TI, TII, and TIII, respectively (see Supplement 2-1A). The mean CoV of the force during hits and nearest misses across all subjects were $15.91 \pm 4.68\%$, $3.76 \pm 0.80\%$, and $6.94 \pm 1.23\%$, for TI, TII, and TIII,

respectively (see Supplement 2-1B). For both the magnitude and variability of the force during hits and nearest misses, an ANOVA revealed a significant difference between the three targets (mean force, Friedman's test, $X^2(2)=6.89$, $p=0.032$, $n=9$; CoV, Friedman's test, $X^2(2)=9.56$, $p=0.008$, $n=9$); however, post-hoc analysis did not reveal significant differences ($p>0.05$). The time-to-target across all subjects did not change during the task with an average time to reach the target-of-interest of $9.47\pm1.43s$, $9.21\pm1.34s$, and $9.72\pm1.46s$ in the first, second, and third trial, respectively (repeated measures ANOVA, $F(2,16)=0.03$, $p=0.968$, $n=9$; see Supplement 2-1C), which suggests no improvement of performance on the small time scale of the experiment. The mean time across all trials to reach *TI*, *TII*, and *TIII* was significantly different with $11.79\pm0.82s$, $12.89\pm1.97s$, and $5.20\pm0.57s$, respectively (repeated measures ANOVA, $F(2,16)=11.77$, $p=0.001$, $n=9$). A post-hoc test revealed that subjects were able to hit *TIII* significantly faster than *TI* (two-sided t-test Bonferroni corrected, $t(8)=6.38$, $P=0.001$, $n=9$) or *TII* (two-sided t-test Bonferroni corrected, $t(8)=3.71$, $p=0.018$, $n=9$).

Since the results presented only informed about the cursor hitting the target-of-interest in the given window of time, an additional analysis was performed to quantify the probability of cursors hitting non-selected targets before the end of each trial. Specifically, unintended hits were defined as the cases when the cursor was navigated and held inside a non-selected target for at least 875ms. Unintended hits of the same target could occur multiple times per trial if the subject re-entered the non-selected target area successively. On average unintended hits of *TIII* occurred multiple times per trial regardless of the current target-of-interest. This observation, as well as the time-to-target analysis, suggest that *TIII*, which required concurrent activation of both features, was the easiest target to reach for all subjects. *TI* and *TII*, which were associated with the increase of one feature while suppressing the other (thus maximally decorrelating the two features), required more effort. Figure 2-5

shows the relative amount of all hits and unintended hits across all targets and subjects. It has to be noted that the presence of unintended hits does not imply that the features could not be decorrelated but only that the subjects needed to extensively explore the 2-D space before hitting the selected target, especially before reaching the targets *TI* and *TII*. Notably, 37.04% of all attempts hitting either *TI* or *TII* were successful without any unintended hits.

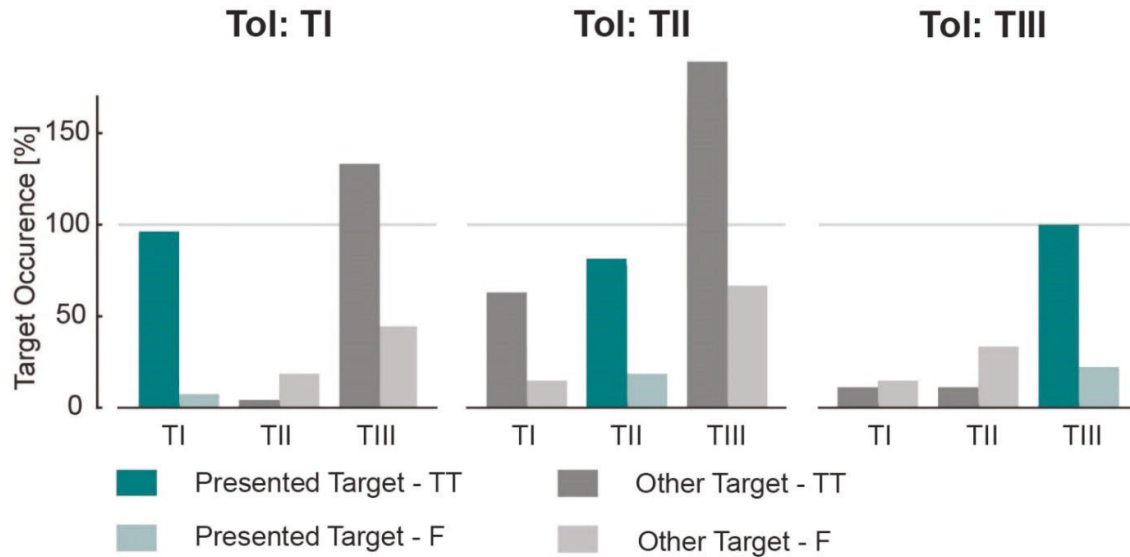


Figure 2-5: Target hits during target- and force tracking task. Relative occurrence of target hits (green) and unintended hits (preceding hit of a non-selected target prior to the hit of the target-of-interest; grey), i.e. the absolute number of (false) hits divided by the total number of trials per target-of-interest (*Tol*). Results are shown across all subjects for targets *TI* (left), *TII* (middle), and *TIII* (right) for the target-tracking task (TT). Corresponding results of the force-tracking task (F) (control condition; see text) are shown by the lighter colors.

In order to assess the importance of providing feedback on both spectral features enabling subjects to perform the target-tracking task, a control condition, i.e. the force-tracking task (see section 2.2.6), was included. In a post-processing step, the cursor movement was simulated using the underlying neural activity recorded during the force-tracking task. Figure 2-5 compares the percentage of target hits in the initial target-tracking task and the subsequent control force-tracking task. Using the activity of motor neurons during the force-tracking task, *TI*, *TII*, and *TIII* were hit in 7.41%, 18.52% and 22.22% of all

trials, which indicates that without feedback the subjects did not naturally explore the two-dimensional space.

TI and *TII* are the two targets that have the most distinct placement inside the feature space. However, Figure 2-5 shows that regardless of which of those two targets was presented as the target-of-interest to the subject, in certain trials, unintended hits of the other target occurred. Therefore, the chance level of hitting *TI* and *TII* was estimated, which is the cutoff probability that one of these targets was intentionally hit without navigating the cursor in the other non-selected target. All hits that happened without preceding unintended hits of the other non-selected target were marked as *True*, while no hits or preceding unintended hits as *False* (see Figure 2-6). The results during the force task were used as an estimation of the chance level. From this analysis, across all trials, subjects managed to move and keep the cursor as instructed insight the target area of *TI* and *TII* by exceeding the chance level (see Figure 2-6)

Target - Tracking			Force - Tracking		
	True	False		True	False
TI	92.59 %	7.41 %	TI	11.11 %	88.89 %
TII	62.96 %	37.04 %	TII	14.81 %	85.19 %

Figure 2-6: Chance level analysis for target- and force-tracking task. Chance level analysis of a hit in target *TI* or *TII* in the target-tracking task (left) and force-tracking task (right). ‘True’ implies that the target-of-interest, i.e. either *TI* or *TII*, was hit without a preceding hit of the other target whereas ‘False’ includes all cases where the target-of-interest was missed, or the other target was hit before the trial finished.

2.3.3 Experiment 2: Beta modulation during steady contractions

Experiment 1 showed that common 1-6Hz and beta frequency features in the motor neuron pool can be partially uncorrelated with biofeedback. However, these results were

obtained by using variable force contractions (i.e., force was not constrained; see supplementary material). As subjects received no visual feedback on force, these uncontrolled dynamics in the motor output might have influenced the activation of the beta frequency feature. Therefore, we designed experiment 2 to assess whether the beta component present in the tibialis anterior during sustained contractions could be modulated while the contraction force was kept constant (i.e., isolated changes in the beta component present in the motor neuron pool; see section 2.2.6).

The beta activity, force and global EMG for a single trial are shown in Figure 2-7A. The phases during which the subject needed to modulate muscle beta activity are marked in red and blue for beta up- and down-modulation, respectively. Importantly, the subject was able to modulate the beta activity in the muscle as instructed while force and global EMG were kept unchanged (see Figure 2-7B). The mean beta feature amplitude across all subjects, normalized against the amplitude in the control condition, decreased significantly from 1.47 ± 0.40 to 1.09 ± 0.20 during beta up-modulation to beta down-modulation (two-sided paired sample t-test, $t(7)=3.44$, $p=0.011$, $n=8$). Hence, the task led to a substantial increase of almost 50% in the mean beta feature amplitude during up-modulation compared to the control condition, without any changes in force. On the contrary, in the down-modulation phases, no significant changes of beta activity were observed (repeated measures ANOVA, $F(2,14)=11.13$, $p=0.001$, $n=8$; post-hoc comparison between beta down-modulation and control set: two-sided t-test Bonferroni corrected, $t(7)=1.46$, $p=0.561$, $n=8$; post-hoc comparison between beta up-modulation and control set: two-sided t-test Bonferroni corrected, $t(7)=3.47$, $p=0.031$, $n=8$).

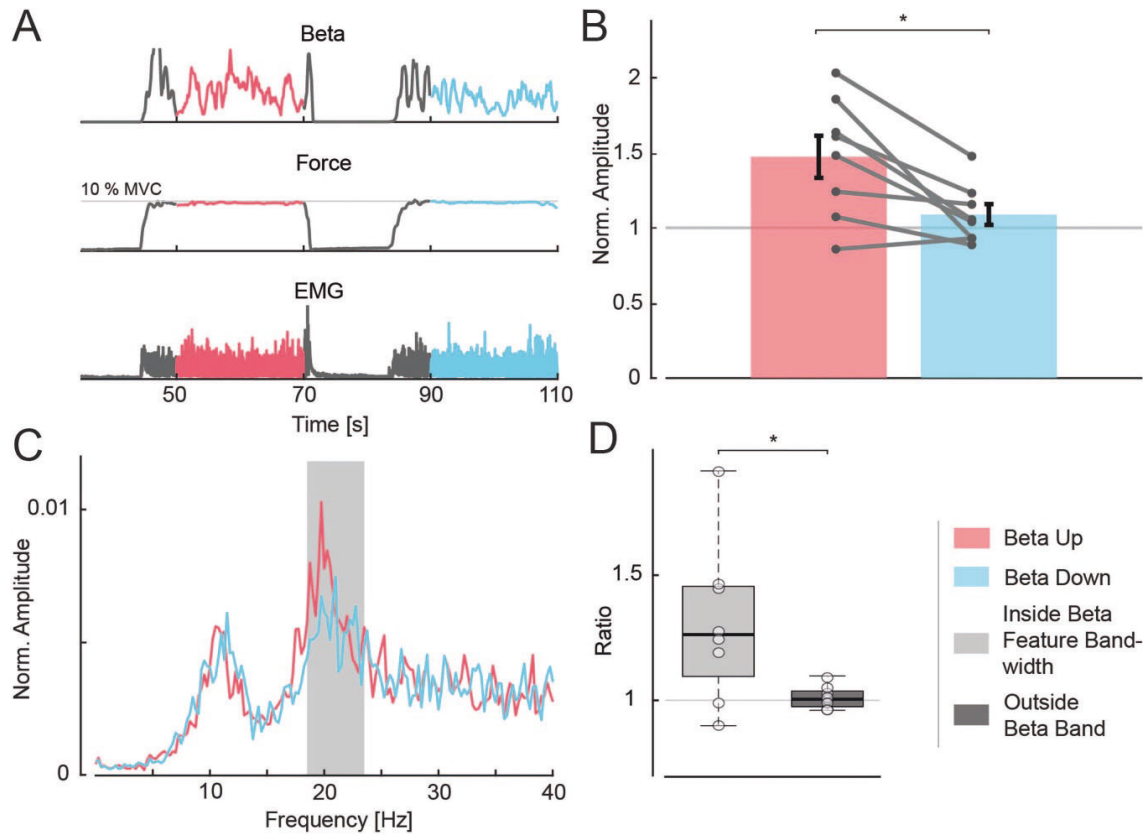


Figure 2-7: Overview of beta modulation during steady contractions. A: Beta feature amplitude, force and rectified EMG trajectories for a single trial and subject in the beta modulation task (experiment 2). Red indicates the beta up-modulation interval whereas blue the beta down-modulation one. B: Normalised beta feature amplitude for both modulation conditions. Black bars indicate the standard error of the mean, grey points the mean value for each subject (n=8), while the grey line combines data points of the same subject. C: Mean power spectral density of motor neuron CST during beta up- and down-modulation for a single representative subject. Grey box indicates the beta feature bandwidth. D: Ratio of beta up-modulation power to beta down-modulation power inside the beta feature bandwidth (light grey) and outside the beta band (up to 100Hz; dark grey) for PSD across all subjects (n=8), shown by their median and quartiles. * $p < 0.05$.

Control measurements of various variables apart from the beta activity, i.e. force, global EMG power, and the number of decoded motor neurons (firing rate above 4 and below 30 pulses-per-second [pps]), did not suggest any other significant changes apart from the one in beta activity. Only the motor neuron discharge rate changed (although minimally) from 11.78 ± 2.70 pps to 11.38 ± 2.65 pps for up- and down-modulation, respectively, with borderline significance (two-sided paired sample t-test on the normalized discharge rate [1.17 ± 0.11 and 1.13 ± 0.13], $t(7) = 2.37$, $p = 0.050$, $n = 8$). Further details are given in the supplementary materials

(see Supplement 2-2). Although the number of active motor neurons was reduced in certain blocks, the relationship between beta feature amplitudes during up- and down-modulation remained unaffected when recalculated with the shrunk set of motor neurons (two-sided t-test of normalized beta up- and down-modulation with reduced set of motor neurons [1.46 ± 0.74 and 1.06 ± 0.44], $t(7)=3.03$, $p=0.019$, $n=8$; see section 2.2.7).

The modulation of the beta content in the decomposed motor neuron pools resulted in changes in the spectral properties of the CST. Figure 2-7C exemplifies this effect by illustrating the mean PSD of the CST for a representative subject during beta up- and down-modulation. The grey box indicates the beta feature bandwidth estimated before the target-tracking experiment. The power spectrum was raised in the feature bandwidth while the subject increased the overall beta feature activity. Figure 2-7D compares the ratios of the mean PSD during beta up- to beta down-modulation intervals inside the feature bandwidth relative to the spectral content outside of the beta band (up to 100Hz). A rise in activity inside the feature bandwidth (1.31 ± 0.32) occurred during increased beta activity while an absence of this effect was observed outside the beta range (1.01 ± 0.05 ; two-sided paired sample t-test, $t(7)=3.00$, $p=0.020$, $n=8$).

2.4 Discussion

Concomitant control of supernumerary and natural limbs requires using independent command signals. Ultimately, human augmentation through artificial devices should ideally rely on the control of biological signals that have no direct involvement in the natural motor repertoire (Makin *et al.* 2017). Here we characterized in real-time the spiking activity of pools

of motor neurons projecting to a single muscle to test if the activity in the beta band (13-30Hz) shared by the neurons could be volitionally controlled independently from voluntary muscle contractions. With the two sets of experiments run here we show for the first time that beta activity in the motor neuron pool of a muscle can be partially uncorrelated from the effective drive to the muscles at low frequencies that causes force production, and that this could potentially be used to expand the degrees-of-freedom of a muscle for human-machine interface applications.

We designed a feedback paradigm which used as inputs the spiking activity of tens of motor neurons projecting to a single muscle and decomposed in real-time from HDsEMG. The designed paradigm allowed us to provide feedback to the subjects about both the contents of the decomposed motor neurons within the beta range and in the low (1-6Hz) frequency band. Importantly, these two spectral components have been suggested to have different links to movements: while low-frequency components have been shown to be highly correlated with voluntary muscle contractions, beta oscillations have been associated with cortical projections to the muscles without a clear or direct role in motor function (Baker 2007). Results showed that most subjects controlled a cursor in a 2-D space to hit specific targets above chance levels by partially decorrelating the low- and beta-band components in the motor neuron pool. The distribution of the end point trajectories suggested that subjects were able to volitionally place and hold the cursor in the designated target areas. Despite TIII having intersection areas with TI and TII, the end points were separated across targets (only three end points of TIII also belonged to the cluster of TII; see Figure 2-4). The target hit and time-to-target analyses revealed that the simultaneous up-modulation of both features (i.e., increasing the power of the motor neuron activity within the low and beta frequency bands) was successfully achieved in most cases. On the contrary, subjects took longer to reach

towards target areas when the selective promotion of one feature only was required, i.e. the decorrelation of the two features. In particular, subjects showed a limited ability to move the cursor inside the target area that required concurrent suppression of the beta activity while promoting the low-frequency feature. To eventually navigate the cursor towards the target area, the force analysis presented in Supplement 2-1 revealed that subjects had used less-steady contractions for the targets that required pronounced beta activity. Thus, subjects used this compensatory movements as a strategy to overcome the limitations of suppressing beta activity during constant contractions. This attenuation of activity inside the beta band during the performance of variations in movement is in agreement with previous studies (see Kilavik, *et al.*, 2013 for a review (Kilavik *et al.* 2013)). Furthermore, when we then asked subjects to perform a constant force output, they failed to suppress beta activity.

The frequency components inside the beta range of the motor neuron pool are cancelled-out completely in the force output due to the low-pass filter characteristics of the muscle (Farina & Negro 2015). In fact, their functional role in movement generation is not fully understood (Baker 2007; Engel & Fries 2010; Jenkinson & Brown 2011; Little *et al.* 2019). While in other cortical structures, e.g. the primary visual cortex, different frequency bands comprise well-described decoupled functionalities (Belitski *et al.* 2008; Kayser & König 2004), such clear assignments are yet to be defined for motor cortical structures. Motor cortical activity within the beta range has been suggested to act as a regulator to maintain the current state of the force output (Engel & Fries 2010), a probability estimator rather than a motor control signal *per se* (Jenkinson & Brown 2011), or a state-informing signal enabling sensorimotor integration (Baker 2007). Our results indicate that beta activity may not be fully linked to motor function: in our experiments, subjects were able to modulate beta activity while keeping the force output unchanged. Furthermore, the accuracy of force control did not

decrease when subjects voluntarily promoted beta activity. Recent studies have indicated that beta oscillations may not be directly linked with motor function but rather with a form of state definition when updates in the motor system are needed (Little *et al.* 2019; Shin *et al.* 2017). While it still remains unclear how coherent corticomuscular beta is originated in the latter case (Thompson *et al.* 2019), our findings are aligned with this type of processing: beta modulations do not directly affect motor function and thus beta activity can be promoted probably by increasing the occurrence of beta events.

During both experiments run in this study, subjects were required to gain, at least partially, control over a biological signal (beta activity in motor neurons) that is outside the dynamics of the neuromuscular system (Farina *et al.* 2014d). To train subjects to modulate the beta activity in the common neural commands to the muscle, we used an operant conditioning framework. Subjects received a reward or punishment (through visual feedback), for example, when they were able or failed to navigate the cursor into the correct target during experiment 1. Previous brain-computer interface studies have shown that such frameworks can enable subjects to gain control over spectral features extracted from cortical signals during periods of no motor activity (Bashford *et al.* 2018; Wolpaw & McFarland 2004). Further experiments are needed to investigate the relevance of using visual feedback and the influence of the type of feedback given to subjects to allow volitional modulation of common beta activity in motor neurons.

Recent studies have shown that motor cortical oscillations can be used to control supernumerary degrees-of-freedom while maintaining motor activity by using highly invasive brain implants (Bashford *et al.* 2018) or by triggering predefined motions of a robotic arm during motor imagination tasks (Penaloza & Nishio 2018). In our study, however, we proposed a novel communication channel, which is accessible non-invasively via a wearable neural

interface that could allow healthy individuals to expand the boundaries of human motor control. We showed that most subjects were able to explore a large section of the 2-D space, and to place and hold the cursor inside certain targets during the target-tracking task. Yet, despite the existence of a favoured direction in which subjects moved the cursor, the PCA revealed that a continuous cursor movement along two orthogonal axes inside the 2-D space was possible. This demonstrates the potential of controlling supernumerary degrees-of-freedom non-invasively by partially decorrelating spectral features present in the motor neuron pool and decoded with wearable (non-invasive) peripheral interfaces.

The novel neural interface, including a real-time decomposition algorithm (Barsakcioglu *et al.* 2021; Barsakcioglu & Farina 2018), enabled us to study the activity of a single motor neuron pool in isolation. Hence, all physiological observations gained throughout the experiment are truly generated by the observed muscle and its motor neuron pool. In addition, we restricted the leg into position to allow dorsiflexion of the ankle only and instructed subjects to keep a stable body position throughout the experiments. Although, we did not track the movement of other body parts, e.g. hip, knee and trunk, and the activity in the related motor neuron pools which may affect the behaviour of the single pool we have observed, the force and global EMG profiles provided no evidence for co-contracted antagonist muscles during the beta modulation task. Thus, the analysis presented here most likely revealed a command signal inside the neural input to the motor neuron pool examined that is not translated into movement of the corresponding muscle but, to some extent, can be modulated in isolation.

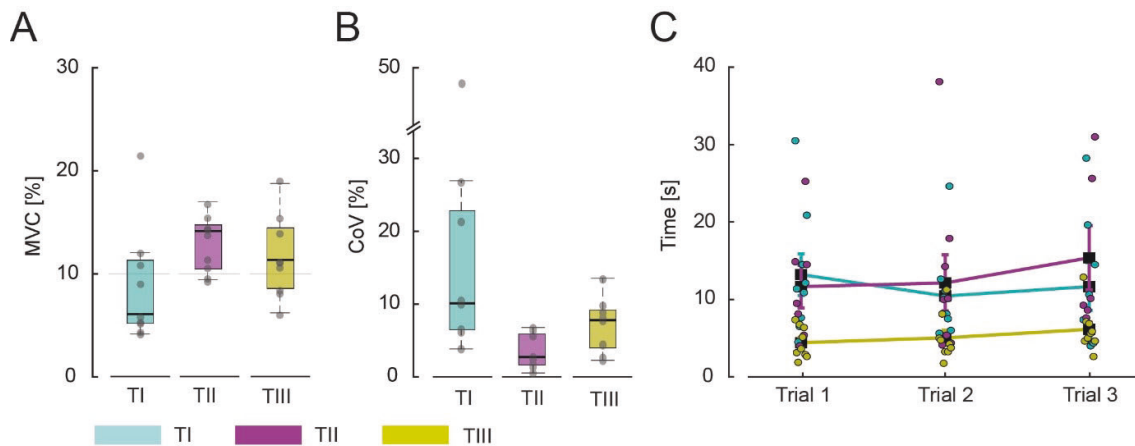
The decomposition algorithm employed in this study uses a separation matrix to deconvolute EMG to the underlying neural activity in real-time. The initialization of this separation matrix is performed on a set of EMG data recorded prior to the experiment (see section 2.2.4) and is

not altered for the duration of the experiment (Barsakcioglu *et al.* 2021). However, the quality of the EMG signal may worsen over time, for example, due to the accumulation of sweat between the skin and the electrode (Ribeiro *et al.* 2019). Such negative influences on the electrode-skin contact may result in misclassification of neural spiking activity. This issue can be overcome by adaptive decomposition (as in (Chen *et al.* 2020)), but in this study we have not implemented adaptive strategies to assure that the same motor neurons were sampled during the experiments for each subject. For this reason, we have chosen to limit the number of trials per experiment to ensure subjects having enough time to explore the augmented control strategies as well as to reach a high quality in neural spike detection throughout the experimental session.

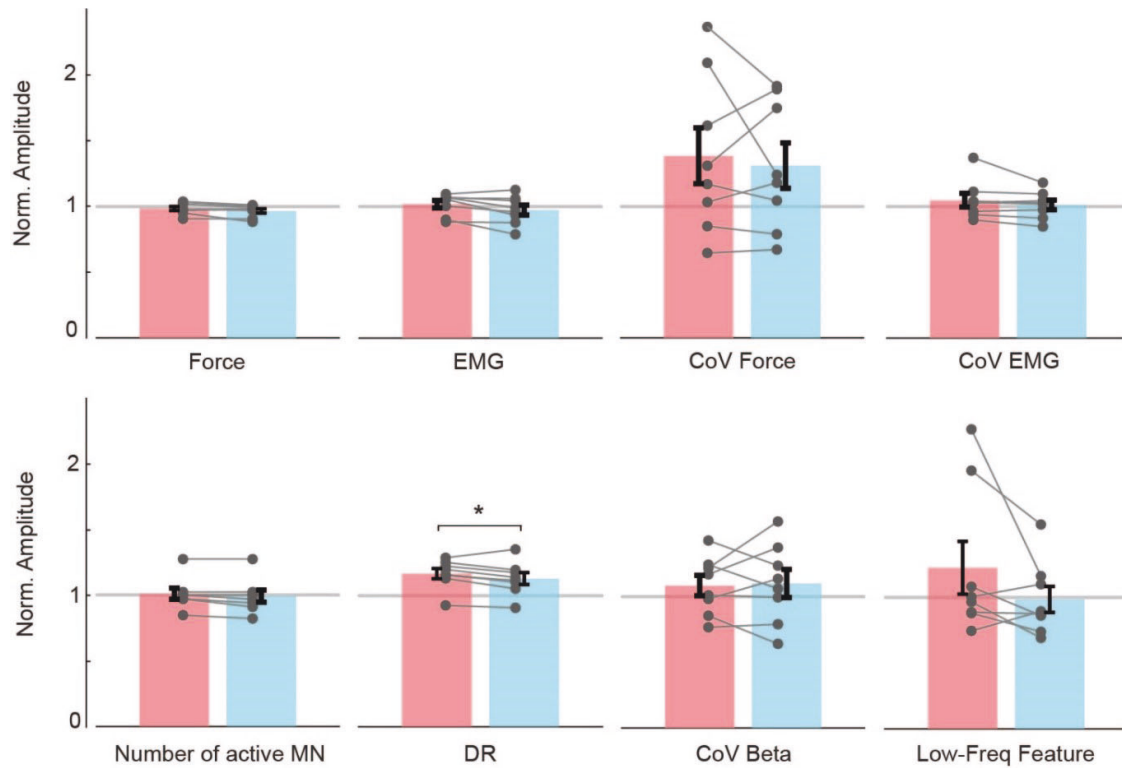
The method proposed here based on extracting high-frequency components from motor neuron activity inherently relies on the recorded muscle being active (Barsakcioglu *et al.* 2021), as it depends on the characterization of motor neuron action potentials. Nevertheless, the results of experiment 2 indicate that natural force control was not critically altered while subjects gained partially control over the high-frequency feature. Furthermore, the independent modulation of high-frequency components present in the motor neuron pool was tested in isometric conditions only. Future research aims to probe the feasibility of using beta activity to drive supernumerary degrees-of-freedom should include additional investigations during dynamic muscle contractions. Moreover, the aspect of learning should be addressed by focusing on the temporal evolution of beta control. The current results, however, present the first evidence of a potential resource for controlling additional degrees-of-freedom without the need of an invasive signal acquisition.

In conclusion, we demonstrated the ability to decorrelate high-frequency (inside the beta range) from low-frequency components of the neural code in the motor neuron pool by using a wearable neural interface. We showed that subjects were able to modulate activity within the beta band while maintaining a steady force output. Further investigations on whether the observed behaviour of beta rhythms in the periphery are also present at the cortical level might generate a better understanding of the underpinning phenomena. Nevertheless, these results provide first evidence for the potential of beta activity in non-invasive human augmentation applications to create the basis for a control channel parallel to natural movements.

2.5 Supplementary Material



Supplement 2-1: A: Mean force per subject during the hit and nearest miss time interval (875 ms) shown with its median and quartiles for all three targets. B: Mean coefficient of variation of force per subject during hit and nearest miss interval (875 ms) shown with its median and quartiles for all three targets. C: Mean time to reach each target in each trial across all subjects. Vertical bars indicate the standard error of the mean.



Supplement 2-2: Various functional parameters (find the explanation below) normed by the control condition for beta up- and down-modulation phases averaged across all subjects. Black bars indicate the standard error of the mean, grey points the mean value for each subject, while the grey line combines data points of the same subject. Force: force amplitude; EMG: rectified EMG; CoV Force: CoV of force amplitude; CoV EMG: CoV of rectified EMG; Number of active motor neuron: number of motor neuron with a discharge rate between 4-30 spikes-per-second; DR: discharge rate; CoV Beta: CoV of beta feature amplitude; Low-Freq Feature: low-frequency feature (CST filtered in 1-6 Hz) amplitude. * $p < 0.05$.

CHAPTER 3 THE ORIGIN AND NATURE OF BETA IN THE MOTOR NEURON POOL¹

Beta oscillations (13-30Hz) are ubiquitous in the human motor nervous system. In Chapter 2, I have shown that beta components present in a motor neuron pool can be modulated volitionally without significantly altering the force output of the innervated muscle. Thus, these oscillations may provide a resource for extending human motor function. Yet, their origin and role in movement generation are unclear. Traditionally, beta activity has been treated as a stationary signal. However, recent studies observed that cortical beta occurs in ‘bursting events’, which are transmitted to muscles. This short-lived nature of beta events makes it possible to study the relationship between beta activity found in the muscles and cortical beta. Here, we assessed if muscle beta activity mainly results from cortical projections. We ran two experiments in healthy humans of both sexes (N=15 and N=13,

¹ This chapter was published as “*Reading and Modulating Cortical β Bursts from Motor Unit Spiking Activity*” in *Journal of Neuroscience* (2022) with the co-authors Deren Y. Barsakcioglu², Alessandro Del Vecchio³, Jaime Ibañez^{2,5,6,*} and Dario Farina^{2,*}; see (Bräcklein *et al.* 2022b).

Data acquisition of *Experiment I* was performed by Alessandro Del Vecchio and Jaime Ibañez.

The abstract was slightly altered to ensure logical flow of the thesis.

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respectively) to characterize beta activity at the cortical and motor unit (MU) levels during isometric contractions of the tibialis anterior muscle. We found that beta rhythms observed at the cortical and MU levels are indeed in bursts. These bursts appeared to be time-locked and had comparable average durations (40-80ms) and rates (~3-4 bursts/second). To further confirm that cortical and MU beta have the same source, we used a novel operant conditioning framework to allow subjects to volitionally modulate MU beta. We showed that volitional modulation of beta activity at the MU level was possible with minimal subject learning and was paralleled by similar changes in cortical beta activity. These results support the hypothesis that MU beta mainly results from cortical projections. Moreover, they demonstrate the possibility of decoding cortical beta activity from MU recordings, potentially translating it to future neural interfaces that use peripheral information to identify and modulate activity in the central nervous system.

3.1 Introduction

Neural oscillations of brain activity in the beta range (13-30Hz) are ubiquitous in the motor nervous system (Kilavik *et al.* 2013). Alongside their pervasive appearance in the brain, beta oscillations with cortical origin are transmitted linearly and at fast and stable speeds to tonically active muscles (Ibáñez *et al.* 2021; Witham *et al.* 2011). Beta activity can indeed represent an important portion of the neural inputs received by spinal motor neurons and their innervated muscle fibres, i.e. MUs (Dideriksen *et al.* 2018; Farina *et al.* 2014d; Grosse *et al.* 2002). However, the prominence of beta activity at the MU level contrasts with the fact that, so far, it has been difficult to find a direct link between these oscillations and motor function (Baker 2007; Davis *et al.* 2012; Engel & Fries 2010; Jenkinson & Brown 2011; Little *et*

al. 2019). One aspect of beta inputs to MU that makes them hard to study is not knowing which main sources are contributing to these inputs. Are the characteristics of beta activity in MUs similar to the non-stationary features of beta oscillations at the cortical level? Is the motor cortex the main structure projecting common beta inputs to muscles? Or are there other relevant sources elsewhere in the central nervous system?

An interesting recent observation is that cortical beta activity is not a continuous signal, but it appears in short-lived bursts (Bonaiuto *et al.* 2021; Feingold *et al.* 2015; Little *et al.* 2019; Pfurtscheller *et al.* 2005; Shin *et al.* 2017). Such temporal non-stationary characteristics of beta activity require new approaches, based on joint time and frequency analysis, to study these oscillations (Jones 2016; Tal *et al.* 2020; van Ede *et al.* 2018) and their possible links to motor function (Bonaiuto *et al.* 2021; Little *et al.* 2019; Shin *et al.* 2017; Wessel 2020). The tracking of the non-stationary, burst-like behavior of cortical beta allows for directly following its propagation to the peripheral nervous system by identifying its main characteristics, such as burst duration and frequency, at the cortical and peripheral level. The analysis of the transmission of beta from the central to the peripheral nervous system would provide new insights into the role of beta oscillations on motor control. Moreover, understanding beta transmission would enable the development of neural interfaces to monitor and extract cortical activity non-invasively from the periphery to supplement and overcome current limitations of traditional brain monitoring interfaces.

Here we ran two experiments to characterize beta oscillations present at the level of MUs in the tibialis anterior muscle and their association with cortical beta rhythms in the context of mild isometric contractions. In the first experiment, we asked subjects to hold a constant force level while concurrently recording cortical activity via electroencephalography (EEG) and muscle activity via high-density electromyography (EMG). The EMG was

decomposed into the underlying MU activity associated with force generation. Then, in the second experiment, we used a decomposition algorithm to extract MU activity from the EMG in real-time (Barsakcioglu *et al.* 2021) and a novel neural feedback paradigm to operantly conditioning beta in the MUs (Bräcklein *et al.* 2021). By doing this, we were able to assess how the relationship between cortical and peripheral beta rhythms is influenced by volitional modulation of MU beta power. Overall, our results demonstrate that beta activity in the MUs is short-lived, mainly driven by cortical bursts, and can be volitionally modulated, imposing parallel modulation at the cortical level.

3.2 Materials and methods

3.2.1 Subjects

In this study, 28 healthy subjects (3 females, all subjects between 24 and 35 years old) participated, of whom 15 (2 females) in Experiment 1 and 13 (1 female) in Experiment 2. All subjects were naïve to the experimental paradigms. None of the subjects reported any history of severe neuronal or lower limb injuries. Experiment 1 was approved by the University College London Ethics Committee (Ethics Application 10037/001) and Experiment 2 by the ethics committee at Imperial College London (reference number: 18IC4685).

3.2.2 Data acquisition

High-density surface EMG (HDsEMG) from the tibialis anterior muscle of the dominant leg (self-reported) was acquired via a 64-electrode grid (5 columns and 13 rows; gold-coated; 1 mm diameter; 8 mm interelectrode distance; OT Bioelettronica, Torino, Italy). The electrode grid was placed over the muscle belly aligned to the muscle's fiber direction. In addition,

single-channel EMG of the medial and lateral head of the gastrocnemius muscle was recorded via wet electrodes (Ambu Ltd, St Ives, United Kingdom) placed above the muscle belly throughout Experiment 2. The EMG signals were monopolar recorded, amplified via a Quattrocento Amplifier system (OT Bioelettronica, Torino, Italy), sampled at 2048Hz, A/D converted to 16 bits, and digitally band-pass filtered (10-500Hz). Subjects were seated throughout the experiments while the foot of the dominant leg was locked into position to allow dorsiflexion of the ankle only. The force due to ankle dorsiflexion was recorded via a CCT TF-022 force transducer, amplified (OT Bioelettronica, Torino, Italy), and low-pass filtered at 33Hz. The communication between the amplifier and the computer was conducted via data packages of 256 samples (one buffer corresponds to a signal length of 125ms). All incoming EMG signals were band-pass filtered between 20-500 Hz using a 4th order Butterworth filter.

Furthermore, EEG signals were acquired from 31 positions according to the International 10-20 system via active Ag/AgCl electrodes (actiCAP, Brain Products GmbH, Munich, Germany). FCz was used as a reference. The signal was amplified (BrainVision actiCHamp Plus, Brain Products GmbH, Munich, Germany) and sampled at 1000 Hz. The EEG was offline band-pass filtered between 0.5 and 45 Hz (4th order Butterworth filter). A surface Laplacian filter covering the central part of the brain by taking the neighboring positions of Cz into account was applied (Kayser & Tenke 2015). Both EMG and EEG signals were offline resampled at 512 Hz and synchronized with a common digital trigger signal.

For one subject, no EMG of the lateral nor medial head of the gastrocnemius muscle was recorded due to a material failure.

3.2.3 Experimental paradigm

The experimental paradigm for both experiments is visualized in Figure 3-1A.

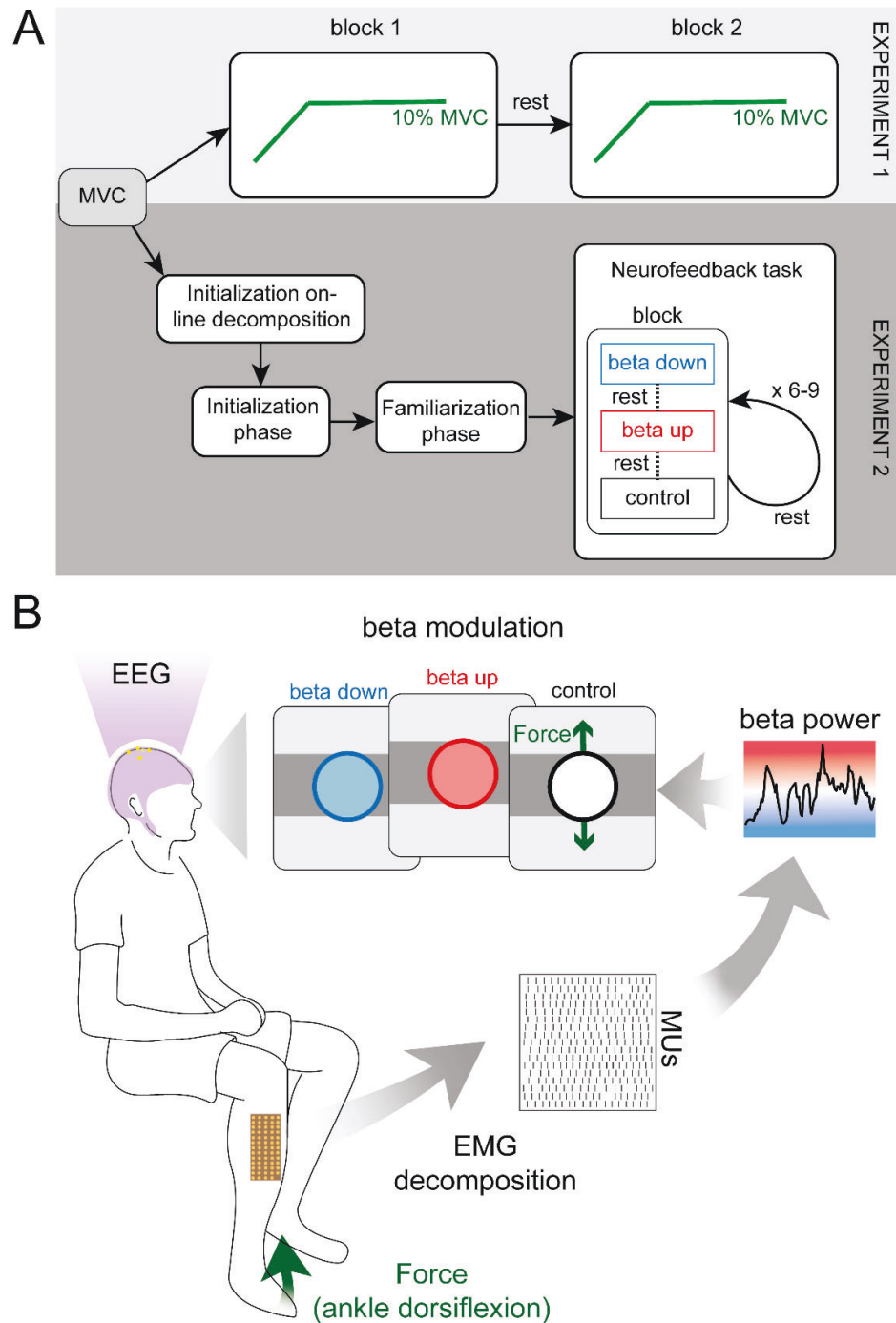


Figure 3-1: Schematic overview of the experimental paradigms used in both experiments. A: Experimental flow-chart for Experiment 1 and 2. Both experiments start with estimating the maximum voluntary contraction level (MVC). In Experiment 1, subjects are asked to repeat two blocks of ramp-and-hold force task at 10% MVC separated by a rest period. Experiment 2 continues with two initialization steps in which the online decomposition (“Initialization online decomposition”) and the neurofeedback parameters (“Initialization phase”) are initialized. In “Familiarization phase” subjects are exposed to the neurofeedback paradigm used during the “Neurofeedback task”. A single block of the “Neurofeedback task” consisted of three trials: beta down, beta up, and control. The trials were presented in randomized order and separated by a rest period. A minimum of six and a maximum of

nine blocks were presented to each subject separated by a rest period while only the last three blocks were used for the analysis. B: Schematic overview of Experiment 2. HDsEMG of the tibialis anterior muscle was decomposed into the underlying neural activity while, concurrently, the force due to ankle dorsiflexion and the EEG were recorded. Subjects were asked to navigate a cursor inside a target rectangle by performing ankle dorsiflexion at $10\% \pm .5\%$ MVC. Color of the cursor changed based on the beta power in the MU pool. Subjects were asked to keep the cursor inside the force target and change the cursor color to either blue (down-modulation of the beta activity) or red (up-modulation of the beta activity). In a control condition, no feedback on the beta feature was provided and, instead, the cursor turned white when placed inside the target.

Pre-experimental processing

Before the start of the experiments, subjects were asked to perform a single maximum dorsiflexion of the ankle to estimate the maximum voluntary contraction level (MVC). The obtained MVC was set as a reference for the following experiment to ensure that stable forces were produced by the tibialis anterior muscle.

In addition to force feedback, Experiment 2 also informed the subjects about the amount of beta activity in the MU innervating the tibialis anterior muscle. For this, an online decomposition algorithm was used to decode MU activity in real-time (Barsakcioglu *et al.* 2021). In order to estimate the separation matrix used to decode MU activity from the HDsEMG recordings, subjects were instructed to perform an additional ramp and hold task. This involved a 4s period of linear increase in the contraction level departing from a relaxed position and reaching a contraction level of 10% of the MVC (ramp phase) and steady contraction at 10% of the MVC level held for 40s (hold phase). The decomposed MU discharge behavior was visually inspected following established guidelines (Del Vecchio *et al.* 2020) while subjects were instructed to gradually increase the force due to dorsiflexion up to 10% MVC to recruit MUs.

Experiment 1 – force task

Experiment 1 aimed to assess the characteristics of cortical and MU beta activity during constant isometric contraction at a mild force level. This experiment consisted of two blocks. In each block, subjects were provided with visually guided feedback on the exerted force and asked to follow a ramp and hold trajectory for 40s at 10% MVC presented on a screen while EEG was recorded concurrently. Between blocks, subjects were instructed to rest to avoid muscle fatigue.

Experiment 2 – beta modulation

In Experiment 2, the relationship between cortical and MU beta was assessed while subjects were allowed control over MU beta. For this, subjects were instructed to move a cursor inside a target rectangle by exerting a force due to ankle dorsiflexion at 10% MVC. While holding the cursor inside the rectangle, i.e. exerting a constant force at 10% MVC, subjects were asked to change the color of the cursor to match a presented target by modulating the MU beta power at ~20Hz. Similar to Experiment 1, EEG was recorded throughout Experiment 2.

Experiment 2 consisted of three parts: i) an *initialization phase* to determine all parameters necessary for real-time neurofeedback on the MU beta activity, ii) *familiarization phase* to allow subjects to get familiar with the experimental neurofeedback environment and task, and iii) the *neurofeedback task* in which subjects were exposed to real-time feedback on the exerted force and MU beta activity.

Initialization phase

The initialization phase mimicked the paradigm previously performed in (Bräcklein *et al.* 2021). Subjects were asked to exert a force at 10% MVC for 40s guided visually by a force trajectory. During this period, the underlying MU activity was used to identify the most prominent peak inside the beta band of the intramuscular coherence (IMC). The IMC was used in this case as it allowed us to estimate the common input to the MU pool at a given frequency (Castronovo *et al.* 2015; Dideriksen *et al.* 2018). The power inside a 5Hz band of the cumulative MU spike train (CST) centered around the IMC peak in the beta band was extracted online using a 3rd-order Butterworth filter. The mean of this beta feature in the initial training block was used for normalization during the neurofeedback part in Experiment 2. The logarithm of this normalized beta feature was then fitted to a Gaussian distribution to provide feedback on the beta activity using a color code. Specifically, a blue-to-white-to-red colormap was mapped to the logarithmical beta feature ranging from two standard deviations below the mean (blue) to two standard deviations above the mean (red), while the mean was coded via the color white (see Figure 3-1B). If the beta feature value was outside the range of the colormap, i.e. more than two standard deviations off the mean, the displayed color was set to the closest extrema (either blue or red).

Familiarization phase

The familiarization phase provided subjects with the same feedback environment as they experienced later in the neurofeedback task. Subjects were instructed to move a cursor up into a target rectangle by modulating the force exerted during dorsiflexion of the ankle. This target rectangle was centered at 10% MVC with a lower and upper bound at 9.5% and 10.5% MVC, respectively. The cursor's color changed accordingly to the underlying beta

feature and its corresponding value in the blue-white-red colourmap. If the cursor was outside the target rectangle, its color was changed to black. Hence, subjects only received feedback on the underlying beta feature when the cursor was inside the target. By doing this, subjects were encouraged to exert stable forces. Cursor position and color were updated every 125ms. The beta feature amplitude was averaged across the amplitudes observed in the seven most recent 125ms buffers analyzed as previously performed by (Bräcklein *et al.* 2021). Subjects had approximately 10min to get themselves familiar with this neurofeedback environment.

Neurofeedback task

The neurofeedback task was divided into multiple blocks. Subjects were asked to perform a minimum of three and a maximum of six blocks of training before three last consecutive blocks were used for further analysis. Each block consisted of three trials. Each trial started with subjects contracting their tibialis anterior muscle to produce ankle dorsiflexion forces that moved the cursor inside the target rectangle at 10% of the MVC. Once the cursor was within the target rectangle, the force produced had to be kept constant for 30s while beta activity had to be modulated. Specifically, subjects were asked to either keep the cursor blue for as long as possible (beta down-modulation condition), or red (up-modulation condition). In a third condition, no feedback on the underlying beta activity was given (the cursor stayed white when held inside the target; see Figure 3-1B). The color target indicating the modulation condition of each trial, was provided verbally by the experimental instructor and as visual clues by the color of the cursor edge. Hence, the cursor edge was blue when subjects were asked to keep the cursor blue (down-modulating MU beta), red (up-modulating beta), or black if no neurofeedback on MU beta was provided. Per block, each

modulation condition was presented once in a randomized order. Between each trial, subjects rested for at least 1 min to minimize muscle fatigue.

3.2.4 Analysis

Spectral analysis

The time-frequency representation of the CST and the surface Laplacian EEG was obtained using the continuous wavelet transform implemented via the `cwt` function in MATLAB (Version 2018b, MathWorks Inc., MA, USA). The corticomuscular coherence (CMC) was estimated using magnitude-squared wavelet coherence implemented via the MATLAB function `wcoherence`. A similar approach was chosen to estimate the temporal evolution of the IMC via a custom MATLAB script built upon the `wcoherence` function. To estimate the IMC, the MU pool was split into two randomly selected sub-pools of equal size. The magnitude-squared wavelet coherence between the CSTs of both MU sub-pools was calculated. This step was repeated over 100 iterations, always choosing a different configuration of MU sub-pools. The IMC was obtained by averaging the coherence estimates obtained during the 100 iterations.

The beta bursting activity present in the CST and EEG signals was extracted using a band-pass filter (13-30Hz, 4th-order Butterworth). The envelopes of the band-pass-filtered signals were used to determine when beta bursts occurred. The threshold above which the envelope was classified as a bursting event was empirically determined similar to the methods used in (Little *et al.* 2019; Shin *et al.* 2017). For Experiment 1, the envelopes from EEG and CST in each block were split into 1s windows. In each window, the power of the signal and the percentage of signal above the threshold was determined. The correlation between all concatenated windows was calculated using the Pearson correlation coefficient and averaged across blocks.

Hereby, the threshold was increased from 0 to 6 times the median in .25 steps. The threshold that resulted in the maximum correlation between power and percentage of signal above threshold was used to identify beta events. This procedure was repeated for Experiment 2 on block-level for the non-beta power feedback trials. The results are visualized in Figure 3-2. For Experiment 1, the empirically determined threshold was 2.50 and 2.75 times the median for CST and EEG, respectively. For Experiment 2, it was 2.25 and 2.75 times the median for CST and EEG. Consecutive periods where the envelopes were above the threshold were marked as ON periods (beta bursting events), similarly as previously performed in (Echeverria-Altuna *et al.* 2021). Hence, the length of ON periods was used to estimate the duration of beta events. The beta event power was calculated as sum of all ON events divided by the recording time. The remaining periods, i.e. when the envelope was below the threshold, were identified as OFF periods. The time points of ON and OFF events were set to the center of the respective periods. To analyze neural activity around ON and OFF periods, the wavelet transposed spectra of CST and EEG, the wavelet CMC and IMC were averaged in 500ms windows centered at the times of ON and OFF events. Furthermore, the percental mismatch between ON and OFF events was calculated as: $((ON - OFF) / OFF) * 100$.

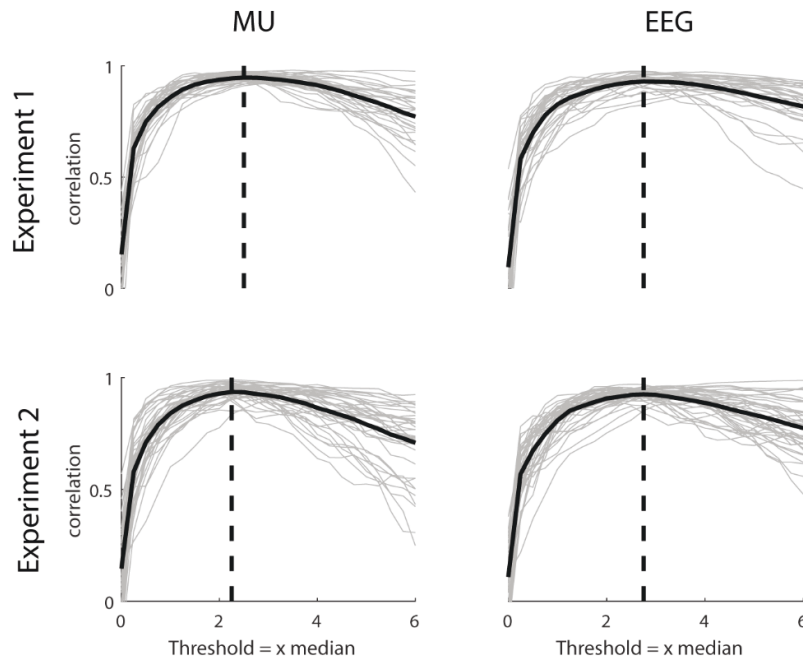


Figure 3-2: Beta burst threshold estimation. Correlation between beta band power and number of samples above threshold for Experiment 1 (top) and Experiment 2 (bottom) for the MU (left) and EEG (right) data. Grey lines indicate single blocks while solid black line indicate mean across blocks. For Experiment 2, only the control condition was used. Dashed black lines indicate maximum correlation value and corresponding threshold.

Experiment 1 – force task

The HDsEMG recorded during 40s of isometric ankle dorsiflexion at 10% MVC was offline decomposed into the underlying MU activity using the algorithm proposed in (Negro *et al.* 2016a). The decomposition results were manually inspected as detailed in (Del Vecchio *et al.* 2020). To control if the identified bursts in the EEG and the MU pool result from underlying amplitude modulations or in contrast from isolated bursting events, the lagged coherence method was employed (Fransen *et al.* 2015) using the NeuroDSP Python toolbox (Cole *et al.* 2019). This spectral measure examines coherence between the signal and a delayed version of the same signal at each frequency. If the lagged coherence is large, it provides evidence that the observed bursting events occur periodically and thus may be due to an underlying modulation. However, when the examined signal occurs in de-coupled

events, detached from any ongoing modulations, the lagged coherence is smaller. The power spectral density was calculated using Welch's method (2s window, 50% overlap) and normalized between 1 and 40Hz.

Experiment 2 – beta modulation

The online decomposed MU activity was post-hoc cleaned from artefacts. Action potentials that were fired with an instantaneous discharge rate above 30 pulses-per-second (pps) were neglected. Only the 30s-time interval during which subjects were instructed to modulate the beta activity while keeping the force constant were analyzed. In addition, the beta activity and discharge rate were recalculated by neglecting MUs that had an average discharge rate below 5pps or above 30pps or a discharge rate coefficient of variation (CoV) above 0.5 in any of the recorded blocks. The resulting cleaned pools of MUs were used in the subsequent analysis, also for example, to recalculate the beta feature and wavelet transformed CST activity, CMC, and IMC.

Functional values obtained during up- and down-modulation of MU beta activity, such as the mean force, beta amplitude, average rectified EMG, i.e. global EMG, bipolar EMG, and the corresponding CoVs to all values mentioned before, and the mean MU discharge rates were normalized by the averaged values obtained during the control condition (when no neurofeedback on the MU beta activity was provided). The wavelet transformed CST and EEG, CMC, and IMC were interpolated to transform the logarithmical frequency scale into a linear one for further analysis to ensure an equally weighted representation of all frequencies. The results were averaged inside the entire beta band (13-30Hz) and within in 500ms window centered around the ON-triggered averaged. The values obtained during neurofeedback were normalized by the corresponding values obtained during the control condition.

3.2.5 Statistics

Statistical analysis was performed via SPSS (IBM, Armonk, NY, USA) and custom MATLAB routines. Results were reported as mean $\pm$ standard deviation. Significant clusters of beta activity in the difference in the time-frequency representation of beta ON and OFF events were determined using the cluster-level analysis proposed by (Maris & Oostenveld 2007). In brief, this approach assessed clusters of adjacent samples in both frequency and time dimensions under a single permutation distribution (we used 10 000 permutations and an univariant clustering threshold of .05). This approach allows to bypass multi-comparison issues present in multi-dimensional data. The characteristics of beta bursting events in the MUs and the EEG were compared by using two-sided paired t-tests. The effect of volitional beta modulation on multiple motor behavioral properties of the innervated leg were tested by a repeated measures MANOVA. Hereby, the independent variables were the different modulation conditions, i.e. beta down- and up-modulation. Dependent variables were the mean force, mean rectified EMGs of agonist and antagonist muscles, the CoV of these values and the mean discharge rates of the decomposed MUs across subjects. Differences in the mean beta feature amplitude were assessed by two-sided paired t-tests. To assess whether the temporal evolution of the modulated beta feature correlated with muscle activation, the correlation coefficient between the exerted force, the rectified EMG of the agonist muscle or the discharge rate of the identified MU pool, and the beta feature were estimated using the Pearson correlation coefficient. To do this, force, rectified EMG and discharge rate were post-processed in a similar fashion as the beta feature, i.e. corresponding values were averaged per each recording buffer.

The difference in beta event features at the cortical and MU level was assessed using linear mixed models. Linear mixed models were also used to evaluate the effect of volitional beta modulation at MU level on the beta bursting characteristics and spectral values, such as wavelet-transformed CST and EEG, CMC, and IMC, on single blocks, in which the difference between beta down- and up-modulation was the dependent variable and the subject-wise grouping a random effect. Values during up- and down-modulation were normalized using data from the non-feedback condition as described in 3.2.3. The partial eta-squared (η_p^2) was used to assess the effect size of the changes between beta modulations. Values greater than 0.14 indicate that a “large” effect can be observed in the particular comparison (Cohen 1988). The threshold for statistical significance was set to $p < .05$.

3.3 Results

3.3.1 Experiment 1 – force task

In total, 22.73 ± 7.95 MUs per block were identified in Experiment 1. Figure 3-3 visualizes the time-frequency spectra inside the beta band of cortical (EEG signals) and muscle (the CST generated with the decomposed MUs) signals during a period of isometric ankle dorsiflexion at 10% MVC. Both spectra indicated that beta activity at the cortical and muscle levels occurred in short intervals, i.e. bursts of activity, while subjects held constant forces. The zoomed-in plot (Figure 3-3, bottom) suggested that some bursts observed in the muscle overlapped with bursts observed in the EEG.

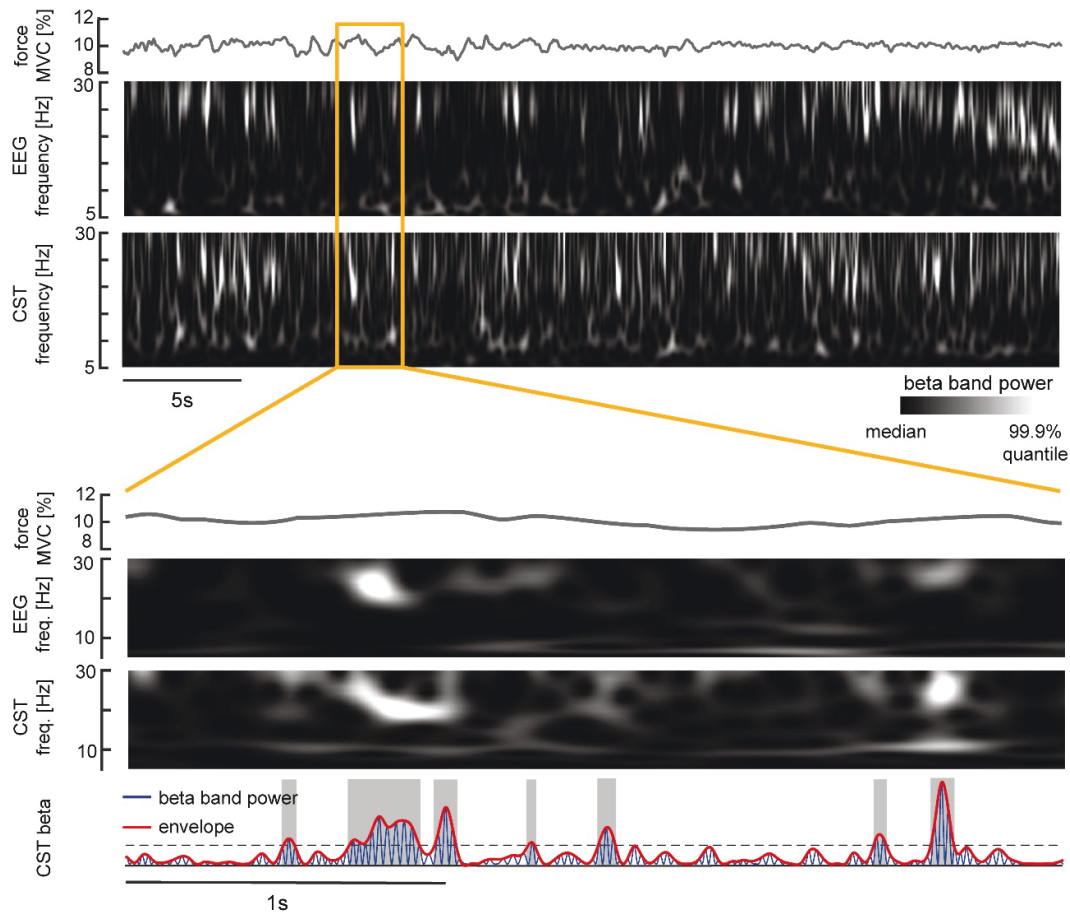


Figure 3-3: Beta power present in the EEG and MU pool shown in a representative subject. TOP: Force due to dorsiflexion of the ankle, interpolated time-frequency spectrum inside the beta band for surface Laplacian EEG and CST via continuous wavelet transform. BOTTOM: Zoom-into force, interpolated time-frequency-spectra of surface Laplacian EEG and CST, and beta band power (blue) and maxima envelope (red) extracted from the band-pass filtered CST. The Black dashed line indicates the threshold used to identify beta bursts (ON, grey shaded areas) and valleys in between bursts (OFF).

While the observed beta burst might occur as infrequent uncoupled bursting events, they could also result from an underlying amplitude-modulated oscillation. Hence, we conducted a control analysis to assess whether beta bursts in MUs and EEG result from a sustained amplitude modulation. In this case, the phase inside the beta band should predict the phase in upcoming cycles. In contrast, if these bursts do not originate from an underlying sustained modulation, the current phase inside beta should correlate less with future cycles (Fransen *et al.* 2015). Figure 3-4 illustrates that although both cortical and MU show

prominent beta activity in their spectra, the lagged coherence decreases inside this range compared to other spectral components. Further, this effect seems prolonged across multiple cycles. This indicates that in both EEG and MU activity, beta bursting events seem to be isolated, thus not resulting from underlying modulation.

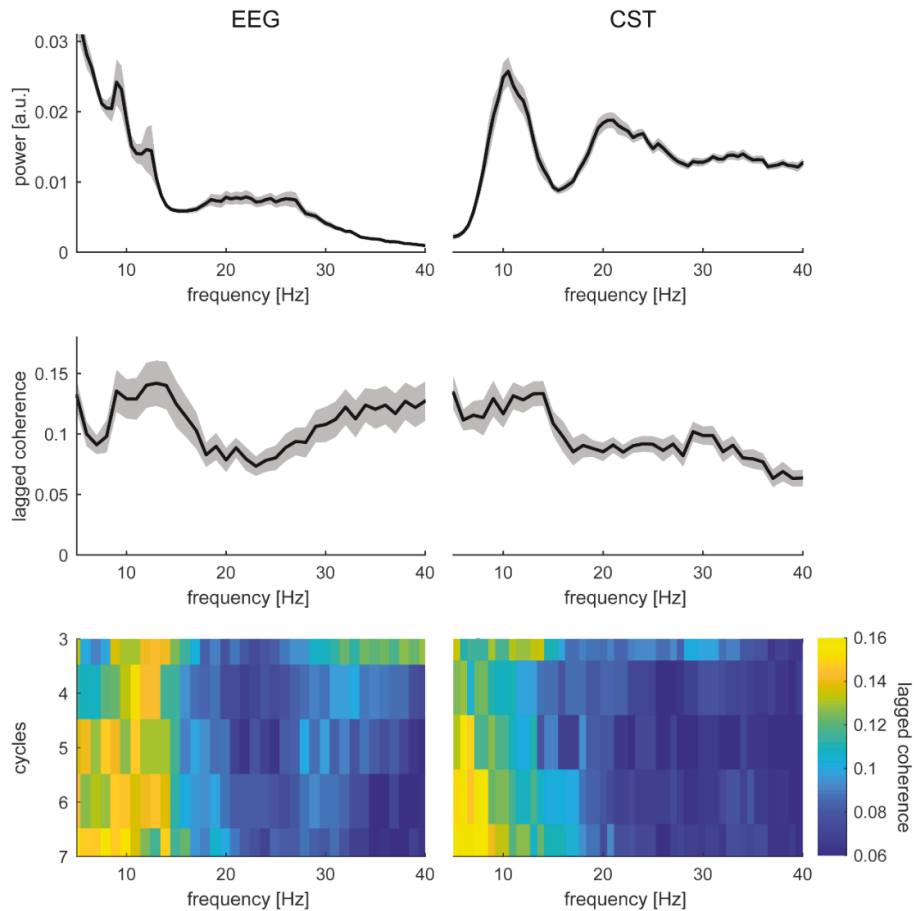


Figure 3-4: Lagged coherence analysis for EEG (left) and MU activity (right). Top: Mean power spectral density normalized between 1 and 40Hz across all blocks. Shaded areas indicate standard error of the mean. Middle: Mean lagged coherence at three cycles across all blocks. Shaded areas indicate standard error of the mean. Bottom: Mean lagged coherence for cycles 3 to 7 across blocks.

To understand activity around the short-lived beta bursts found in the EEG and CST signals, the wavelet-transformed data were averaged at the center of ON and OFF periods found in the EEG across blocks. Figure 3-5 visualizes these triggered averages for the wavelet-transformed EEG, CST, the CST-EEG coherence (CMC), the intramuscular coherence (IMC),

and the force profile at respective time intervals. While the exerted force did not significantly change between ON and OFF periods (no significant clusters, always $p > .05$), beta activity present in the EEG was significantly pronounced during ON relative to OFF periods in a cluster at the center of EEG beta events ($p = .024$). Also, beta activity in the CST was pronounced during ON periods compared to OFF, despite the time points of ON and OFF being determined by the EEG activity ($p = .042$). It is worth noting that the maximum difference between ON and OFF in the EEG was around time lag 0 (-.49ms), while the maximum difference in the CST was delayed by 24.41ms. Furthermore, a significantly pronounced bursting activity in the CMC was observed ($p = .001$). Similarly, the results suggested that the IMC was also of transient behavior inside the beta band (IMC, $p = .026$).

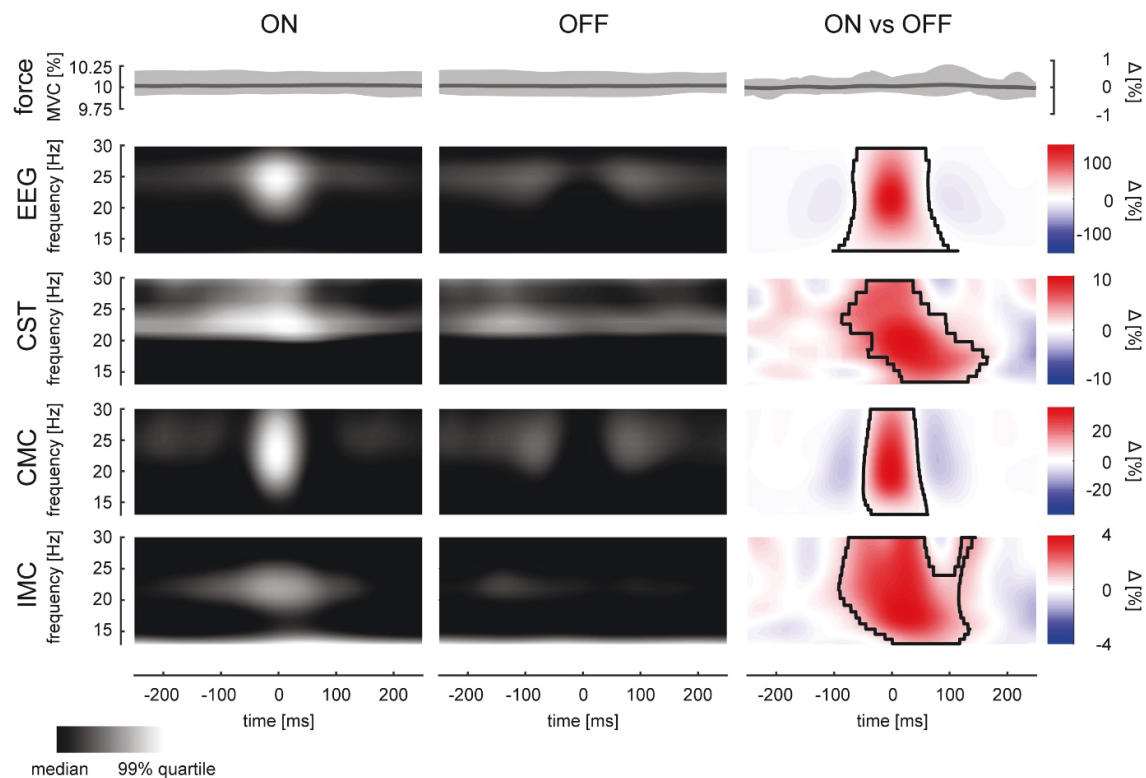


Figure 3-5: Neural activity during beta bursting events present in the EEG. ON and OFF periods were aligned and averaged across blocks. From top row to bottom: force (shading indicates 95% percentile), interpolated wavelet-transformed EEG, wavelet-transformed-MU activity, CMC, and IMC, at the center time points of ON

periods (left), OFF periods (center), and percental mismatch (right). Black boundaries indicate significant clusters ($p < .05$).

The previous results indicated that beta activity observed in cortical and muscle recordings occurred in bursts. Moreover, the significant beta activity in the CST identified during EEG beta bursting events suggested that beta bursts in the MU overlapped with those present at the cortical level. This confirms previous observations made using surface EMG signals (Echeverria-Altuna *et al.* 2021). In addition, we observed that the common input inside the beta range to the MU pool was of bursting behavior and appeared to be time-locked to cortical beta bursts. To further assess how beta bursts observed in the MU pool matched with the beta bursts in the EEG we compared the rate and duration of the beta bursts extracted from the CST and EEG (Figure 3-6). Beta events observed at the MU level appeared at a rate of $3.56 \pm .41$ events per second while beta events in EEG at a slightly but significantly lower rate of $3.23 \pm .30$ ($p = .003$, $\eta_p^2 = .469$). There was no significant difference detected between the average duration of the beta bursts observed on the MU level ($55.61 \pm 11.27\text{ms}$) and the bursts in the EEG (53.10 ± 9.23 ; $p = .398$, $\eta_p^2 = .052$).

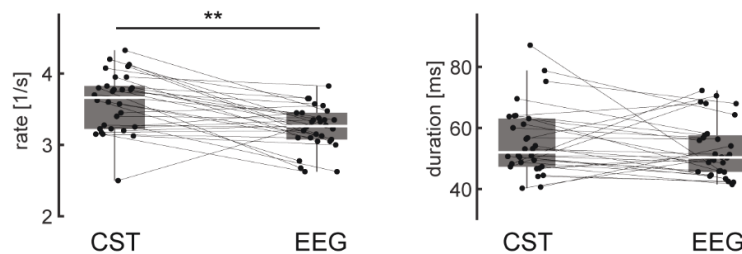


Figure 3-6: Relationship between beta bursts observed at the cortical and muscle levels. The rate at which beta events occurred (left) and their mean duration (right) are shown for cortical (EEG) and peripheral (CST) signals across blocks by their median and quantiles. Values for individual blocks are marked in grey and connected observation sides of beta events (i.e. CST and EEG). ** $p < .01$

3.3.2 Experiment 2 – beta modulation

Results from Experiment 1 showed that beta activity occurs in bursts both at the cortical and muscle levels. Moreover, the bursts observed at both levels are similar in features such as duration and rate of events and appear to be temporarily aligned with a small offset. These results therefore support the notion that beta activity in the EEG and CST have a shared underlying source. If this is the case, it is expected that modulation of beta activity at the MU level should correspond to a similar modulation of cortical beta observed in the EEG. To test this, Experiment 2 used a novel neural interface based on real-time decomposition of MU activity from the interference EMG.

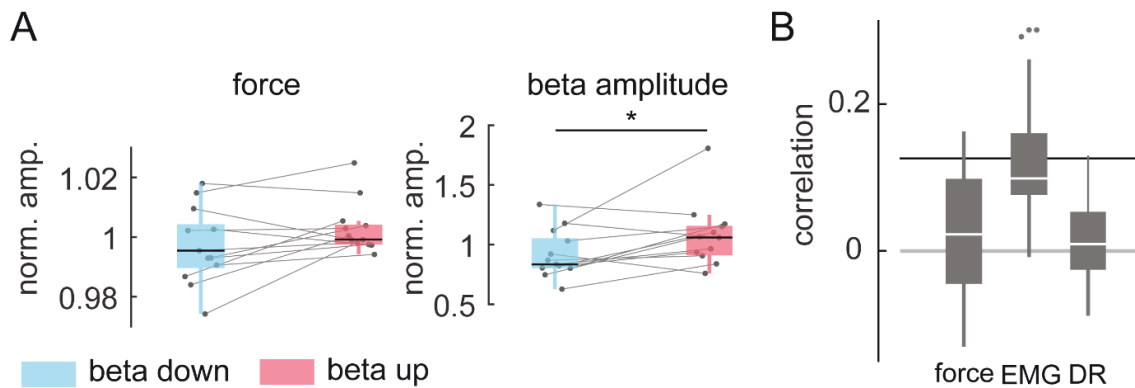


Figure 3-7: Functional values during beta power modulation. A: Mean force and beta feature amplitude (normalized by mean amplitude during non-feedback condition) during down- and up-modulation conditions (blue and red, respectively) shown by their median and quantiles all subjects. Grey points indicate the mean value per subject, while grey lines combine data of the same subject. * $p < .05$. B: Temporal correlation between the beta power feature and the force, global EMG of the tibialis anterior and the mean discharge rate (DR) shown across subjects with their median and quantiles. Black bar indicates significance level of correlation.

In this online experiment 11.92 ± 2.48 MUs per subject were identified and tracked in real time. Subjects could significantly reduce the normalized mean beta amplitude during down-modulation to 0.91 ± 0.20 , compared to up-modulation at 1.07 ± 0.26 (two-sided paired t-test, $t(12) = -2.454$, $p = .030$; see Figure 3-7A). In the context of volitional MU beta modulation, neither the mean exerted force nor other functional measures of the innervated

leg changed significantly (repeated measures MANOVA Wilks' Lambda corrected, $p = .424$, $\eta_p^2 = .811$). Furthermore, across all subjects, no temporal correlation between the beta feature and the force, rectified EMG of the tibialis anterior muscle, and discharge rate of MUs were detected (Figure 3-7B; all medians are below the significance level). Taken together, these results suggested that subjects were able to modulate the beta band activity present in a MU pool without critically altering the motor output.

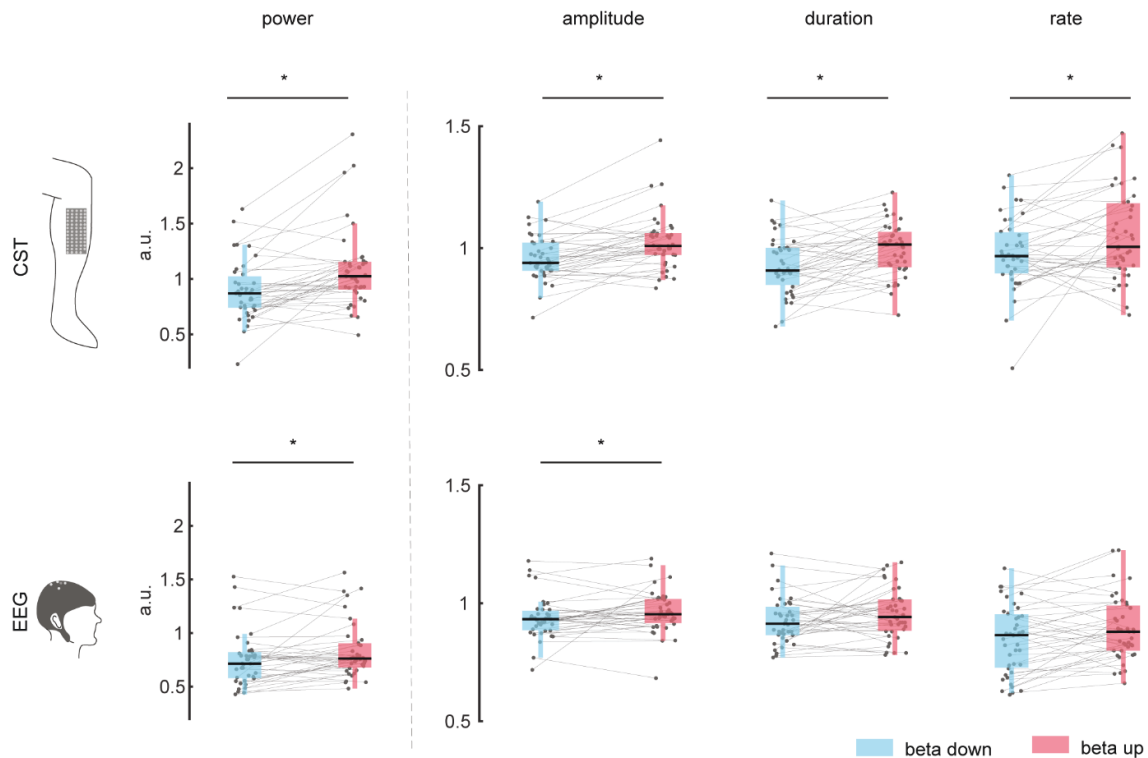


Figure 3-8: Normalized beta events features during modulation. Mean power, amplitude, duration and rates of beta events are shown across blocks. Corresponding values for beta down-modulation (blue), and up-modulation (red) are normalized by the control condition (no neurofeedback on beta activity). The top row shows values observed on the MU level (CST) and the bottom one for EEG level. Grey dots indicate values for single blocks. Grey lines combine values corresponding to the same block. $*p < .05$

To study the impact that modulation of beta activity in the MU pool has on cortical beta activity, we compared the burst power and the three burst features that contribute to the power estimate, i.e. peak amplitudes of the beta bursts, the bursts durations, and the number of bursts, between beta down- and up-modulation conditions normalized by the

corresponding values obtained when no beta feedback was provided (Figure 3-8). The power of the beta bursts in both CST and EEG increased during up-modulation compared to down modulation from $0.89 \pm .27$ to $1.09 \pm .37$ ($p = .003$, $\eta_p^2 = .540$) in the CST, and from $0.75 \pm .25$ to $.83 \pm .26$ ($p = .013$, $\eta_p^2 = .415$) in the EEG. The amplitudes of beta bursts in the CST and in the EEG were significantly higher in the up-regulation condition than in the down-modulation condition (CST: from $0.96 \pm .09$ to $1.02 \pm .12$, $p = .002$, $\eta_p^2 = .581$; EEG: from $0.94 \pm .09$ to $0.96 \pm .09$, $p = .038$, $\eta_p^2 = .311$). The duration of the beta events did also change between conditions at the MU level from $0.93 \pm .12$ to $1.00 \pm .11$ during down- and up-modulation, respectively ($p < .001$, $\eta_p^2 = .652$) but was not significant at the cortical level with longer durations of beta events during up-modulation (from $.92 \pm 0.10$ to 0.96 ± 0.11 , $p = .079$, $\eta_p^2 = .235$). The rate of observed beta events at the MU level increased significantly from $0.98 \pm .15$ to $1.05 \pm .18$ ($p = .023$, $\eta_p^2 = .363$). On average, the rate of beta events did also increase at the cortical from $.85 \pm .14$ to $0.89 \pm .14$, but this effect was marginally not significant ($p = .058$, $\eta_p^2 = .268$).

The appearance of beta bursts in the EEG and MU activity changed during volitional beta feature modulation. Figure 3-9 shows the impact of volitional beta modulation on the MU and EEG beta activity during beta ON events. The spectral power in the beta band during ON events increased significantly in the CST from $.99 \pm .20$ to $1.09 \pm .23$ ($p = .019$, $\eta_p^2 = .378$) and in the EEG from $.90 \pm .11$ to $.94 \pm .11$ ($p = .026$, $\eta_p^2 = .351$) during down- and up-modulation conditions, respectively. Similarly, the IMC increased significantly during up-modulation from $0.97 \pm .06$ to $0.98 \pm .05$ ($p = .034$, $\eta_p^2 = .321$) suggesting a stronger common input in the beta band during the up-regulation condition. Interestingly, the CMC did not change significantly from $1.00 \pm .06$ to $1.00 \pm .06$ between conditions ($p = .994$, $\eta_p^2 = .000$), which implies that while the common input to the MU inside the beta range increased during beta up-modulation relative to down-modulation, the spectral connectivity between cortical

beta and MU beta remained unaffected. These results indicated that cortical beta power mirrored the changes in the MU. Finally, it should be noted that the same overall effects were observed when using beta bursting events in the CST to define the timing of ON periods (see Figure 3-9).

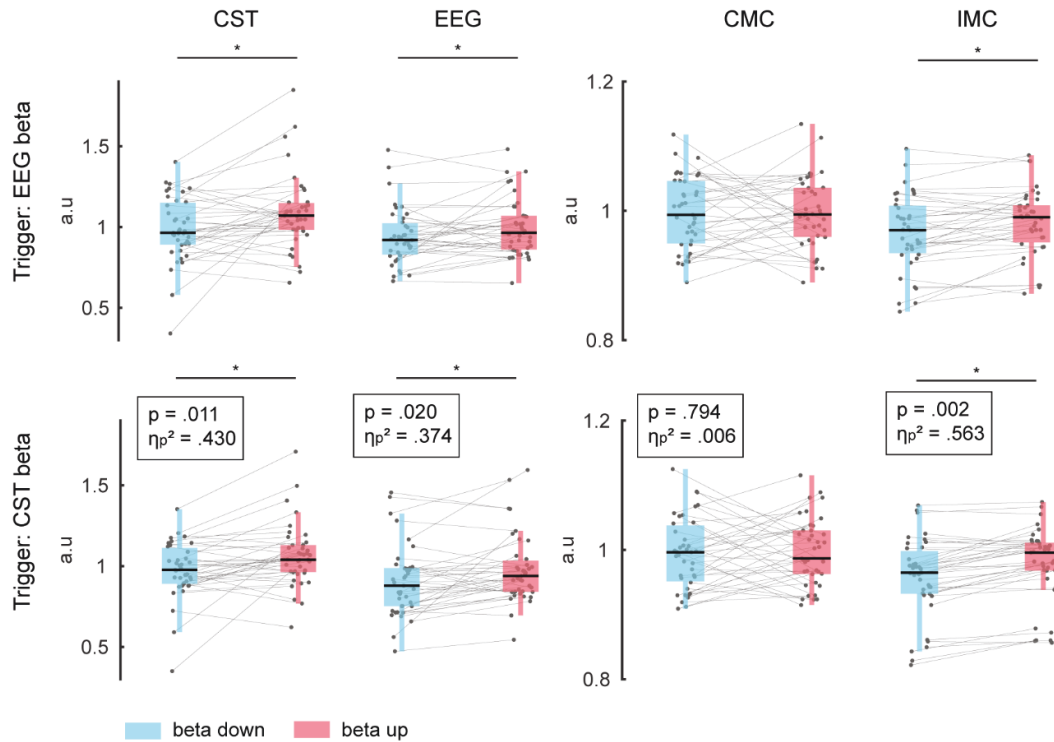


Figure 3-9: Impact of volitional beta feature modulation on spectral measures. From left to right: beta band power extracted from the MU activity and EEG, beta-band coherence in the CMC and IMC across subjects during beta feature down- (blue) and up-modulation (red). Mean values were extracted from a 500ms window centered around the ON periods identified in the EEG (top) and MU activity (CST, bottom) and were normalized by the corresponding values obtained during the control condition (no beta neurofeedback). Grey dots indicate values for single block, while grey lines combine values corresponding to the same block. * $p < .05$

3.4 Discussion

We studied the correspondence of cortical beta activity with beta oscillations found in the output of spinal motor neurons. To do this, we assessed how cortical and peripheral beta bursting events relate to each other during muscle contractions. We then used a MU-driven

neurofeedback approach to modulate the beta inputs to muscles to test if cortical beta activity followed the modulation of peripheral beta activity. Our results demonstrate, for the first time, that beta activity present in a MU pool appears in isolated bursts that closely correspond to the beta activity observed at the cortical level. In addition, when modulated at the periphery, cortical beta showed the same modulation pattern. We conclude that beta activity in the periphery is mainly determined by cortical projections.

The common beta activity present in the MU population strongly corresponded to the cortical beta projections. We showed that beta activity present in a MU pool is short-lived and shares the characteristics of the cortical beta rhythms, i.e. rate and duration of beta events. Moreover, the common input to the MU pool inside the beta range and the resulting MU beta activity were time-locked and followed cortical beta rhythms by tens of milliseconds. Although determining the transmission delay by only analyzing the beta power is not robust against noise that may mask the underlying shape of beta bursts, our observation is in strong agreement with previous investigations using the averaged CMC (Ibáñez *et al.* 2021; Mima *et al.* 2000). When we asked subjects to perform volitional modulations of the beta activity present in the MUs via a novel neurofeedback paradigm (Bräcklein *et al.* 2021), changes in the cortical beta power were shown to be coherent with those induced in the periphery. These findings suggest a strong and stable correspondence between peripheral and cortical beta oscillations during steady force contractions.

Although the effective beta activity at the MU level could potentially result from other neural centers (Thompson *et al.* 2019), as it was suggested to be the case for MU activity in the alpha range (8-12Hz) during tremor (Christakos *et al.* 2006), it seems that these non-cortical contributions may be minimized or suppressed in the context of cortical inputs during isometric contractions. If their contribution would have superseded the presence of cortical

projections at the MU level, the resulting beta activity in the periphery would be expected to differ from beta patterns observed at the cortical level. Moreover, the common input to the MUs inside the beta band was increased during volitional up-modulation of the MU beta power while the connectivity between cortical and peripheral sites remained unaffected (Figure 3-9). Hence, the coherence between the cortical regions and the MU pool inside the beta band (CMC) did not change, but the strength of the common input received by the MU pool (IMC) did. This provides additional evidence for MU beta signals mainly emerging from the cortical sites: if successful beta modulation resulted from additional modulation of non-cortical sources, the CMC would have been affected by the volitional beta feature modulation (Negro & Farina 2011a).

The dominance of cortical beta inputs to muscles contrasts with the observed lack of direct influence on the produced force. No significant relationship between the force output of the tibialis anterior muscle and the presence of beta rhythms in the innervating MU pool was detected. Still, despite the absence of any evidence for a direct link between beta bursts and the motor output, beta oscillations at the MU level could determine a non-linear effect on the neural drive to the innervated muscle and therefore on the force output (Watanabe & Kohn 2015). Our results show, however, that these beta events at the MU level are infrequent, i.e. approximately four events per second (Figure 3-6). While a stationary beta that changes amplitude continuously, as simulated in (Watanabe & Kohn 2015), may influence force control, a bursting beta is very unlikely to do so since the corrections in force would be far too slow to improve steadiness. Alternatively, the motor system could utilize the observed beta events as a sonar signal integrating sensory information from the muscle (Baker *et al.* 2006), yet this hypothesis requires further experimental validation. During Experiment 2, when subjects were instructed to modulate MU beta power, and cortical beta

changed coherently, the exerted force remained unchanged. This provides further evidence that apart from the timing of beta bursting events, also the modulation of the beta event amplitude does lie inside a motor null-space relative to force production. Hence, the strong link between cortical and spinal neurons via beta activity observed in this study did not seem to have any direct influence on motor output.

When subjects were exposed to neurofeedback on the MU beta activity, beta modulations at the cortical and MU levels were mainly driven by altering the amplitude. Also rate and duration of beta events increased during beta-up modulation, however, this effect was only significant at MU level. It yet remains unknown what underlying mechanism led to a volitional increase in beta power via increase in the amplitude of beta bursts. One possible explanation would be that subjects were able to recruit larger cortical networks involved in the projection of beta activity to the muscle. It was previously shown that the duration of beta bursts was not affected by the performed motor task in normal conditions (Echeverria-Altuna *et al.* 2021). Here, we observed, although not always significant, slightly longer periods of beta events during beta up-modulation compared to down-modulation of MU beta. Subjects did not receive feedback on the instantaneous amplitude of beta events, nor about their duration or rate. Instead, the feedback provided on the beta feature amplitude during Experiment 2 was smoothed with a moving average and aimed to motivate subjects to modulate the beta activity across the entire duration of the trial, i.e. suppressing or promoting beta activity as long and as often as possible. Further experiments with different neurofeedback approaches (e.g., using the instantaneous behavior of beta events) are necessary to investigate whether subjects could learn to modulate other characteristics of beta activity in the brain and the muscles. This would be highly useful to advance our understanding of the possible roles of beta oscillations in movement.

Finally, the strong presence of cortical projections at the MU level opens up new means of studying cortical beta: peripheral neural interfaces, such as presented in (Barsakcioglu *et al.* 2021), would allow an indirect yet reliable window into cortical activity and may contribute to an advanced understanding of the functional role of beta oscillations in the human motor nervous system by complementing traditional interfaces, such as based on EEG or magnetoencephalography. We showed that by closing the loop with a peripheral neural interface based on MU activity, subjects could volitionally modulate the power of cortical beta bursts. This could provide new possibilities to exploit cortical beta, for example, as a control signal for virtual or robotic effectors (Dominijanni *et al.* 2021; Eden *et al.* 2022).

In conclusion, we have shown for the first time that the final neural drive to muscles contains bursting beta activity. Moreover, these beta bursts in the MU behavior shared the appearance and were time-locked to those observed on the cortical level. Volitional modulation of MU beta activity was accompanied by coherent changes in cortical beta manifesting the strong correspondence between cortical and MU beta. The observed bursting activity inside the beta band appeared in infrequent events at low rate and thus may, at most, influence force generation as a disturbing factor rather than supporting accurate force control. Cortical beta oscillations seem to be the main contribution to MU beta activity and the strong correspondence between cortical and peripheral beta suggests the potential use of peripheral neural interfaces to track and modulate cortical activity.

CHAPTER 4 FLEXIBLE CONTROL OF MOTOR UNIT PAIRS¹

Recent developments in neural interfaces enable the real-time and non-invasive tracking of motor neuron spiking activity. Such novel interfaces could provide a promising basis for human motor augmentation by extracting potentially high-dimensional control signals directly from the human nervous system. However, it is unclear how flexibly humans can control the activity of individual motor neurons to effectively increase the number of degrees-of-freedom available to coordinate multiple effectors simultaneously. Here, we provided human subjects (N=7) with real-time feedback on the discharge patterns of pairs of motor units (MUs) innervating a single muscle (tibialis anterior) and encouraged them to independently control the MUs by tracking targets in a 2D space. Subjects learned control

¹ This chapter was published as “*The control and training of single motor units in isometric tasks are constrained by a common input signal*” in *eLife* (2022) with the co-authors Deren Y. Barsakcioglu², Jaime Ibañez^{2,3,4}, Jonathan Eden², Etienne Burdet², Carsten Mehring^{5,6}, and Dario Farina²; see (Bräcklein *et al.* 2022a)

The work presented in sections 4.2.6 and 4.3.2 mainly represents work conducted by Deren Y. Barsakcioglu but is still included in this thesis to support the remaining findings. The introduction was slightly altered to ensure a logical flow of the thesis. Furthermore, this chapter follows the IMRD layout (Introduction-Methods-Results-Discussion) like Chapter 2 and 3, opposing to the corresponding article which was published in the IRDM format. Hence, minor changes were performed to the Results and Methods section to ensure a logical flow.

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strategies to achieve the target-tracking task for various combinations of MUs. These strategies rarely corresponded to a volitional control of independent input signals to individual MUs during the onset of neural activity. Conversely, MU activation was consistent with a common input to the MU pair, while individual activation of the MUs in the pair was predominantly achieved by alterations in de-recruitment order that could be explained with history-dependent changes in motor neuron excitability. These results suggest that flexible MU recruitment based on independent synaptic inputs to single MUs is unlikely, although de-recruitment might reflect varying inputs or modulations in the neuron's intrinsic excitability.

4.1 Introduction

In the preceding chapters, I analysed the nature and origin of high-frequency components present in populations of motor neurons and their potential use as a resource for human motor augmentation. As introduced in Chapter 1, section 1.4, flexible control of individual motor neurons could also be a viable approach to coordinating natural and sDoF. While the CNS seems to orchestrate motor neuron behaviour on a low-dimensional manifold by recruiting smaller motor neurons before larger ones, i.e. size principle, previous works have tried to challenge the perspective of single MUs only being activated in a pre-determined fashion (Basmajian 1963; Formento *et al.* 2021)¹. A recent study even provided evidence that, indeed there may exist a neural substrate that allows selective cortical control of MUs via descending pathways (Marshall *et al.* 2021). However, it is unclear whether humans can learn to leverage such a potential neural structure for selectively activating MUs by converting the

¹ although, limitations due to the use of multifunctional muscles apply as discussed in section 1.4.2

common neural input received by a MU pool to independent inputs to individual MUs and thus changing the original MU recruitment.

This study examined whether humans could control pairs of MUs with different recruitment threshold innervating the same muscle flexibly. Further, it addressed how this potential ability depends on the similarity of the MU pairs in size or, equivalently, in the recruitment threshold. For this purpose, we used a neural interface that provided subjects with biofeedback on the activity of individual MUs (Barsakcioglu *et al.* 2021). Subjects were encouraged to navigate a cursor inside a 2D space into different targets as quickly as possible by selectively recruiting different MUs using feedback received on both MU activity and resulting cursor movement. This allowed us to assess if subjects could selectively change the net excitatory inputs individual MUs or if, instead, they had to rely on control strategies based on a common input to the MU pair. After several days of training, all subjects were able to perform the target-tracking task. However, the control strategies used that allowed individual MU activation did not leverage potential selective inputs to single MUs. Instead, subjects strongly favoured control strategies based on a common input signal combined with changes in intrinsic motor neuron excitability due to history-dependent physiological properties of the activated MUs.

4.2 Materials and methods

4.2.1 Subjects

Seven healthy subjects (two females and five males, age: 27.86 ± 4.06 years [mean $\pm$ standard deviation]) were recruited for the study of whom three are authors of this article. Four subjects were naïve to the experimental paradigm, while the remaining three were

recently exposed to single MU feedback. Experiments were carried out over 14 days in blocks of four to five consecutive days with never more than two days of break between blocks. Each experimental session lasted approximately two hours. One subject withdrew from the experiment after only ten sessions due to time constraints. The study was approved by the ethics committee at Imperial College London (reference number: 18IC4685).

4.2.2 Data acquisition

High-density surface EMG (HDsEMG) was acquired from the tibialis anterior muscle of the dominant leg via a 64-electrode grid (5 columns and 13 rows; gold-coated; 1 mm diameter; 8 mm interelectrode distance; OT Bioelettronica, Torino, Italy). The adhesive electrode grid was placed over the muscle belly aligned to the fibre direction. In addition, EMG from the fibularis longus and the lateral and medial head of the gastrocnemius muscles were recorded throughout the experiment via pairs of wet gel electrodes (20 mm interelectrode distance; Ambu Ltd, St Ives, United Kingdom) placed over the muscle belly. All EMG signals were monopolar recorded, amplified via a Quattrocento Amplifier system (OT Bioelettronica, Torino, Italy), sampled at 2048Hz, A/D converted to 16 bits, and digitally band-pass filtered (10-500Hz). The foot of the dominant leg was locked into position to allow dorsiflexion of the ankle only. The force due to ankle dorsiflexion (single degree-of-freedom) was recorded via a CCT TF-022 force transducer, amplified (OT Bioelettronica, Torino, Italy), and low-pass filtered at 33Hz. The communication between the amplifier and the computer was conducted via buffers, i.e. data packages of 256 samples corresponding to a signal length of 125ms. All incoming EMG signals were band-pass filtered between 20-500 Hz using a 4th order Butterworth filter. Bipolar derivations were extracted from the filtered EMG signals obtained from the fibularis longus, and medial and lateral head of the gastrocnemius.

4.2.3 Experimental paradigm

Pre-experimental calibration

Subjects were instructed to perform maximum isometric dorsiflexion of the ankle to estimate the maximum voluntary contraction level (MVC). The obtained MVC was then set as a reference value for the subsequent experimental session. In a sub-MVC task, subjects were instructed to follow a 4s ramp trajectory (2.5% MVC per second) followed by a constant phase at 10% MVC of 40s. In both tasks, visual feedback of the force produced by isometric tibialis anterior contractions was provided. Based on the EMG of the tibialis anterior recorded during this sub-MVC task, the separation matrix used by an online decomposition algorithm was generated to extract MU discharge behaviour in real-time (see Barsakcioglu *et al.*, 2021 for further explanation). The decomposition results were visually inspected while subjects were instructed to recruit MUs one after another based on the visual feedback provided.

Force feedback task

After initialising the real-time decomposition algorithm, subjects were instructed to follow ramp trajectories consisting of a 10s incline (1% MVC per second) followed by a 10s plateau at 10% MVC and a 10s ramp decline (-1% MVC per second) guided by visual feedback of the force. This ramp trajectory was repeated five times with 5s rest period in between ramps. Only the first ramp was used for further analysis since repeated activation of MUs within a short time may influence the recruitment order (Gorassini *et al.* 2002). If a subject failed to follow the ramp during the initial ramp (which happened in 20.2% of all cases), the consecutive ramp was chosen as the basis for further processing and analysis. Based on the recorded force and underlying MU activity during the incline phases, the recruitment order of the decomposed MUs was estimated as suggested in (Del Vecchio *et al.* 2020). The onset

of MU recruitment was defined as the time when a MU started to discharge action potentials at 5 pulses-per-second (pps) or above. The averaged force values, extracted from a 100ms window centred around this onset of MU activity during the initial ramp, was used to establish the MU recruitment order by ranking MUs based on their corresponding force values in ascending order. The plateau phases of the ramps were used to estimate the average discharge rate of each MU during 10% MVC. These values were later used during the target task to normalise the discharge rates. The decline phase was used to determine the force values associated with MU de-recruitment. The time point of MU de-recruitment was defined by the last action potential discharged before a MU turned "silent" for at least 1.5s. Similar to the calculation of the force level needed for MU recruitment, the force level at MU de-recruitment was estimated by the average force value extracted from a 100ms window at the offset of MU activity across the initial ramp.

MU selection

Subjects were provided with visual feedback on the ranked MU activity. In an exploration phase (approximately 10 minutes), subjects were instructed to recruit MU one-by-one by gradually increasing the contraction level of the tibialis anterior until all identified MUs were discharging action potentials. The entire pool was divided into two sub-pools comprising the first and last recruited half of MUs, respectively. One pair of MUs with a similar recruitment threshold from each sub-pool was randomly selected. Hereby, MU pairs were excluded from the selection if subjects could not recruit these two MUs one by one even after the initial exploration phase. The MU recruited first in each pair was labelled as *MU1*, while the MU recruited last as *MU2*. The selected MUs and their decoded spiking activity are exemplarily shown in Supplement 4-1.

Target task

In the main experiment, subjects navigated a cursor in a 2D space by modulating the discharge rate of MU1 and MU2. The normalised discharge rate of these two MUs was used to span the manifold with units ranging from 0 to 1 along both axes. This target space included three targets of equal size (radius of 10% of the normalised discharge rate) placed along the axes (TI [1 normalised discharge rate MU1; 0 normalised discharge rate MU2], TIII [0; 1]) and the diagonal (TII [1; 1]). In addition, each target was framed by an angle space comprised of a triangle with one corner in the coordinate origin [0; 0] and two sides to be tangent at the circumcircle of the corresponding target. Towards the coordinate origin, the angle area was cropped by a circle centred at the origin with a radius of 40% of the normalised discharge rate. In order to navigate the cursor inside this angle space, subjects would need to generate the same discharge relationship between MU1 and MU2 as for reaching towards the target area but without necessarily matching the exact discharge rate determined during 10% MVC. For example, to place and hold the cursor inside the angle space of TI, subjects would need to keep MU1 active while MU2 was inactive. However, the discharge rate of MU1 could be different from its discharge rate at 10% MVC required to place the cursor on the centre of TI. This means the cursor could be placed at (.7; 0), resulting in an angle hit but not a target hit.

The discharge behaviour of MU1 and MU2 was decomposed from the acquired HDsEMG in real-time. The obtained discharge rates were averaged over the preceding eight buffers (corresponding to 1s) and normalised by the average discharge rate at 10% MVC of the respective MUs (see 4.2.3). The cursor movement was updated every buffer (corresponding to 125ms) and smoothed over six buffers (corresponding to 750ms) using a moving average. In total, the moving average on the discharge rates and the cursor position

resulted in a weighted average on the discharge behaviour of MU1 and MU2 over an effective window of 1625ms. However, the visual feedback was updated every 125ms. Because of the update rate, as soon as the discharge behaviour of the selected MU changed, subjects were aware of the change due to the visual display of MU discharges and the resulting cursor movement. In an initial familiarisation phase, subjects could freely move inside the target space and explore different control strategies. During this period, the gain along each axis was set manually to enable subjects to reach the target areas without overexerting themselves to prevent symptoms of muscle fatigue. On average, across all subjects, the gain was increased to $1.15 \pm .01$ for MU1 and $1.16 \pm .01$ for MU2, respectively.

After familiarisation with the target environment, subjects were asked to rest at the coordinate origin. Once the target-of-interest appeared, indicated in blue, a trial started, and subjects were instructed to navigate the cursor as quickly and as directly as possible into the target area. If the cursor was kept inside the target-of-interest for at least seven consecutive buffers (corresponds to 875ms; one buffer size longer than the moving average), subjects were granted a target hit, and the trial ended. The trial was also terminated if subjects failed in navigating and holding the cursor inside the target area within 20s. If the subjects kept the cursor inside the angle area of the target-of-interest for seven consecutive buffers before the trial was terminated, the subjects were granted an angle hit. Therefore, in a single trial, subjects could achieve both an angle and target hit. A target hit was indicated via colour change of the target-of-interest to green, an angle hit to yellow, and a failure (no target nor angle hit within 20s) to black. Once the trial ended either after 20s or a target hit, the subject was instructed to navigate the cursor back into the coordinate origin and rest there for at least 2s. The entire trial cycle is illustrated in Figure 4-1. In total, every target was presented ten times in randomised order. Moreover, this target task was repeated three times for three

different conditions. In Condition I, MU1 and MU2 were coming from the lower threshold pair while, in Condition II, they were taken from the higher threshold pair. In Condition III, MU1 was the lower threshold unit from the low-threshold pair and MU2 the higher threshold MU from the high-threshold pair. After the target task was completed, subjects repeated the force task.

During the familiarisation phase and the experiment, subjects received both feedback on the cursor movement and the underlying discharge behaviour of the selected MU pair updated for every buffer of incoming data, i.e. 125ms. Figure 4-1 visualises a simplified version of the biofeedback environment presented to the subjects.

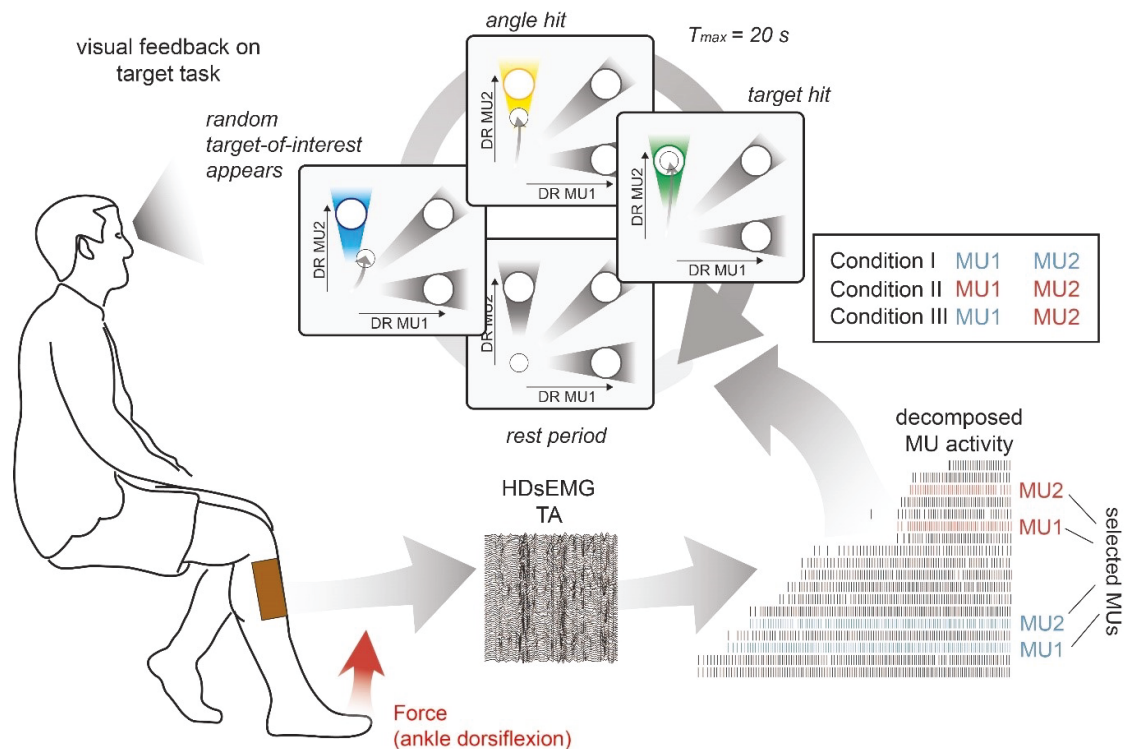


Figure 4-1: Schematic overview of the target task. HDsEMG of tibialis anterior was acquired and decomposed from the underlying neural activity in real-time. Concurrently, the force due to dorsiflexion of the ankle (red arrow) and bipolar EMG of FL, GL, and GM were recorded. The identified MU pool was ranked accordingly to the recruitment order. Two pairs of MUs with a similar recruitment threshold were selected from the initial (blue) and the latter recruited half (red). During the target task, subjects were instructed to navigate a cursor inside a 2D space by modulating the normalised discharge rate (DR) of MU1 and MU2. The selection of MU1 and MU2 was determined by three different conditions. In Condition I, MU1 and MU2 were coming from the low

recruitment threshold pair (blue), in Condition II from the high recruitment threshold pair (red), while in Condition III, the lowest threshold MU of the low threshold pair was pooled with the highest threshold MU of the high threshold pair. During the target task, subjects were asked to stay inside the origin until the target-of-interest (blue) appeared (randomly selected). By navigating the cursor inside the angle area around the target-of-interest, subjects were granted an angle hit (yellow). The trial was terminated when either the subject managed to place and hold the cursor inside the target area (target hit, green) or more than 20s had passed since the target-of-interest appeared. In each condition, 30 targets were shown, i.e. each target ten times.

4.2.4 Analysis

Force task

The EMG of the tibialis anterior, fibularis longus, and medial and lateral head of the gastrocnemius acquired during both force tasks before and after the target task were rectified and low-pass filtered at 10Hz with a 4th-order Butterworth filter. Similar to the estimation of the force level at the on- and offset of MU activity, the average global EMG values to MU de-/recruitment of all muscles were calculated. The average value inside a 100ms window centred around the time point of recruitment and de-recruitment for each MU and muscle was calculated across all ramps. This was separately repeated for all values acquired before and after the target task. Three subjects did not repeat the force task after the target task and only followed a single ramp at the beginning of the experiment. Moreover, no de-recruitment threshold was determined for those subjects.

Target task

If subjects failed to navigate and hold the cursor for 875ms inside the target-of-interest within the 20s-time window, the nearest miss was calculated. The nearest miss was defined as the average cursor position over 875ms with the shortest Euclidean distance towards the centre of the target-of-interest. In addition, as previously described in (Bräcklein

et al. 2021), unintended hits were used as a metric to assess the effectiveness of subjects directly hitting the target-of-interest without unintentionally hitting unselected targets before. Therefore, an unintended hit was classified as the case when subjects navigated and held the cursor inside an unselected target for at least 875ms. Unintended hits of the same target could occur multiple times within a single trial if the cursor re-entered the unselected target on several occasions before the trial was terminated. Similarly, unintended angle hits were counted when the cursor was navigated into unselected angle areas, respectively.

TI and TIII required the sole activation of either MU1 or MU2. To assess subjects' performance in navigating the cursor towards these two targets even when neither the target nor the angle area was reached but for example, the discharge rate of the target MU was greater than the one of the other, a new performance metric was introduced that compensates shortcomings of traditional metrics, e.g. time-to-target and distance to target. This performance metric was defined as:

$$performance = \sum_{n=1}^N \sqrt{\left(\frac{d(n)}{d_{max}}\right)^2 + \left(\frac{\varphi(n)}{\varphi_{max}}\right)^2}, \quad Eq. 4-1$$

where $d(n)$ is the Euclidean distance between the centres of the cursor and target-of-interest and $\varphi(n)$ the angle between the cursor and the target-of-interest at the buffer n . N is the total number of buffers recorded in one trial. For TI and TIII, d_{max} was set as the Euclidean distance between the centres of TI and TIII, and φ_{max} to 90°. For TII, the target along the diagonal, d_{max} was set to the distance between the centre of TII and the origin, and φ_{max} to 45°. When the cursor was held in the origin, i.e. no activation of either MU1 or MU2, $\varphi(n)$ was set to φ_{max} . By summing across all recorded buffers, this metric incorporates the time-to-target and favours those trials in which the cursor was kept close to the target-of-interest even when neither the angle nor the target area was hit. To scale the *performance values*

between 0 and 1, the obtained result was normalised with the *worst* and *best performance values* estimated per target. The *best performance value* for each target across subjects was obtained by simulating cursor movement based on artificially generated MU discharge patterns that match the required activation of MU1 and MU2 to hit the respective target. For example, the *best performance* for T1 was estimated based on discharge behaviour for MU1 that matched a normalised discharge rate equal to 1 and MU2 equals 0. The performance value during the idealised cursor movement until the target hit was used as the corresponding *best performance value*. For the *worst performance value*, the performance was calculated as if the cursor was kept in the origin for the entire 20s.

The described metrics were calculated across all conditions and subjects. Three subjects started with Condition III only from day 10 onwards.

4.2.5 Questionnaire

After every condition, subjects were provided with a questionnaire. Subjects were asked to indicate the level of control they had over MU1 when reaching towards T1, over MU2 when going towards TIII, and both MUs when reaching towards TII. Moreover, whether subjects felt they were using a specific strategy when going to the selected target-of-interest and how cognitively demanding it was to control the MUs together and independently was assessed. When applicable, they were asked to explain their strategy. Three subjects did not fill out the questionnaire.

4.2.6 Validation of online EMG decomposition during recruitment/de-recruitment

A new validation study was performed on 3 subjects (1 female and 2 male, age 32, 24, 25 years) to compare the on and offset activity of MUs detected via surface and intramuscular EMG recordings. For this purpose, surface EMG signals were recorded in the same way as in the main experimental task by a 64-channel high-density grid placed over the tibialis anterior muscle and aligned to the fibre direction of the dominant leg. In addition to the non-invasive grid, two fine-wire electrodes were inserted with an insertion angle of 45° to a depth of a few mm below fascia, 10-mm proximal with respect to the top of the electrode grid. The foot was locked into position to allow for the ankle dorsiflexion only, as for the main experimental session. All signals were acquired synchronously with a Quattrocento Amplifier system (OT Bioelettronica, Torino, Italy), sampled at 10240 Hz, A/D converted to 16 bits, and digitally band-pass filtered (10-4400Hz).

For the validation test, the participants were provided with visual feedback of the ankle dorsiflexion force and were presented with six target force profiles. All force profiles consisted of a ramp-up trajectory, a 20s constant force phase at 10% MVC, and a ramp-down trajectory. The ramp-up and ramp-down phases had a slope of 0.5%MVC/s, 1%MVC/s, 2%MVC/s, 2.5%MVC/s, 5%MVC/s, and 10%MVC/s. Each target was repeated twice. In this way, the contractions included recruitment and de-recruitment for a broad range of contractions speeds.

The HDsEMG signals were real-time decomposed during the contractions in the same way as done in the main experimental session. The intramuscular EMG signals concurrently recorded were analysed offline and decomposed with the EMGLab software (setting a high-

pass filtering at 1000 Hz) (McGill *et al.* 2005). The surface EMG online decomposition was compared to the intramuscular EMG decomposition by the rate of agreement, i.e. the percentage of discharges identified by both methods within a maximum time difference of 1ms. For this purpose, there was the need to identify the motor units that were commonly identified by intramuscular and surface EMG decomposition. The identification of the pairs of common motor units was performed with a procedure fully independent on the rate of agreement (which was the metrics to be validated). For this purpose, the spike train of each motor unit identified by intramuscular EMG decomposition was used for spike-triggered averaging the multi-channel surface EMG. This provided the estimate of the action potential waveform shape as detected by the surface EMG grid for the motor units identified by intramuscular EMG decomposition. The spike trains of the motor units identified by the surface EMG decomposition were then used for spike-triggered averaging the surface EMG and therefore to obtain the action potential waveform shapes at the skin surface for the motor units identified by surface EMG decomposition. The matching for identifying common units between the two decomposition processes was then performed by comparing the shapes of the action potentials at the skin surface obtained from the averaging processes based on the spike trains identified by intramuscular and surface EMG decomposition. The comparison was based on the correlation coefficient between action potential waveform shapes, with a threshold of 0.9 set to accept two motor units as commonly detected.

4.2.7 Statistics

Statistical analysis was conducted using SPSS (IBM, Armonk, NY, USA) and Matlab (Version 2018b, The Mathworks, Inc., Natick, MA, USA) for the linear mixed model analysis. The threshold for statistical significance was set to $p < .05$. Results were reported as mean $\pm$

standard deviation. To avoid accumulation of Type I errors, non-parametric tests were used to assess the relationship between variables (Rochon *et al.* 2012). To compare recruitment and de-recruitment thresholds, a linear mixed model with the difference between recruitment and de-recruitment threshold as dependent variable, a fixed effect intercept and a participant specific random intercept was applied using restricted maximum likelihood estimation. Significance of the fixed effect was assessed by an F-test using Satterthwaite's approximation for the degrees-of-freedom. For analysing the improvement in target and angle hit rate, performance across and for each target, as well as the relationship between the performance of reaching each target and the difference in recruitment and de-recruitment thresholds between MU2 and MU1, two-sided Wilcoxon signed-rank tests were used. Comparison of mean characteristic forces during indirect hits of TIII and the mean correlation between performance and successive trials were conducted via Friedman test. A two-sided Wilcoxon signed-rank test was used for post-hoc analysis. The correlation between recruitment and de-recruitment thresholds as well as the within-MU-pair difference in recruitment and de-recruitment thresholds, was assessed using the Spearman correlation coefficient.

4.3 Results

4.3.1 Target task

During the main experimental sessions, on average, 11.04 ± 3.34 MUs were reliably decomposed (online surface EMG decomposition) per subject ($N = 7$). An identified MU pool from a subject sorted based on recruitment order is shown in Figure 4-2A. As indicated by the

green and red marks, the order in which MUs are de-recruited often differed from the recruitment order. For example, once recruited, a MU could keep discharging action potentials even when the exerted force level was below the initial recruitment threshold. For the target task (see 4.2.3), two pairs of MUs with a small difference in recruitment threshold were selected out of the entire pool. Each pair was selected either from the first or from the last recruited half of the MU pool. Figure 4-2B visualises the recruitment thresholds of these selected MUs. Within pairs, MU1 was recruited before MU2 and the lower threshold pair (blue) was recruited before the higher threshold one (red). Recruitment and de-recruitment threshold of the selected MUs showed a strong relationship across days and subjects (Spearman correlation coefficient, $R = .53$, $p < .001$; see Figure 4-2C). On average, the de-recruitment threshold was -1.11 ± 2.44 % MVC smaller than the recruitment one, and in 66.7% a selected MU was de-recruited at a force level below its initial recruitment threshold. The overall observed effect, however, was weak (see 4.2.7, $p = .111$).

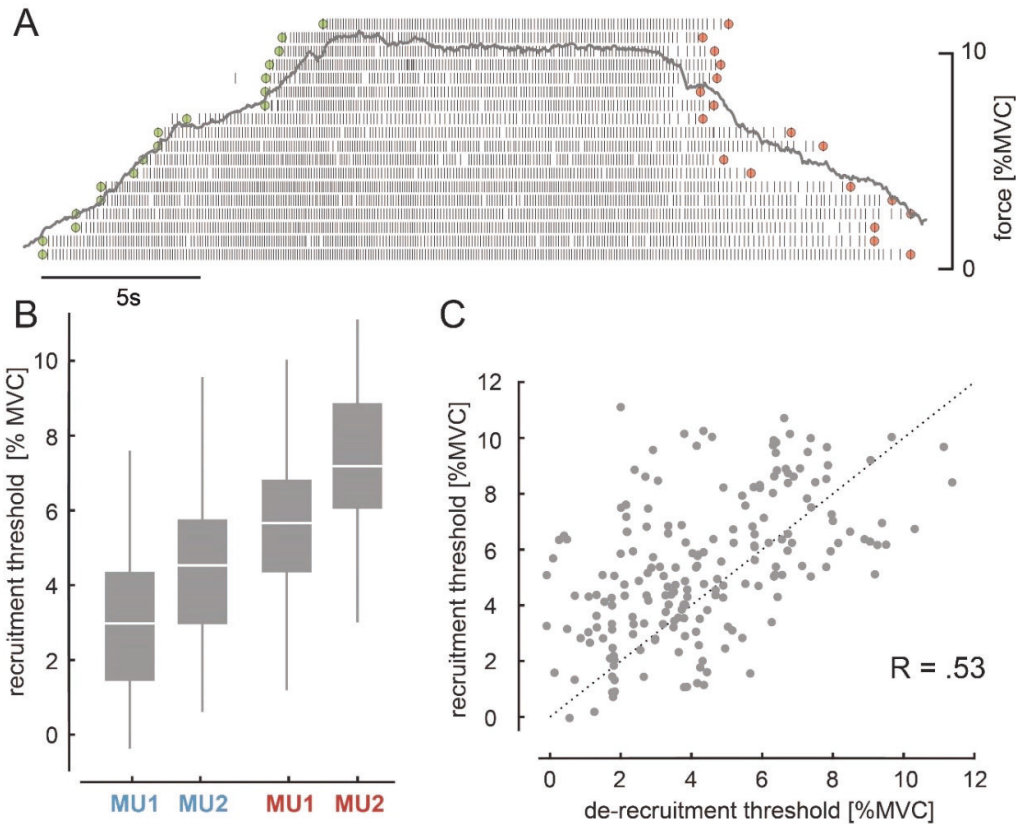


Figure 4-2: MU recruitment and de-recruitment. A: The identified MU pool ranked on the recruitment order of one representative subject is shown with the underlying force profile (grey). Time points of recruitment (green) and de-recruitment (red) for each MU are marked. B: Recruitment level for MU1 and MU2 of the lower (blue) and higher threshold pair (red) across all subjects and days are shown with their median and quartiles. C: Recruitment and de-recruitment threshold for the selected MUs across all days showed a significant relationship ($p < .001$). Dashed line indicates the diagonal. The three subjects for whom no de-recruitment thresholds were determined were neglected in this correlation analysis.

During the target task, subjects were asked to navigate a cursor inside a 2D space by modulating the discharge rate of MU1 and MU2. Three different targets inside the 2D plane were used to encourage subjects to activate both MUs independently despite their different position within the recruitment order. For example, to reach TIII, subjects must keep the higher threshold unit MU2 active while keeping the lower threshold unit MU1 off. Figure 4-3A shows, as an example, the average cursor position during target hits and nearest misses for each target-of-interest across conditions towards the beginning and end of training for a single subject. At the beginning of the task, the subject failed in the majority of trials to place

and hold the cursor inside the target-of-interest. With training, the ability to place the cursor inside the designated target area improved. As shown in Figure 4-3A, TII was hit in all 30 trials, and in only four trials, the subject could not hit TI. Moreover, the nearest misses for TIII in the twelfth training session were closer to the target centre than on day 1. This improvement in hitting targets and angles over several training days was observed across all subjects (see Figure 4-3B). The target hit rate improved from the first to the last day of training from $41.19 \pm 17.76\%$ to $67.04 \pm 18.17\%$ (two-sided Wilcoxon signed-rank test, $p = .028$). A similar trend was observed for the angle hits with an improvement from $64.37 \pm 13.15\%$ to $81.11 \pm 9.84\%$ (two-sided Wilcoxon signed rank test, $p = .016$). According to the distanced-based performance metric (defined in 0), the performance per subject across targets and conditions improved from the first to the last day of training from $.56 \pm .09$ to $.69 \pm .10$ (two-sided Wilcoxon signed rank test, $p = .016$). Despite this clear improvement in performance across days, the ability to move the cursor towards the target-of-interest enhanced differently across targets (see Figure 4-3C). The performance in hitting TI did not significantly improve from the first day of training to the last day (from $.67 \pm .18$ to $.68 \pm .11$; two-sided Wilcoxon signed rank test, $p = .938$). Similarly, no significant improvement was observed for TII (from $.69 \pm .11$ to $.80 \pm .13$; two-sided Wilcoxon signed rank test, $p = .109$). However, a significant improvement in performance was detected when subjects were asked to move towards TIII (from $.33 \pm .12$ to $.59 \pm .17$; two-sided Wilcoxon signed rank test, $p = .016$). Furthermore, across conditions and days, we only found minor within-session learning (mean correlation between performance and consecutive trials across days and condition for TI: $.037 \pm .078$, TII: $.066 \pm .094$, TIII: $.043 \pm .074$, see Supplement 4-2C) with no significant difference across targets (Friedman, $p = .103$).

Taken together, target and angle hits, as well as the performance metric, indicate that subjects improved in navigating the cursor towards the target-of-interest across days. However, the main improvement was observed for reaching TIII, which started from poor initial performance. Moreover, subjects experienced a steep learning curve at the beginning of the experiment, while the learning rate seemed to decrease towards the end. For this reason, further analysis only focuses on the last five days of training to avoid additionally induced variability by greater learning rates at the beginning of training.

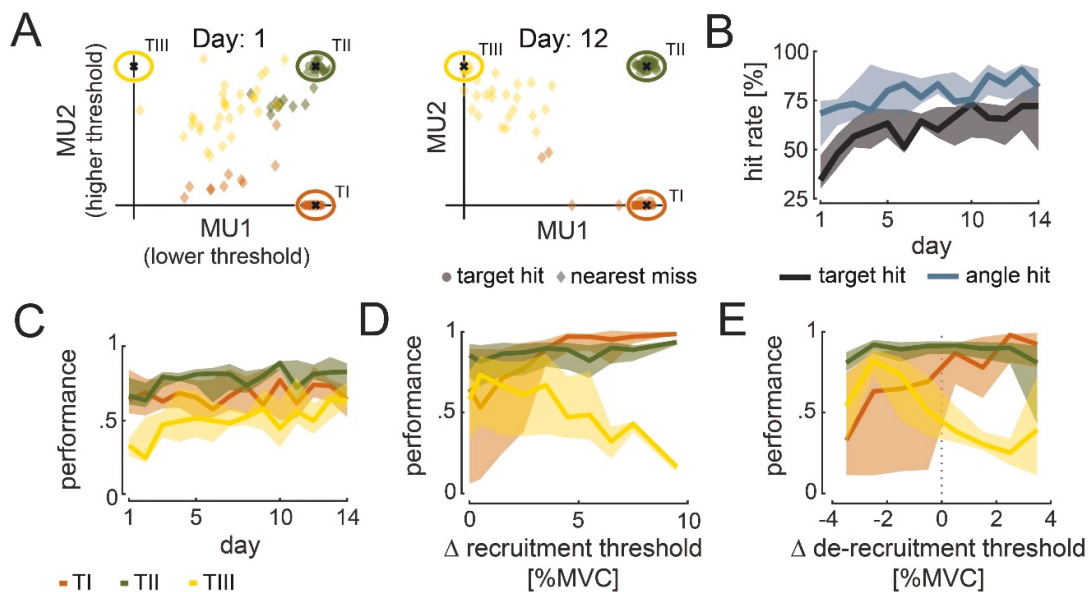


Figure 4-3: Cursor movement and performance during target task. A: Average cursor position during target hits (circle) and nearest misses (diamond) across conditions are shown for the first and twelfth day of experiments for TI (orange), TII (green), and TIII (yellow) of one subject. B: Target (black) and angle hit rate (blue) across subjects, conditions and targets-of-interest are shown with their medians (solid line) and 25% and 75% quartiles (shaded areas) across days. C: Performance values across subjects and conditions for TI (orange), TII (green), and TIII (yellow) are shown with their medians (solid line) and 25% and 75% quartiles (shaded areas) across days. D & E: Performance values corresponding to the difference in recruitment threshold (D) and de-recruitment threshold (E) between MU2 and MU1 are shown across subjects and conditions for the last five days of training. The median (solid line) and 25% and 75% quartiles (shaded area) for TI (orange), TII (green), and TIII (yellow) are illustrated in steps of 1% MVC.

The performance of reaching each target did not solely depend on the target's position but also on the difference in recruitment and de-recruitment threshold within the selected MU pair, i.e. the force difference between the onset and offset of activity between MU2 and

MU1 measured during the initial force task. Figure 4-3D and E illustrate the performance over the within-pair difference in recruitment and de-recruitment threshold, respectively, for each target-of-interest. Performance per subject in reaching TI significantly increased from selected MU pairs with a small difference in recruitment threshold (0-1% MVC) to those with a high difference (3-10% MVC) from $.61 \pm .16$ to $.90 \pm .05$ (two-sided Wilcoxon signed rank test, $p = .016$). To reach TI, only MU1 must be active. Therefore, this result indicates that subjects performed better in keeping only MU1 active while not activating MU2 when the difference in their recruitment threshold was high. This may be due to less accuracy needed in the force generated when the recruitment threshold difference is large. For example, if MU1 gets recruited at 2% MVC while MU2 at 5% MVC, the subject could potentially exert any force between 2 and 5% MVC to keep only MU1 active without MU2 to ultimately hit TI. A more precise force level needs to be generated when this difference is smaller. For TII, no dependency in performance and the within-pair difference in recruitment threshold was detected (from $.79 \pm .07$ to $.72 \pm .08$, two-sided Wilcoxon signed rank test, $p = .578$). On the contrary, the performance in reaching TIII decreased significantly for larger differences in recruitment threshold within the selected MU pair from $.66 \pm .09$ to $.55 \pm .04$ (two-sided Wilcoxon signed rank test, $p = .016$). This indicates that subjects experienced difficulties in keeping MU2 active while MU1 was inactive in order to reach TIII when their difference in recruitment threshold was relatively large (3 – 10% MVC). The difference in recruitment threshold between MU1 and MU2 correlated with the difference in de-recruitment threshold (Spearman correlation coefficient, $R = .46$, $p = .001$; see Supplement 4-2A). Thus, similar implications on the relationship between performance in reaching the targets and the difference in de-recruitment threshold between MU1 and MU2 can be drawn (see Figure 4-3E). Subjects became better in reaching TI for a positive difference in de-recruitment

thresholds (i.e. MU2 is de-recruited before MU1; $.76 \pm .05$) than for negative ones (i.e. MU2 is de-recruited after MU1; $.44 \pm .11$; all four subjects for whom the de-recruitment threshold was recorded increased in their performance from negative to positive). When subjects had difficulties in activating MU1 without activating MU2 to reach TI (for example, when the recruitment threshold of both MUs is similar, see above), they usually tried to switch off MU2 while keeping MU1 active to reach again towards TI. However, if MU2 was de-recruited after MU1, the subjects needed to move back to the coordinate origin to switch off MU2 before they could start a new attempt in reaching towards TI. This required additional time, resulting in lower performance. However, when MU2 was de-recruited before MU1, the subjects only needed to switch off MU2 (while MU1 remained active) before reaching TI again. For TII, no dependency between difference in de-recruitment threshold and performance was observed (from $.83 \pm .05$ to $.83 \pm .04$; the performance never varied more than .02 from a negative to a positive difference in de-recruitment threshold per subject). When asked to reach TIII, subjects decreased in performance from $.57 \pm .04$ to $.40 \pm .02$ for negative and positive differences in the de-recruitment threshold, respectively. The better performance for negative differences in de-recruitment thresholds may be explained by a possible strategy to reach TIII by leveraging the lower de-recruitment threshold of MU2 compared to MU1. Because of this, subjects could activate both MUs first before lowering the force level to switch off MU1 while MU2 remained active due to its lower de-recruitment threshold. This would result in cursor movement towards TIII.

During the last five days of training, the majority subjects reported that they felt having control over MU2 when reaching towards TIII (14% of cases subjects indicated having no control over MU2; see Supplement 4-2D). Moreover, they declared the usage of a clear strategy to establish such control, which varied in cognitive demand across subjects and days.

In 95% of all cases, this control strategy was described as a rapid increase in force due to dorsiflexion of the ankle followed by a slow release until the cursor moves towards the vertical axis.

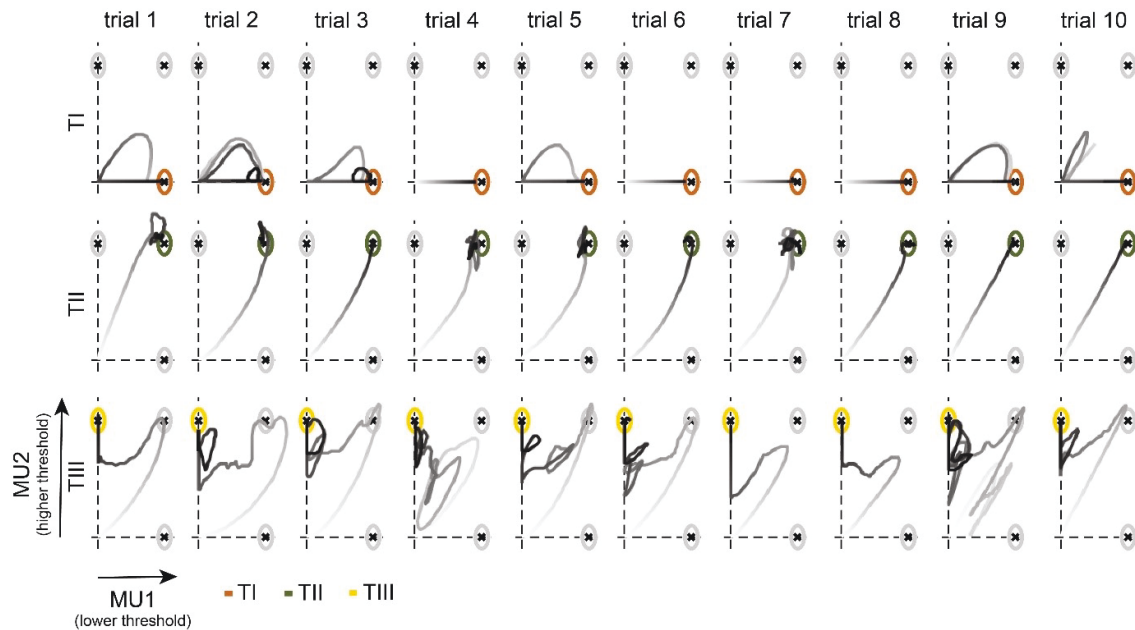


Figure 4-4: Cursor trajectories of one subject during the target task. Cursor movement towards TI (orange, top), TII (green, centre), and TIII (yellow, bottom) in each trial for Condition I on the 14th day of one representative subject is shown. Trial 1 to trial 10 indicate the first to the tenth appearance of each target-of-interest. The grey intensity of the cursor trajectories increases over time within the trial.

All subjects improved their performance during training. To better understand which strategies have emerged, ultimately enabling subjects to recruit and de-recruit single MUs, we analysed the cursor trajectories during the task. The cursor movement for one subject during the last day of training (Condition I) for all 30 trials is visualised in Figure 4-4. While the subject was able to hit all targets before the trials ended, the cursor trajectories did not always mimic the straight path, to the target centre. When asked to move towards TI, the subject moved the cursor along the horizontal axis. In trials 1, 2, 3, 5, 9, and 10, the subject could not hit TI in the first attempt but returned to the origin to then move towards the target centre directly. For TII, in all cases, the subject moved directly along the diagonal towards the target-

of-interest. For TIII, however, instead of moving directly towards the target centre, the subject moved the cursor towards TII first and then towards the vertical axis to finally hit TIII. This observation is in line with the descriptions provided by the questionnaire (see Figure 4-3E), i.e. increase in force to activate both MUs, followed by a decrease in the force until MU1 switches off, and ultimately adjusting the force level to move along the vertical axis towards the target centre.

By analysing the unintended target and angle hits (see Figure 4-5A), the probability that the cursor was moved towards unselected targets while trying to hit the target-of-interest was quantified.

While only a few unintended hits occurred across targets and conditions, unintended angle hits of TII happened multiple times when subjects tried to reach for TIII. In fact, across all conditions, almost all subjects conducted most of their unintended hits when aiming for TIII (with unintended hits in TII). Only one subjects had a higher rate of unintended hits when aiming for TII (with unintended hits in TI) in Condition I. Therefore, the strategy to reach TIII, i.e. moving towards TII first, as illustrated in Figure 4-4 and interpreted by the questionnaire answers, can be observed across subjects. Moreover, the few unintended hits when reaching towards TI and TII suggest that subjects established control strategies that allowed for a direct movement towards the target-of-interest. These clear control strategies, as well as the difference in learning rate across targets, suggest that subjects were able to activate MU1 alone (to reach TI), MU1 and MU2 together (to reach TII) but could not volitionally activate MU2 before MU1 (TIII). In fact, during the last five days of training, 76%, 78%, and 94% of all successful attempts, i.e. at least an angle hit, of going towards TI were achieved without activating MU2 once during the trial while it was only 7%, 2%, and 1% for TIII (MU2 only without MU1) in Condition I, II, and III, respectively. Such rare and occasional deviation from

the normal recruitment of two MUs was already observed in one of the first studies employing single MU biofeedback (Henneman & Mendell 1981). The percentage of these *direct movements* towards TI and TIII with respect to the difference in recruitment threshold within the selected pair and subjects is shown in Figure 4-5B. While direct movements towards TI increased with a larger difference in recruitment threshold, direct movements towards TIII were very rare and only possible with MUs recruited at very similar force levels, i.e. with small differences in recruitment thresholds that led to variable recruitment orders given sudden excitatory inputs at the beginning of the trials. Also, all subjects moved directly towards TI in more than 70% of all successful attempts. Although, only three subjects navigated the cursor directly towards TIII in more than 5% of all successful attempts (see Figure 4-5C). Furthermore, 8.33% of these direct movements towards TIII occurred after TI-instructed trials. In the remaining 91.67% of the trials, direct movements towards TIII occurred after TII- or TIII-instructed trials, which required the activation of MU2 to reach the target. In contrast, direct movements towards TI occurred in 25.93% after TIII-instructed trials or even as the first trial in the session.

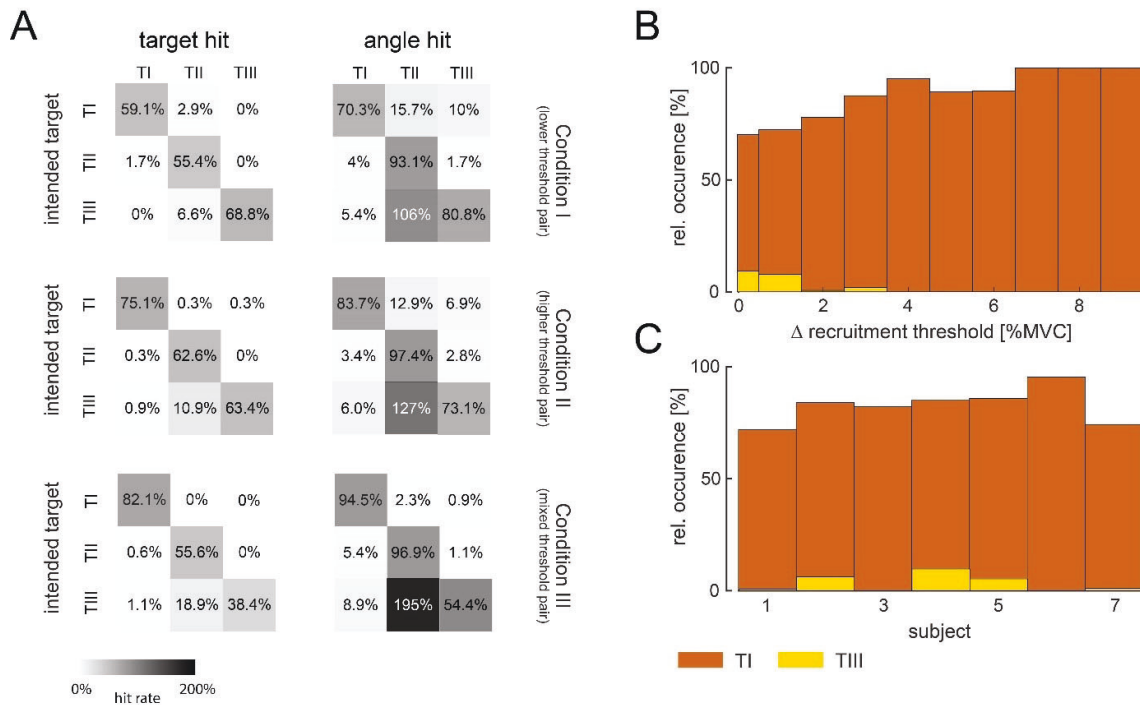


Figure 4-5: Movement towards targets-of-interest. A: Relative hit rate of intended and unintended hits (grey) of targets and angles are shown for the targets-of-interest T1, TII, and TIII during the last five days of training across subjects for Condition I (top), Condition II (centre) and Condition III (bottom). Please note that hit rates above 100% can be reached for unintended hits when subjects re-entered the target before the trial ended. Colour intensity corresponds to the hit rate. Relative occurrence of direct movement towards T1 (orange), i.e. only activating MU1 without MU2, and TIII (yellow), i.e. only activating MU2 without MU1, during successful attempts (at least angle hits), are shown with respect to the difference in recruitment threshold between MU2 and MU1 (B) and across subjects (C).

In these rare cases in which direct movements towards TIII ended, at least, in an angle hit, the level of force and global EMG of tibialis anterior, fibularis longus, and medial and lateral head of the gastrocnemius at the time point of recruitment of MU2 was compared with the corresponding values obtained at normal recruitment of MU2 during the initial force ramps. During direct movement towards TIII, MU2 was recruited on average at a $48.43 \pm 22.46\%$ lower force level than during ramp recruitment. Also, the global EMG values at recruitment of MU2 were, on average, slightly lower during direct movements towards TIII (tibialis anterior: $-15.91 \pm 9.67\%$; fibularis longus: $-18.75 \pm 23.27\%$; gastrocnemius lateral: $-9.99 \pm 31.53\%$; gastrocnemius medial: $-0.02 \pm 27.31\%$). However, in the vast majority of

cases, target hits of TIII were not achieved by direct movements towards the target centre. Instead, subjects used a three-stage approach to place the cursor inside TIII, as observed in Figure 4-4 (for example, TIII, trial 7). First, subjects navigated the cursor along the diagonal towards TII (stage one) before in the second stage moving towards the vertical axis. In the third stage, subjects manoeuvred the cursor along the vertical axis inside TIII. The discharge rate of MU1 and MU2 and the exerted force during indirect movement towards TIII are shown for a representative subject in Figure 4-6A (more single-trial examples for TIII-instructed trials are shown in Supplement 4-3). During the first stage, the subject increased the force to orderly recruit MU1 and MU2. Once both MUs were active, the subject decreased the force level in stage two to a minimum so that MU1 stopped firing while keeping MU2 active. In the third stage, the force was slightly increased to match the necessary discharge rate of MU2 to reach TIII without re-activating MU1. To assess whether such force modulation during indirect hits of TIII could be observed across subjects and conditions, characteristic forces (due to ankle dorsiflexion) for each stage were compared in Figure 4-6B. The characteristic force during stage one was the mean force in a 100ms window around the maximum force when both MUs were active, i.e. discharge rate greater than 5pps. In stage two, the characteristic force was estimated by averaging the force inside a 100ms window at the minimum force level after switching off MU1 while MU2 continued firing action potentials. In stage three, the characteristic force was set as the mean force during the hold period preceding a target hit of TIII. Across conditions, all subjects used significantly different force levels during each stage (Friedman, $p < .001$, two-sided Wilcoxon signed rank test Bonferroni corrected, always $p < .05$). During stage one, the exerted force level was the greatest while being reduced to a minimum in stage two, before being slightly increased again in stage three. Furthermore, for the four subjects for whom the de-recruitment threshold was determined, 24.31% of all

indirect target hits of TIII were achieved while MU2 was de-recruited before MU1 during the initial force ramps. This indicates that this three-stage approach also worked for pairs of MUs for which the de-recruitment threshold was not reversed to the recruitment one (see Supplement 4-3C and D).

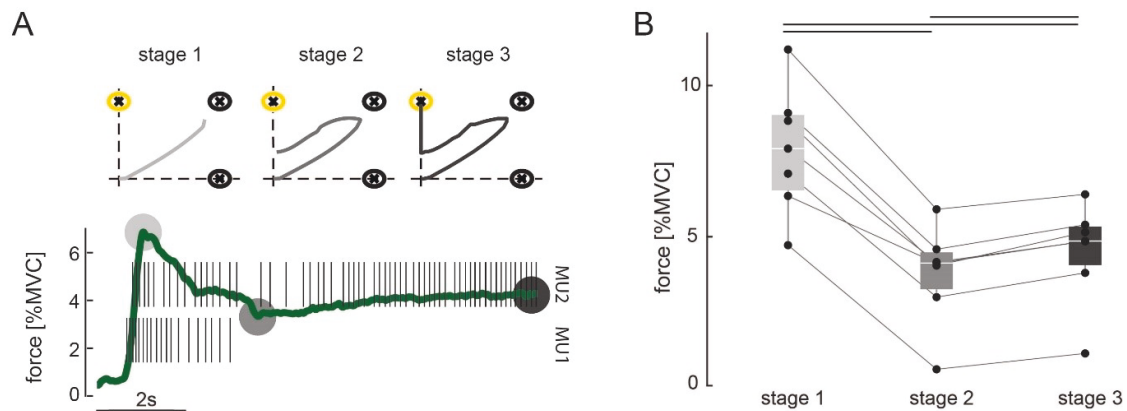


Figure 4-6: Three-stage approach to hit TIII. A: Force due to ankle dorsiflexion (green) and the discharge behaviour of the selected MU pair during a successful attempt of hitting TIII (yellow) for a representative subject. The subject used a three-stage approach to achieve the target task: stage 1: increasing the force to orderly recruit both MUs; stage 2: reducing the force until MU1 stops firing while the cursor is placed along the vertical axis; stage 3: slightly increasing the force again to manoeuvre the cursor inside TIII. Grey circles mark the characteristic force values of each stage. Stage 1: maximum force while both MUs are active; stage 2: minimum force after MU1 stopped firing; stage 3: force during hit of TIII. The corresponding cursor movement for each stage is shown on the top. Grey intensity increases with stages. B: Characteristic forces (due to ankle dorsiflexion) are shown with their median and quartiles at each stage of control for all subjects across all conditions during all TIII hits in the last five days of training. Each dot represents a subject, and corresponding values are connected via the lines. Black bars indicate a significant difference with $p < .05$.

The force, global tibialis anterior, fibularis longus, and medial and lateral head of the gastrocnemius EMG values in 100ms window centred around the onset of MU activity were compared before and after the experiment to investigate potential changes in the recruitment order due to single MU modulation. A subtle decrease in force ($-.18 \pm 2.33\%$ MVC, neither did the de-recruitment force change critically: $-.16 \pm 2.28\%$ MVC) and global tibialis anterior EMG ($-2.35 \pm 22.62\%$) were identified. However, the global EMG from the lower leg muscles not used for the MU decomposition increased slightly (fibularis longus: $5.45 \pm$

28.58%; gastrocnemius lateral: $14.12 \pm 37.53\%$; gastrocnemius medial: $18.41 \pm 26.51\%$).

These changes indicate that the overall recruitment order did not change critically due to the imposed single MU modulation. The increase in activity in the lower leg muscles not directly involved in ankle dorsiflexion relative to the agonist muscle might be explained by induced fatigue towards the end of the experiment (Patikas *et al.* 2002).

4.3.2 Validation of online EMG decomposition during recruitment/de-recruitment

Surface EMG decomposition with methods similar to those used in this study has been previously extensively validated, including by comparison with intramuscular EMG decomposition (Barsakcioglu *et al.* 2021). However, this study involved recruitment and de-recruitment of MUs and the accuracy in detecting the recruitment and de-recruitment times was of crucial importance for interpreting the results. Therefore, a validation study was performed in which the onset and offset of MU activity detected from the surface EMG was compared with the corresponding MU activity detected from concurrent intramuscular recordings (see 4.2.6). Figure 4-7 shows an example of such a comparison. In this example, one MU was commonly detected by the (online) surface and intramuscular EMG decomposition. The rate of agreement over the full interval of activity of the MU in this representative case was >94%, which is similar to previous validation results obtained in stable-force conditions (Barsakcioglu *et al.* 2021). From Figure 4-7, it can be noted that there were no errors in the first discharges at recruitment and in the last discharges at de-recruitment, therefore onset and offset of this MU activity were detected highly accurately. In this example, the rate of agreement was also computed separately for the different phases of the contraction (ramp-up, constant force, ramp-down). The ramp-up phase of this MU

activity lasted between 12.1 s to 15.0 s of the contraction time and in this phase the rate of agreement was 91.7%. The constant-force phase of the contraction for this MU lasted from 15.0 s to 35.0 s, during which the rate of agreement was 94.3%, and the ramp-down phase lasted between 35.0 s to 43.2 s with an estimated accuracy (rate of agreement) of 96.1%.

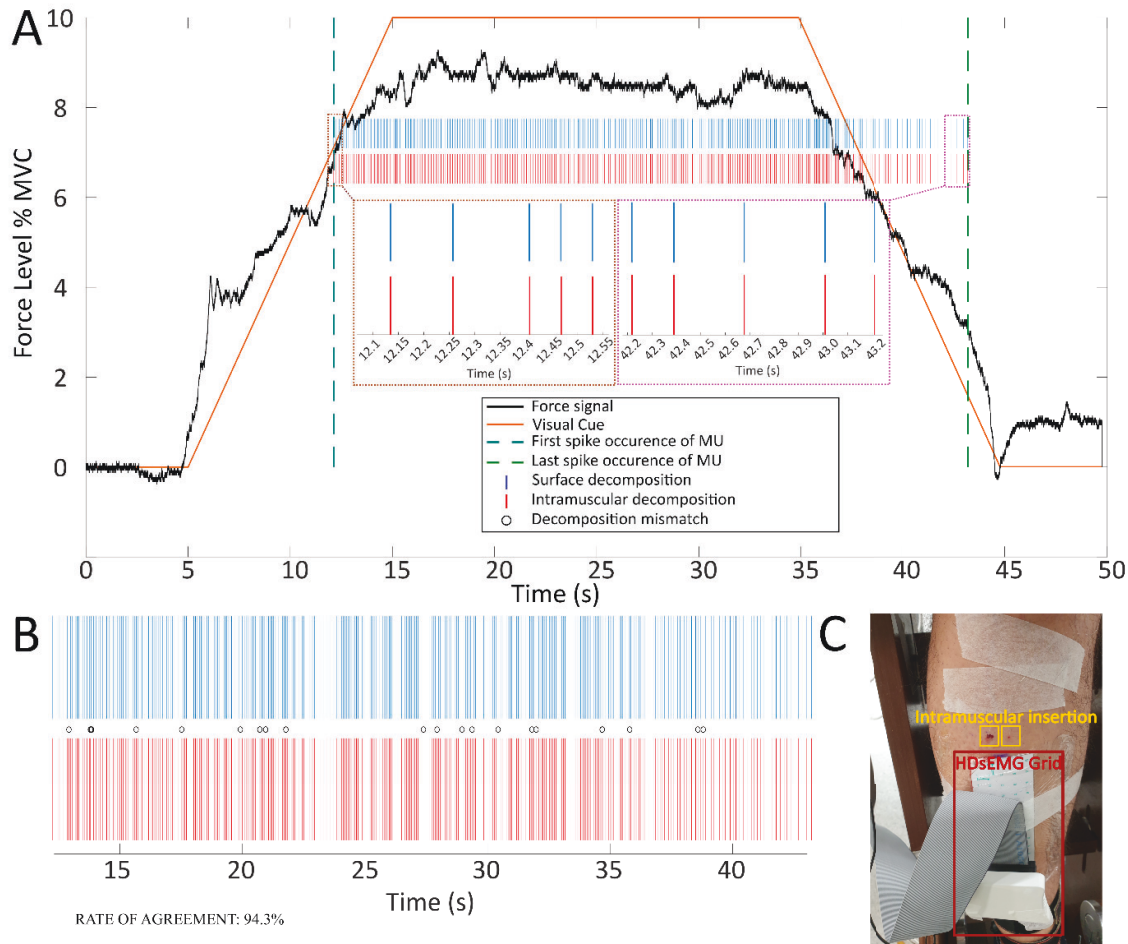


Figure 4-7 An example two-source validation of surface and intramuscular recorded EMG signals during recruitment/de-recruitment. A: The visual cue provided to the user during the trial (orange solid line), the force feedback on the ankle dorsiflexion (black solid line), the first and last occurrence of MU firing (teal and green dashed lines respectively) and two subpanels showing the first and last five MU discharges identified for intramuscular and surface decomposed signals (brown and magenta subpanels). B: The decomposed MU spike train from surface EMG (blue) and intramuscular EMG (red), as well as decomposition mismatches identified (black circles). C: The experimental setup for concurrent intramuscular and surface EMG recordings.

The analysis of the other contractions recorded for validation led to results similar as those presented representatively in Figure 4-2. As in previous validation studies using

intramuscular EMG as reference, the number of MUs commonly detected by the surface and intramuscular EMG was very small (range 0-1 per contraction). This is due to the different detection volume of the two recording methods, as previously discussed (Barsakcioglu *et al.* 2021), and is in agreement with previous validation studies done at constant force. Out of the 36 contractions during which validation signals were recorded (3 subjects, 6 slopes, 2 trials per slope), common MUs were detected in only 16 contractions. In all cases in which common MUs were detected the rate of agreement was >90% and the errors in decomposition were equally distributed in all phases of the contractions (on average, $93.2 \pm 1.3\%$, $92.6 \pm 1.5\%$, and $95.5 \pm 2.0\%$ for the three phases of the contraction, respectively), without any preferential distribution around the intervals of recruitment and de-recruitment. Notably, we never observed an error in the first discharge (onset) or the last discharge (offset), meaning that the recruitment and de-recruitment times were detected by the online decomposition with an accuracy of 1ms (margin used in the time alignment between discharge times detected by the surface and intramuscular decomposition).

From the validation tests, we concluded that the online surface EMG decomposition in variable force contractions with recruitment and de-recruitment achieved similar performance as the decomposition in static contractions.

4.4 Discussion

Volitional and flexible control of single MUs could revolutionise neural-interface applications. Here we used real-time biofeedback on single MU activity to encourage subjects to learn independent control of pairs of MUs. Our results showed that subjects could gain control over four MUs from a single muscle. The control strategies that emerged, allowing for

selective MU control, were limited by the presence of a common input to the MU pool. Therefore, subjects did not exploit potential neural structures with selective inputs to individual MUs.

In this study, the identified MU pools were ranked based on their recruitment order. We have shown that the de-recruitment threshold does not always match the recruitment one. While small deviations between recruitment and de-recruitment threshold may be explained by a slight increase in co-contraction during the declining force ramp (De Luca & Mambrito 1987), larger mismatches might be due to intrinsic neuro-modulatory and inhibitory mechanisms. The latter are triggered following the activation of a MU, which can disrupt the simple dependency of recruitment order from neuron size and input received (Heckman & Enoka 2012). One potential mechanism contributing to this neuromodulation in motor neurons are persistent inward currents (PICs) (Binder *et al.* 2020). PICs, i.e. descending neuromodulation from the brainstem to the spinal cord, can alter the excitability of motor neurons which may lead to a self-sustained firing of action potentials and thus to de-recruitment at a force level lower than the recruitment threshold, i.e. recruitment de-recruitment hysteresis (Binder *et al.* 2020). In this study, we examined the behaviour of pairs of MUs at very low forces, i.e. less than 10% MVC. These low-threshold MUs active at such force levels may be more prone to the effect of sustained PICs (Heckman *et al.* 2008b), which might explain the hysteresis between recruitment and de-recruitment threshold observed for most MUs examined in this experiment.

An inhibitory input is needed to extinguish the impact of PICs on the MU discharge behaviour (Heckman *et al.* 2005). Such an inhibitory signal leads to the reversal of MU activity to the initial state, once a MU stops firing for a prolonged amount of time. This explains why MU recruitment was not critically altered even after extensive single MU control during this

experiment. The very rare cases in which an activation of the higher threshold MU before the lower threshold one occurred may be explained by an incomplete extinction of the PIC effect since in the vast majority of these cases (>90%) the preceding trial required an activation of MU2.

PICs act diffuse across the entire MU pool (Johnson & Heckman 2014). Therefore, the excitability of MU1 and MU2 should be affected similarly by this neuromodulatory mechanism. However, it may be possible that the broad influence of PICs is focalised by local inhibition, which allows suppression of the PIC effect on selective MU pools and sub-pools (Heckman *et al.* 2008a). Inhibitory signals to motor neurons can be facilitated via various mechanisms, including descending pathways, spinal circuitries or pre-synaptic inhibition (Heckman *et al.* 2009). One intensively studied form of inhibition directly influencing PICs is reciprocal inhibition (Heckman *et al.* 2008a; Hultborn *et al.* 2004; Johnson & Heckman 2014), triggered by changes in the length of antagonist muscles, which can occur, for example, by moderately altering joint angles (Hynstrom *et al.* 2007). Further, descending inputs may additionally tune interneurons mediating reciprocal inhibition and thus also influence PICs and the excitability of single motor neurons (Jankowska 1992). Synaptic inhibition received by motor neurons follows the size-principle. Hence, smaller motor neurons tend to show a greater hyperpolarisation than larger ones for the same inhibitory input (Heckman & Binder 1988; Henneman *et al.* 1965). Consequently, a higher threshold MU may continue firing after a smaller threshold MU already became silent. In our experiment, we did not measure PICs nor inhibitory inputs to MUs. Hence, we cannot pinpoint the exact mechanisms explored by subjects to generate the necessary activation patterns to move the cursor towards TIII. However, we consider that it is likely that a mixture of broadly distributed neuromodulatory mechanisms, such as PICs, and locally acting inhibition may have produced the variability in

MU activity that was observed in this study. Similar findings on the increasing variability in MU activation by incorporating neuromodulation and inhibition have been shown in a simulation study (Powers *et al.* 2012).

During a progressive increase in force, recruitment depends only on the MU anatomy and the input received. If humans can learn to leverage potential structures in the CNS that allow selective inputs to MUs (Marshall *et al.* 2021), changes in the recruitment order during this initial phase should be expected. However, it is important to underline that a conclusion of flexible control based on changes in MU recruitment cannot be drawn for time intervals that follow an activation of the MU. In these cases, the de-recruitment of a MU at a force level different from the recruitment threshold could be incorrectly interpreted as an alteration of the recruitment order. Presumably, such changes result from the relative intrinsic excitability of the motor neurons which override the sole impact of the received synaptic input on the recruitment order. Therefore, a direct proof of altered MU recruitment as a consequence of independent input to different MUs needs to be provided during the initial activation phase, i.e. a MU with higher recruitment threshold activated before a MU with lower threshold without preceding activations. It is also worth mentioning that this proof should further include MUs with sufficiently different recruitment thresholds since synaptic noise may influence the relative recruitment order for MUs of very similar thresholds (Heckman & Enoka 2012).

We did not get results supporting a *general* flexible control of MUs, i.e. volitional activation of higher threshold MUs before lower threshold ones at initial recruitment. However, flexible control of individual MUs could still be a framework explaining how subjects were able to reach the different targets in the 2D space. If this was the case then, since control

would be achieved only after MUs were recruited, this would imply that flexible control is state-dependent: it can only be achieved in the context of previously contracted muscle fibres. Such state-dependency restricts the possible neural strategies that could allow flexible control of MUs. One possible strategy consistent with such state-dependent control of individual MUs could be relying on an input signal to MUs not directly linked to motor function and non-homogeneously distributed among the MU pool. For example, cortical oscillations could meet these criteria if descending projections of this activity to large and small MUs in a pool differed (Bräcklein *et al.* 2021, 2022b; Ibáñez *et al.* 2021). A different alternative that may allow for flexible MU control after initial recruitment is volitional modulation of reciprocal inhibition (Thompson *et al.* 2013; Xiang *et al.* 2006) presumably via direct descending commands (Jankowska 1992; Nielsen *et al.* 1995). Such augmentation of synaptic inhibition could potentially lead to changes in the excitability of specific MUs. Such inputs to MUs could provide a certain degree of flexibility to control subgroups of MUs in a muscle volitionally. Future studies are needed to test this hypothesis.

Throughout the 14 days of training, subjects were asked to modulate the discharge rate of MU pairs independently to navigate a cursor as quickly as possible into different targets inside a 2D space. The target and angle hit rates indicate that subjects could achieve control over these single MUs. Moreover, the angle hit analysis (see Figure 4-5A, for example) revealed that during the last five days of training, subjects were able to produce the necessary activation pattern in most trials despite different properties of the selected MUs. Although subjects repeated the target-tracking task with different sets of MUs every day, they consistently reported the use of the same control strategies across days. Hence, these findings suggest that subjects learned to establish universal control strategies that allowed for the achievement of the target task for various combinations of MUs. To reach T1 or T11,

subjects used precise force control to exert either a low enough force that only the low threshold MU (MU1) turned active (TI) or a force above the recruitment threshold of both MU1 and MU2 (TII). To reach TII, subjects used strategies that corresponded to a physiological activation of the two MUs similar to the initial force ramp.

As could be expected, subjects had difficulties in navigating the cursor towards TI when the difference in recruitment threshold between MU1 and MU2 was small ($\leq 1\%$ MVC; see Figure 4-3D). In these cases, subjects could not selectively activate MU1 while keeping MU2 deactivated and still reach a discharge rate of MU1 high enough to place the cursor near the centre of TI. When the difference in recruitment threshold was larger ($\geq 3\%$ MVC), the discharge rate of MU1 reached the desired value before the onset of MU2. It seems the discharge rate of MU1 saturated (Fuglevand *et al.* 1993) due to its intrinsic properties, as recently discussed in (Fuglevand *et al.* 2015). Unlike for TI and TII, TIII required a more cumbersome approach. To reach TIII, subjects mainly mimicked the trajectory of TII first, i.e. activating both MUs, followed by the second stage of control in which the force level was reduced until the lower threshold MU turned off by leveraging the mismatch in the de-recruitment thresholds. In order to then place and hold the cursor inside TIII, subjects gradually increased the force again without re-activating MU1. This second and third stage of control were possible in principle by maintaining a common ionotropic input to the MU pair combined with neuromodulatory input and synaptic inhibition, as described above, even when MU2 was initially de-recruited before MU1. If a direct activation of MU2 would have been possible as it was for MU1, subjects would have chosen to mimic a cursor trajectory along the direct path from the origin to the target centre, as observed for both TI and TII (see Figure 4-4). This almost never occurred in the hundreds of trials tested. Therefore, the sole activation of a higher threshold MU was only possible by exploiting the history-dependent

activation of MUs, i.e. exclusive firing of MU2 follows the combined activation of MU1 and MU2. The results suggest that this three-stage approach to achieve a hit in TIII as quickly as possible was feasible for the subjects while a more efficient strategy of directly activating MU2 without a preceding activation of MU1 was not.

It has been previously shown that subjects can learn to control MUs independently when exposed to biofeedback on the discharge behaviour (Basmajian 1963; Formento *et al.* 2021). In these previous investigations, the subjects were allowed for contractions along multiple directions. Such variations in force directions (Desmedt & Godaux 1981; ter Haar Romeny *et al.* 1984) but also other motor behavioural changes, including alternations in postures (Nardone *et al.* 1989), and contraction speed (Desmedt & Godaux 1977b), potentially have an impact on the recruitment order. Similar changes in a MU pool's discharge activity imposed by such behavioural changes were recently suggested in non-human primates (Marshall *et al.* 2021). Therefore, individual MU control may be triggered by small compensatory movements rather than being the result of a dedicated and volitionally controllable individual synaptic inputs. Indeed, independent control of individual MUs would imply that a MU can be controlled independently *of all other MUs*. The fact that a pair of MUs can be controlled independently when varying the task does not imply that the two MUs are independently controlled in absolute terms. They are simply independently controlled with respect to each other. For example, in some tasks or in some conditions, they may be part of different groups of MUs receiving two different common inputs (Tanzarella *et al.* 2021).

A recent study in humans provided evidence for the existence of MU pool synergies similar to the functional grouping of muscles involved in a single movement (Tanzarella *et al.* 2021). The CNS may send a common input to these MU pool synergies, which are not per se limited to innervating only a single muscle (Laine *et al.* 2015). In our experiment, we chose a simple case

of a MU pool constituting a functional group during ankle dorsiflexion, i.e. MUs innervating the TA. During more complex tasks, for example, movement along multiple directions, the CNS would send different common inputs to certain numbers of groups of MUs. While the synergistic organisation of MUs might be flexible across tasks, e.g. movement along multiple directions, it remains yet to be explored if the input to a single functional MU group can be changed volitionally from common to individual while the performed task is maintained. Hence, it is crucial that initial conditions during MU recruitment, such as posture, contraction speed, and force direction, are kept constant when flexible MU recruitment is investigated. MU activation based on changes in behaviour, or the performed motor task, does not indicate that subjects can volitionally trigger MU activity by a selective synaptic input as it is possible for their cortical counterparts. Furthermore, these constraints need to be considered in neural-machine interface applications relying on flexible MU control. Possible extracted control signals may depend on behavioural changes and not on a designated descending control command, and therefore, may not satisfy the intended application. Similar constraints effectively apply for augmentation when the aim is to extend the degrees-of-freedom that a human can volitional control, i.e. adding supernumerary degrees-of-freedom to the natural ones (Dominijanni *et al.* 2021; Eden *et al.* 2022). In such cases, if the control of a supernumerary degree-of-freedom is based on single MU activation, this activation must be uncoupled from motor behaviour to ensure coordination between natural and supernumerary effectors. This would correspond to breaking common input into multiple inputs. Nevertheless, even based on behavioural changes, single MU control can be a resource for specific neural-interface applications, for example, in the absence of any additional motor information or to augment a specific motor task (see (Eden *et al.* 2022) for *augmentation by transfer*).

Previous studies investigating flexible MU control focused on upper-limb muscles (Basmajian 1963; Formento *et al.* 2021; Marshall *et al.* 2021). Here, we extracted single MU activity from the tibialis anterior since this muscle has properties (e.g., muscle fibres arrangement, proximity to the skin, distribution of innervation zones, etc.) that facilitate a reliable decomposition of MU activity using surface recordings (Barsakcioglu *et al.* 2021; Bräcklein *et al.* 2021, 2022b; Del Vecchio *et al.* 2020; Dideriksen *et al.* 2018; Negro *et al.* 2016b) and high corticomuscular coherence and the latency of the corticomuscular transmission suggests a number of monosynaptic connections with the motor cortex that could potentially be leveraged for direct independent MU control (Dideriksen *et al.* 2018; Ibáñez *et al.* 2021). Our results strongly suggest that subjects do not tend to find or opt for a control strategy that relies on flexible MU recruitment order under constraint isometric conditions. In contrast, the established control strategy seemed to be based on a common synaptic input to the MU pair combined with intrinsic changes of MU excitability due to neuromodulation and inhibition. Although the complexity of tasks in which humans use upper and lower limbs may differ, we are not aware of any evidence suggesting that the CNS employs fundamentally different strategies to orchestrate MU activity in a functional motor neuron pool differently across limbs. Future research on this topic may help to better understand if the CNS may allow for variability in upper-limb MU pools.

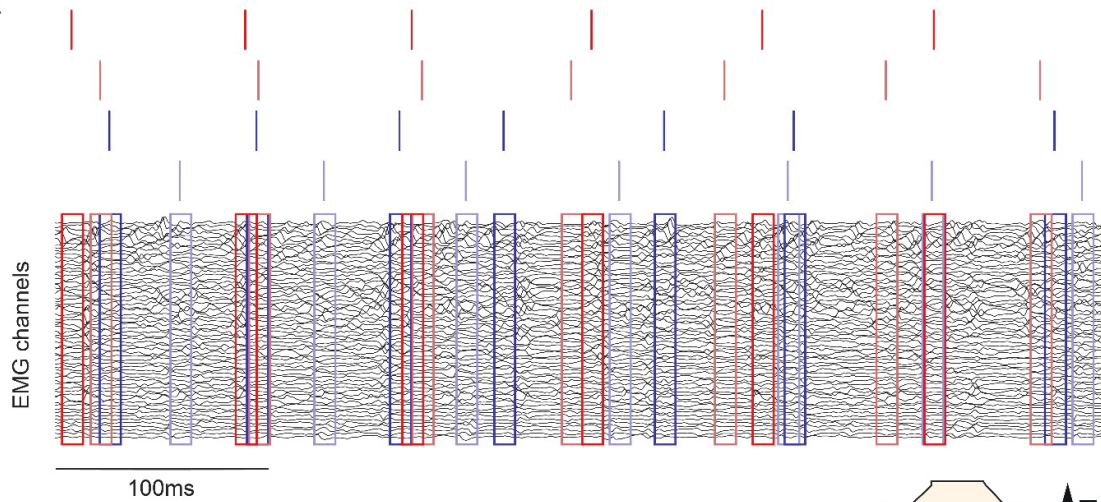
Throughout the experiment, subjects were restricted by an ankle dynamometer and instructed to perform dorsiflexion of the ankle only. We measured force due to dorsiflexion but not, for example, rotational forces due to ankle supination. Modest rotations of the ankle can increase the effect of inhibition in specific MUs and thus might affect their excitability (Hyngstrom *et al.* 2007). Such effects were only observed for modest changes in joint angles ($\sim 20^\circ$) (Hyngstrom *et al.* 2007), which were not possible to perform when the dynamometer

restricted the foot. It may be that even very subtle rotations or slight movements of other body parts, such as the knee or hip, may have influenced the discharge activity of specific MUs (Jankowska 1992), potentially a helpful approach for achieving TIII-instructed trials. However, none of the subjects reported the use of compensatory movements as a control strategy. Furthermore, even if the subjects unknowingly performed very subtle compensatory movements in TIII-instructed trials, they would have discovered and chosen this strategy over directly utilizing potential descending pathways triggering independent MU control.

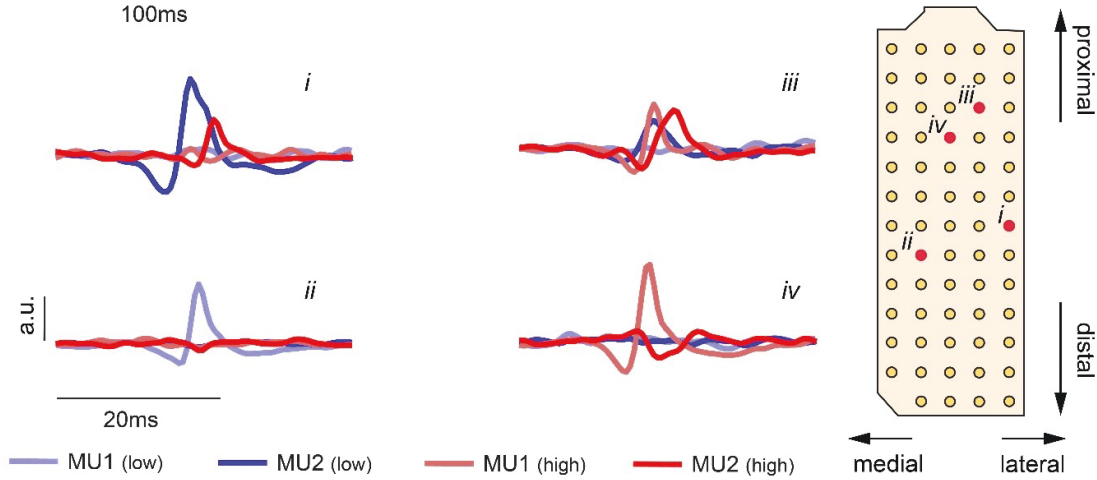
To summarize, we have demonstrated the ability to control up to four MUs from a single muscle using real-time feedback on single MU discharge behaviour. Furthermore, we have shown by operant conditioning that subjects learn concrete control strategies to recruit and de-recruit several MUs volitionally. These strategies exploit orderly recruitment in agreement with the *Henneman's size principle* and a common input to their motor neurons. Conversely, the observed strategies do not leverage potential pathways that may provide selective inputs to single MUs. It is concluded that converting common input to a (synergistic) pool of motor neurons into independent input to single MUs within the same task seems extremely challenging for the CNS.

4.5 Supplementary Material

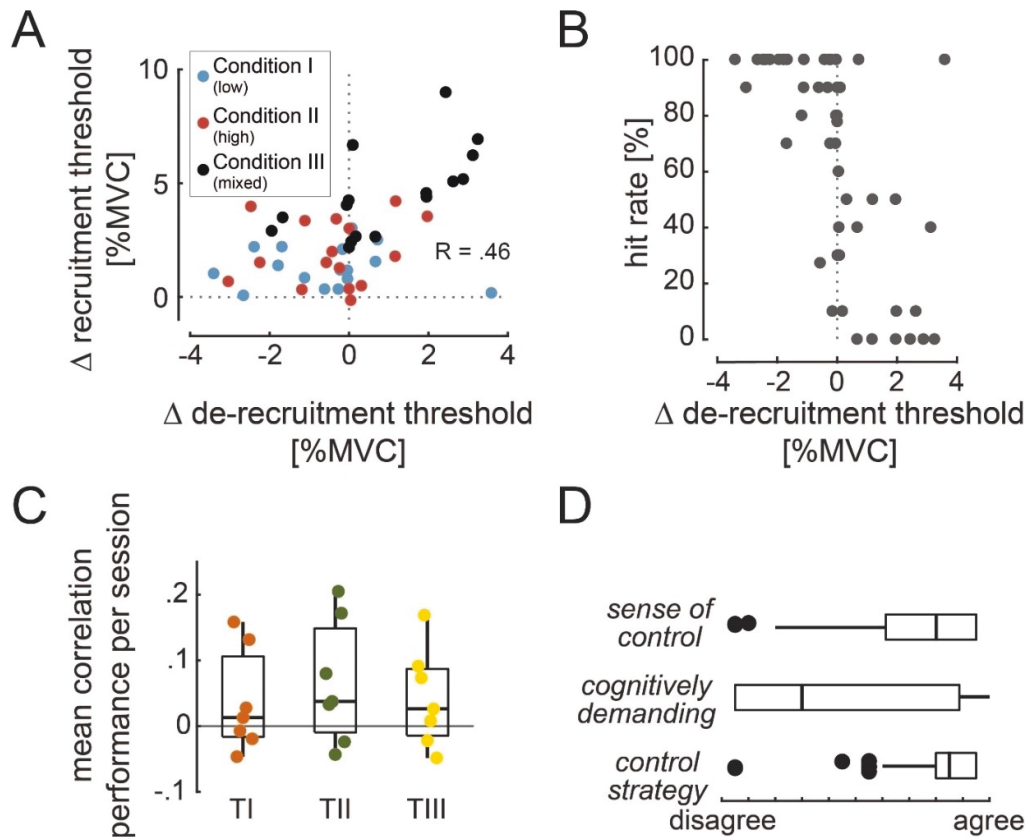
A



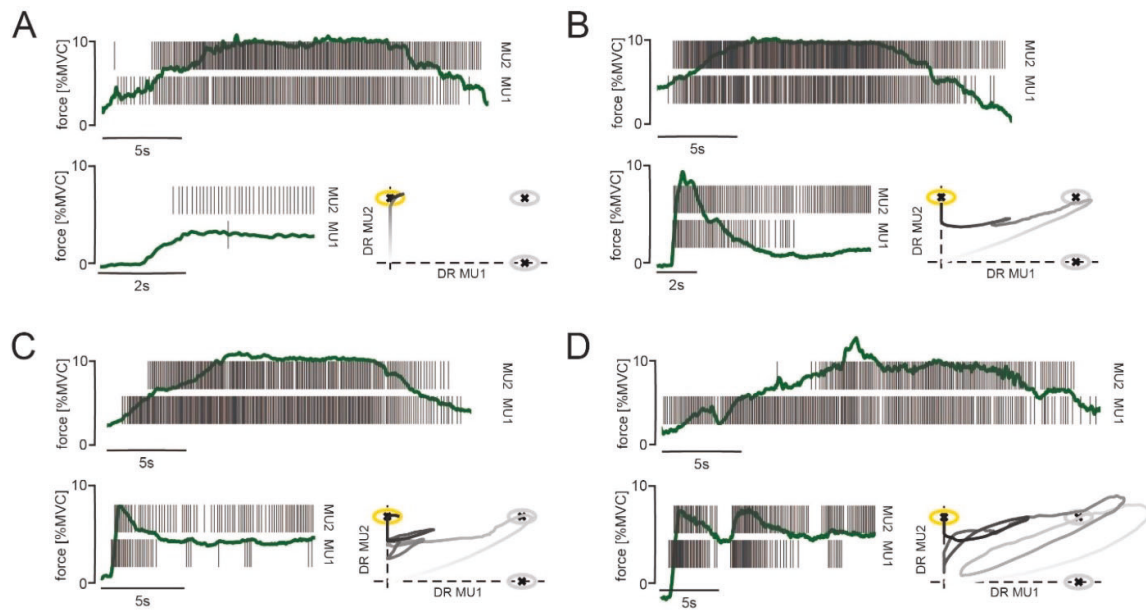
B



Supplement 4-1: HDsEMG from a single subject. A: HDsEMG at 10% MVC during the initial force task overlaid with the spike timing of the four selected MUs. Rectangles indicate a 10ms window centred at the time of a detected MU action potential. B: Spike triggered average of the four selected MUs over 10s at 10% MVC at the initial force task for four different locations i-iv. The locations are indicated on the schematic electrode layout on the right. Double differential signals are displayed for visualisation purposes.



Supplement 4-2: Additional metrics concerning the target-tracking task. A: Difference in recruitment threshold of the selected MU pair versus their difference in the de-recruitment threshold for the last five days of training (Spearman correlation coefficient $R = 0.46$, $p = .001$). Each dot represents a MU pair coloured based on the condition. B: Angle hit rate of all TIII instructed trials during the last five days of training versus the within-pair difference in the de-recruitment threshold. C: Estimation of within-session learning grouped by the three targets T1 (orange), T2 (green), and T3 (yellow). Learning was estimated by calculating the Spearman correlation coefficient between performance and consecutive trials per target and condition. Each dot represents the mean correlation coefficient for one subject averaged across conditions and all 14 days of training. A Friedman test revealed no difference between targets ($p = .103$). The positive median values indicate small learning within one session across subjects for each target. D: Subjective experience of controlling MU2 when reaching towards TIII based on the questionnaire response during the last five days of training across subjects and conditions are shown by their medians and quartiles.



Supplement 4-3: Various successful trials of hitting TIII for different subjects, conditions, and training days. The top figure in each segment shows the discharge activity of MU1 and MU2 (selected MU pair) and force due to dorsiflexion (green) during the initial force task. The bottom left figure shows the discharge activity of MU1 and MU2 and the force (green) during a single successful TIII instructed trial, while the bottom right figure shows the corresponding cursor movement towards TIII (yellow). The grey intensity of the cursor trajectories increases over time within the trial. A: Direct movement towards TIII besides the initial difference in recruitment threshold. B: Indirect movement towards TIII. C & D: Indirect movement towards TIII for a MU pair in which the higher threshold MU (MU2) was initially recruited after and de-recruited before the lower threshold one (MU1). DR: discharge rate.

CHAPTER 5 CONCLUSION AND OUTLOOK

5.1 Summary

Human motor augmentation has travelled a long way from being just science fiction decades ago to an entire research field which only now outlines how much this technology could change the way we all live and interact with the world around us. One of the biggest challenges in this novel human-machine interaction paradigm is extracting a bodily control signal that allows seamless coordination of natural limbs and sDoF. In this dissertation, I analysed the potential use of motor neuron activity as a resource for filling this gap.

In Chapter 2, I introduced a novel approach to extracting potential supernumerary control signals by using a non-invasive neural interface to facilitate spectral separation of the neural code of spinal motor neurons. I demonstrated that spectral components outside the bandwidth of musculoskeletal control, i.e. inside the beta range (13-30 Hz), can be partially uncoupled from the resulting force output. Even though the control over the novel beta featured remained weak, these high-frequency components could potentially lay in a null space of natural control of the human body.

While the investigations in Chapter 2 contributed towards testing the potential of spectral separation of the neural code received by a muscle as a resource to achieve human motor augmentation, in Chapter 3, I focused on the overall nature and potential origin of

these beta oscillations present in motor neurons. I showed for the first time that high-frequency components inside the beta range on the motor neuron level occur in short-lived transient events. These bursts present in populations of motor neurons seem to be time-locked and share similar characteristics as these beta bursts observed at the cortical level. When I used the operant conditioning framework to volitionally modulate beta activity at the motor neuron level (developed and introduced in Chapter 2), I observed that cortical beta bursts changed in a similar direction as the conditioned bursts in the periphery. These results provide evidence for the cortical origin of beta bursts at the motor neuron level.

In Chapter 4, I focused on single motor neuron activity as a resource for controlling sDoF. To investigate potential control mechanisms that selectively drive individual motor neurons, I provided subjects real-time feedback on discharge patterns of pairs of motor neurons innervating the same muscle. Over 14 days of training, all subjects learned control strategies that allowed partially flexible control for various combinations of motor neuron pairs. However, these control strategies leveraged aspects of motor neuron firing that were not based on flexible and independent control of single motor neurons. Instead, these results suggest that a strong common input signal constrains the control of multiple motor neurons. These results provide new insights into using neural interfaces for human-machine interaction by characterising the control of multiple motor neurons innervating a single muscle.

5.2 Future perspectives

The ultimate goal of motor augmentation is the extensions of human motor capacities in general purpose applications. In his dissertation, I explored motor neurons as resource for

such a novel human-machine interaction. For this, it is essential to understand how motor neurons and the extracted features for driving sDoF behave outside highly constrained lab environments. One crucial component needed for such a translation is a decomposition method that allows a robust and reliable tracking of motor neurons during everyday life conditions, including, for example, dynamic contractions (Oliveira & Negro 2021) and fatigue (Martinez-Valdes *et al.* 2020), and also to gain a better understanding on how movement is orchestrated during these scenarios (Hodson-Tole & Wakeling 2008). Moreover, especially when using single motor neurons, training of supernumerary control could potentially benefit if advanced decomposition methods would allow the tracking of the same motor neuron pool across sessions and days, for example, as was shown in previous investigations of motor neuron behaviour (Cescon & Gazzoni 2010; Cogliati *et al.* 2020; Martinez-Valdes *et al.* 2017). Moreover, when aiming to use motor neuron activity in a wide range of applications, decomposing from more convenient locations, such as the wrist (Guerra *et al.* 2022), may play a crucial role.

An additional factor concerning the improvement of decomposition techniques from which motor neuron control for human motor augmentation could benefit is their sensitivity, i.e. the detection of more active motor neurons. Tracking larger motor neuron populations would allow the real-time analysis of higher frequency ranges¹, such as the higher gamma band. Bashford and colleagues have shown that cortical gamma oscillations could provide a promising resource for augmenting motor function (Bashford *et al.* 2018). Such gamma oscillations are also present in the periphery of the motor system (Ulloa 2022). Hence, more sensitive decomposition techniques would allow us to study if gamma present in a motor

¹ currently undersampled when only <20 MU are identified

neuron pool shows similar promising behaviour for human augmentation as its cortical counterpart.

Similarly, flexible individual motor neuron control could benefit from an increased number of tracked motor neurons. In Chapter 4, I have shown a relationship between a subject's performance in individual motor neuron control and the neuron's recruitment and de-recruitment threshold. A pool of many motor neurons would allow further investigation of this relationship and could increase the probability in finding an *ideal* pair, triplet, quartet for individual control.

To translate the findings from this dissertation into a control paradigm that drives sDoF effectively, questions concerning optimal human-machine interaction need to be addressed (Karray *et al.* 2017; Te'eni *et al.* 2007), such as: How to map motor neuron activity into a control scheme to improve *functionality* and *usability* resulting in the effective use of human motor augmentation?

This dissertation was mainly concerned with the *functionality* of using motor neurons as a resource for human motor augmentation; for example, do motor neurons provide the potential for an independent supernumerary control channel? On the other hand, *usability* describes how effectively this functionality can be leveraged (Karray *et al.* 2017). One important aspect of *usability* is the robustness and reliability of how one can extract a supernumerary control signal from motor neuron populations. In the case of beta oscillations, I observed that subjects could modulate the overall trend of the averaged beta amplitude. However, instantaneous control of beta remained weak. In Chapter 3, I found, unlike how beta oscillations were modelled in the control scheme of Chapter 2, beta activity occurs in infrequent bursting events explaining why instantaneous control of its averaged signal has proven difficult. To use such high-frequency bursting events outside the motor bandwidth for

augmentation, we need to identify features of these bursts that can be modulated reliably, for instance, burst duration, amplitude, peak frequency, and occurrence rate. Thus, further investigations on these high-frequency components could improve their usability in augmentation contexts. This may relate to understanding their role in movement generation, origins, how volitional modulation can shape their appearance, and which feature is best suited for neuromodulation (Ramot & Martin 2022).

Furthermore, to effectively exploit the potential functionality of a supernumerary control channel, it is crucial to design a human-machine interaction that suits the underlying characteristics of the bodily control signal – here, the characteristics of motor neuron activity. In the case of single motor neuron control, one possibility is to continuously map the discharge rate of individual neurons into a control signal, as done by Formento and colleagues (Formento *et al.* 2021). A different approach could be to explore interactions based on discrete events. In Chapter 4, I observed that humans could learn to keep larger motor neurons discharge action potentials while smaller ones stay silent, contrasting to their initial activation during orderly recruitment. Such groups of active and inactive motor neurons could be modelled in *activation patterns*, i.e. binary trains, in which each digit stands for a single motor neuron. If the discharge rate of a single neuron surpasses a pre-defined threshold, the motor neuron can be modelled as active (“1”) or otherwise as inactive (“0”). An activation pattern held for a specific time could trigger a control command in human-machine interaction. While such a mapping presumably reduces the impact synaptic noise has on control when directly mapping individual neurons' discharge rates into sDoF navigation, similar to using beta bursting events, it does not allow for an instantaneous interaction (i.e. it takes a certain amount of time to form such activation patterns). Yet, extracting events from bursting beta activity or using activation patterns of groups of motor neurons could result in

a robust way of utilizing motor neurons in augmentation interaction that does not necessarily require an immediate control signal. Thus, such events could be used to trigger pre-defined actions of artificial effectors or act as a virtual button click overt to natural limb movement in virtual and augmented realities. In addition, human motor augmentation facilitated by motor neuron activity is based on control signals that are mainly located inside a motor null space. Hence, these interactions can be private, i.e. the surrounding does not notice when the augmented signal is generated. This feature could promote the use of motor neuron based signals for discrete and non-instantaneous interactions. However, further research is necessary to understand what means of interaction best suits motor neuron-driven supernumerary control channels.

5.3 Final comments

Taken together all this work presented in my dissertation and the pursued research on motor neurons and their role in human motor augmentation throughout the last years, I would like to provide my view on the following question:

Do motor neurons provide a resource towards *augmentation by extension* that can be accessed non-invasively?

Fully flexible coordination of natural and sDoF requires a volitional and independent control scheme for artificial actuators also when natural limbs are at total rest. Therefore, when using motor neuron activity to provide such a control scheme, the operation of the sDoF should be possible even when the innervated muscle is fully rested. Extraction of single motor neuron activity relies on measuring muscle activity via EMG. Hence, in contrast to individual

control of neurons in the cortex (Patel *et al.* 2012), a potential supernumerary control signal based on motor neuron activity can only be extracted when the innervated muscle is contracted.

In conclusion, under current considerations, do motor neurons provide the missing resource to achieve *augmentation by extension*? – I would say *No*. The underlying control structure orchestrating motor neurons appears too rigid to allow for a supernumerary control channel fulfilling all requirements of *augmentation by extension*, including flexible and concurrent activation of natural and sDoF. But, hence, is using motor neurons for enhancing motor capacities simply *augmentation by transfer*? – I would also say *No*.

Augmentation by transfer would entirely block the muscle from which motor neuron activity is extracted when sDoF are orchestrated. In Chapter 2, I showed that the beta component could be modulated partially independent from the low-frequency components translated into the force. Furthermore, in Chapter 4, depending on the history of muscle activation, different combinations of motor neurons could discharge action potentials at the same force level. Based on this, there is a possibility that motor neurons could provide a superior supernumerary control than current *augmentation by transfer* approaches. They may provide the potential of a partially independent control channel that, in my opinion, could advance the application spectrum of current *augmentation by transfer* approaches. However, fully flexible coordination of both natural and sDoF seems to be a challenge too big for solely using spinal motor neurons. Nevertheless, if we *ever* accomplish *augmentation by extension* for general purpose applications remains to be demonstrated (Eden *et al.* 2022).

Human motor augmentation research is just at its beginning and already provides an exciting glimpse of what capabilities lie in it. This dissertation solely dealt with finding an

adequate resource for controlling sDoF using motor neuron activity. Yet, the successful realization of novel human machine interaction within the human augmentation context depends on many more aspects outside of the scope of this book. Effective control is not just limited to control itself but also how the sDoF is integrated into the sensorimotor system. Future human motor augmentation research should therefore be accompanied by investigations that tie in with studies exploring how learning of sDoF control can be facilitated, how sDoF are embodied (Bashford & Mehring 2016; Giummarra *et al.* 2011) and how the CNS integrates additional artificial effectors (Di Pino *et al.* 2014; Kieliba *et al.* 2021; van den Heiligenberg *et al.* 2018), to just name a few. And yet, it remains crucial that further development of such potentially disrupting technology should be accompanied by ethical considerations and constant reflection of its usage (Blanke & Aspell 2009; Johnson 2014; Mirza *et al.* 2019). Opposing its comic book introduction in 1963, human motor augmentation could be a technology supporting everyone to advance our lives for the better.

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