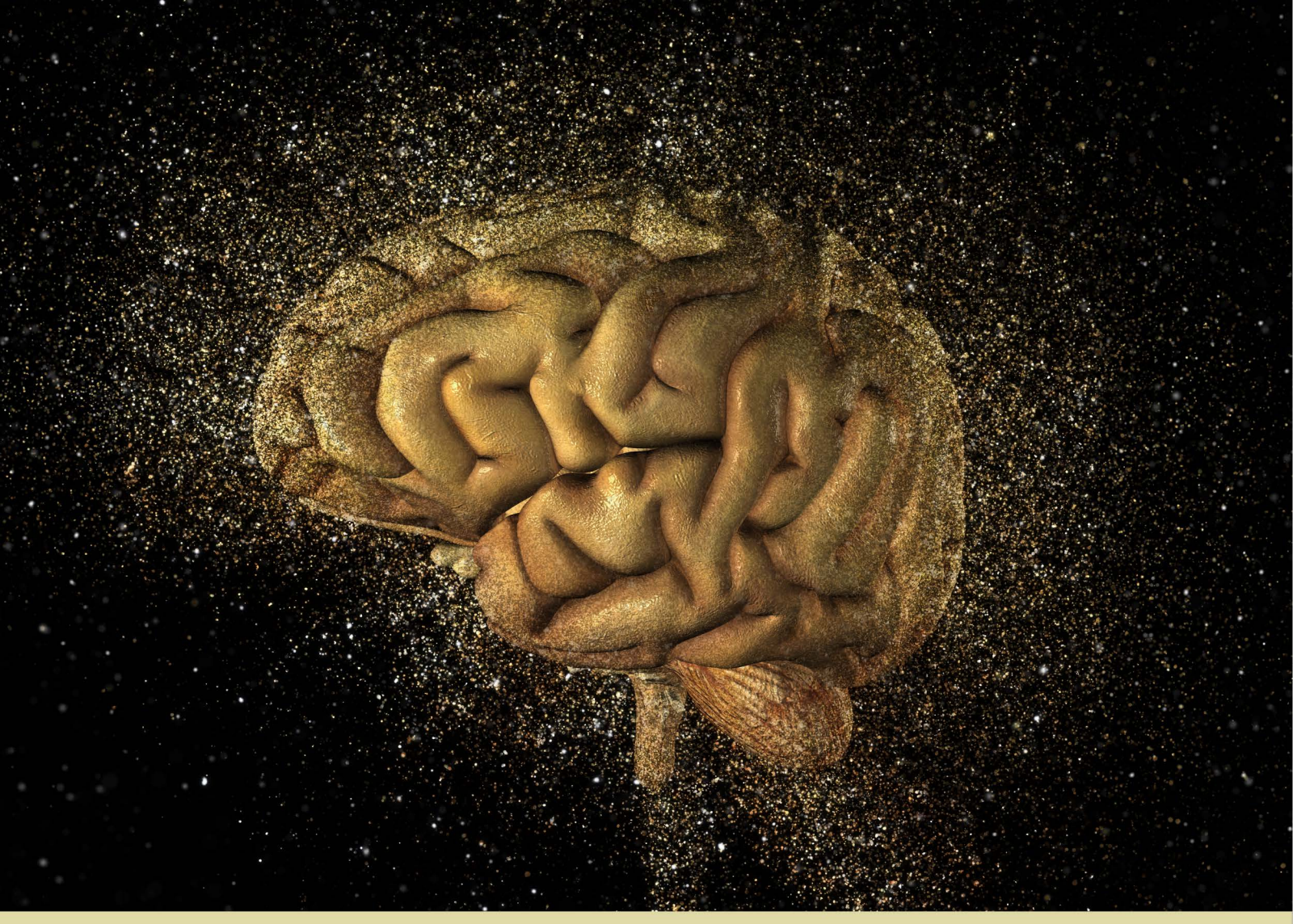

Andreia Martins de Almeida Verdade

Motor excitability modulation during the delay period - a TMS and pupillometry study

Dissertation presented to the University of Coimbra
in fulfilment of the requirements necessary for obtaining a MSc degree in Biomedical Engineering

September 2017

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Universidade de Coimbra

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FCTUC FACULDADE DE CIÊNCIAS
E TECNOLOGIA
UNIVERSIDADE DE COIMBRA

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Dissertation presented to the University of Coimbra in fulfilment of the requirements necessary for obtaining a MSc degree in Biomedical Engineering.

Andreia Martins de Almeida Verdade

Supervisor:
Maria José Braga Ribeiro, Ph.D.

Coimbra, 2017

This project was developed in collaboration with:

Center for Neuroscience and Cell Biology & Institute for Biomedical Imaging and Life Sciences

Instituto de Ciências Nucleares Aplicadas à Saúde

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MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

Para ser grande, sê inteiro: nada
Teu exagera ou exclui.
Sê todo em cada coisa. Põe quanto és
No mínimo que fazes.
Assim em cada lago a lua toda
Brilha, porque alta vive.

Fernando Pessoa, em Odes de Ricardo Reis, 1933.

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À Maria, por me ter acolhido neste projeto já tão tardiamente mas ter sido sempre paciente com a minha impaciência. Por toda a ajuda, toda a experiência e conhecimentos transmitidos ao longo desta fase crucial e por me ter permitido, desde cedo, confiando em mim, evoluir. O meu mais sincero obrigado por me ter guiado nesta jornada, sem a Maria não teria sido possível.

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Abstract

All aspects of motor behavior are ensured by the motor cortex. The efficiency of motor behavior has been widely suggested to depend on the ability to use prior information to prepare the motor system for a later response, during a short interval known as motor preparation period. The fine control of movement execution is critically dependent on the primary motor cortex (M1) and its healthy excitatory and inhibitory systems. The functional organization of M1 has allowed the assessment of corticospinal (CS) excitability and the investigation of adaptive changes in human motor cortex through transcranial magnetic stimulation (TMS). Several studies using TMS have shown CS inhibition earlier than the actual movement execution: during the movement preparation period. This motor inhibition seems to have a role withholding automatic or inappropriate responses and, therefore, favoring the upcoming motor response.

CS excitability has been closely linked to the locus coeruleus-norepinephrine (LC-NE) system and its crucial role in neuronal plasticity in the human brain. The activity of the LC-NE system, closely related to arousal and stress states, is reflected by pupil dilation and, therefore, pupillary response has become a well-established indirect indicator of NE-release through LC activation. Due to its important role in synaptic plasticity, the LC-NE system has been also related to cognitive functions as learning and memory; and pupil diameter, known to reflect LC-NE activity, has been proved to increase during mental activities. These evidences have allowed to raise crucial questions regarding the crosstalk between cortical excitability and pupil dilation, as the specific role of norepinephrine (NE) in these processes and, more importantly, how parameters known to have an effect on NE levels are able to differently modulate CS states.

Accordingly, in the present study, in Part I, the effect of decision complexity on pupillary response was investigated recording pupil diameter fluctuations in 5 young, healthy participants using an eye-tracker system while performing a cued-choice reaction time (cued-CRT) task with two different decision complexity conditions and a passive-viewing condition. In Part II, the hypothesis of pupillary response fluctuation being an indirect indicator of motor cortex excitability modulation was evaluated using

TMS and crossing pupillometry data with the evoked responses amplitude. TMS pulses were delivered over the left primary motor cortex in 5 healthy subjects to induce motor evoked responses (MEPs) in a right hand muscle at various timings during the visual task performance.

Pupillometry results confirmed that decision complexity modulated pupil response with a greater pupillary dilation locked to the higher complexity condition. The passive-viewing condition did not elicit a significant pupillary response suggesting that pupillary response may therefore be a good indicator of the subject's engagement in a task.

The MEPs amplitude across subjects assessed during the motor preparation interval, between the cue and the target stimuli, was also modulated by task engagement. Surprisingly, and contrary to what was initially hypothesized, MEPs amplitudes during this period did not reveal an inhibition of cortical tract and, in fact, a facilitation was observed for the three conditions. Greater cortical excitabilities were associated with the active task engagement. Thus, the preparation of a motor response had an effect on CS excitability baseline levels. However, no effect of decision complexity during this period was observed.

Finally, the analysis revealed a correlation between the pupil fluctuations and MEPs peak-to-peak amplitudes relative to MEP baseline values for all three conditions. Thus, greater pupil size variations appear to be related to higher excitability levels of the CS tract during the preparatory period, in accordance to the proposed hypothesis of pupil response as a good and reliable indicator of motor cortex excitability.

Keywords: CS excitability, motor preparation, decision complexity, pupillary response, TMS

Resumo

Todos os aspetos do comportamento motor são garantidos pelo córtex motor. A eficiência deste comportamento tem sido amplamente sugerida depender da capacidade do uso de informação prévia para preparar o sistema motor para uma resposta tardia, durante um intervalo de tempo fugaz conhecido como período de preparação motora. O controlo preciso da execução do movimento é dependente do córtex motor primário (M1) e do adequado funcionamento dos seus sistemas excitatório e inibitório. A organização funcional do M1 tem permitido o acesso à excitabilidade corticoespinhal e a investigação das mudanças adaptativas no córtex motor humano através do uso da estimulação magnética transcraniana (EMT). Estudos recorrendo ao uso de TMS têm revelado uma inibição corticomotora muito antes da execução de uma resposta motora: durante o período de preparação do movimento. Esta inibição motora parece ter um papel ao evitar respostas automáticas ou inapropriadas e, portanto, favorecendo a resposta motora futura.

A excitabilidade corticoespinhal tem sido relacionada com o sistema locus coeruleus-norepinefrina (LC-NE) e o seu papel crucial na plasticidade neuronal no cérebro humano. A atividade do sistema LC-NE, intimamente relacionada com os estados de alerta e stress, é refletida pela dilatação da pupila e, consequentemente, a resposta pupilar tem-se tornado um indicador indireto bem fundamentado da libertação de norepinefrina (NE) pela ativação do LC. Devido à sua importante função no processo de plasticidade sináptica, o sistema LC-NE tem também sido relacionado com funções cognitivas como aprendizagem e memória; e o diâmetro da pupila, que se sabe refletir a atividade do sistema LC-NE, tem sido demonstrado aumentar durante atividades mentais. Estas evidências têm assim permitido levantar questões crucias relativas à interação entre a excitabilidade cortical e a dilatação da pupila, como o papel específico da norepinefrina nestes processos e, especialmente, como parâmetros que afetam os níveis de NE modulam diferencialmente estados de excitabilidade corticoespinhal.

Consequentemente, no presente estudo, na Parte I, o efeito da complexidade da decisão na resposta pupilar foi estudado através do registro das flutuações do

diâmetro da pupila, usando um sistema de *eye tracking*, em 5 jovens saudáveis enquanto estes executavam uma tarefa de tempo de reação com duas condições de diferente complexidade de decisão e uma condição de visualização passiva. Na Parte II, a hipótese da oscilação resposta pupilar ser um indicador indireto da modulação da excitabilidade do córtex motor foi avaliada usando TMS e combinando os dados de pupilometria com a amplitude dos potenciais evocados motores (PEMs). Os impulsos de TMS foram dados a 5 jovens saudáveis sobre o córtex motor primário esquerdo, de forma a induzir potenciais evocados motores num músculo da mão direita, em diferentes momentos durante a execução da tarefa visual.

Os resultados de pupilometria confirmaram que a complexidade de decisão modula a resposta pupilar, com uma maior dilatação da pupila associada a condições de maior complexidade. O uso da condição visual passiva revelou também a resposta pupilar como um bom indicador do envolvimento ativo na tarefa.

As amplitudes dos PEMs obtidas durante o intervalo de preparação motora, entre a pista preparatória e o estímulo, mostraram também, em concordância com os dados de pupilometria, serem moduladas pelo envolvimento do sujeito na tarefa. Contrariamente ao inicialmente esperado, as amplitudes das respostas motoras evocadas registadas durante este período não revelaram uma inibição do tracto corticoespinhal e, na verdade, uma facilitação cortical foi vista para as três condições. Elevados valores de excitabilidade cortical foram registados para as condições de envolvimento motor ativo na tarefa. Consequentemente, a preparação da resposta motora parece ter tido um efeito nos níveis basais da excitabilidade corticomotora. Contudo, nenhum efeito da complexidade de decisão foi vista nestes resultados.

Por fim, a análise cruzada dos dois tipos de dados revelou uma correlação entre as oscilações da resposta pupilar e as amplitudes dos PEMs medidas, para as três condições. Por conseguinte, estes dados parecem sugerir que maiores variações no diâmetro da pupila estão relacionadas com maiores níveis de excitabilidade do tracto corticoespinhal durante o período de preparação motora, de acordo com a hipótese inicialmente proposta da resposta pupila ser um bom indicador do nível de excitabilidade corticomotora.

Palavras-chave: Excitabilidade corticoespinhal, preparação motora, complexidade da decisão, resposta pupilar, EMT

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ADHD attention-deficit/hyperactivity disorder

AMPA *α*-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid

AMT active motor threshold

AP anterior-posterior

cAMP cyclic adenosine monophosphate

CREB cAMP response element-binding

CRT choice reaction time

CS corticospinal

cued-CRT cued choice reaction time

EMG electromyography

EPSP excitatory postsynaptic potential

FDI first dorsal interosseous

GABA γ-Aminobutyric acid

Glu glutamate

HD Huntington disease

IPSP inhibitory postsynaptic potential

LC locus coeruleus

LC-NE locus coeruleus-norepinephrine

LM lateral-medial

LTD long-term depression

LTP long-term potentiation

M1 primary motor cortex

MEP motor evoked response

mGluRs metabotropic glutamate receptors

MRI magnetic resonance imaging

NE norepinephrine

NMDARs N-methyl-D-aspartate receptors

NS nervous system

PA Posterior-anterior

PET positron emission tomography

PFC prefrontal cortex

PI3K-Akt phosphoinositide-3-kinase – protein kinase B

PKA protein kinase A

PLC phospholipase Cβ

PMd dorsal premotor cortex

p-t-p peak-to-peak

RMT resting motor threshold

RT Reaction-time

rTMS repetitive TMS

SMA supplementary motor area

SP cortical silent period

SRT simple reaction time

VGA Video Graphics Array

ε electromotive force

$\vec{F}$ force

ΦB magnetic flux

1. CHAPTER I. INTRODUCTION

1.1. Cerebral cortex

The nervous system (NS) is a complex cellular network composed of highly specialized neural circuits in which every aspect of behavior relies. The complex processes performed by these circuits depend on the interaction of neurotransmitters and cellular receptors to determine the level of neuronal excitability [1].

Different NS areas are demonstrated to be responsible for different functions of human behavior [2]. The cerebral cortex is a thin convoluted sheet of neuronal cells covering the outer portion of the cerebrum. It is typically 2-3 mm thick, consisting of small folds called sulci, large grooves called fissures, and bulges between them called gyri [3]. The cortex is functionally divided into three separate groups: sensory, motor and association cortices.

Initially examined by Korbinian Brodmann in 1909 [4], the cerebral cortex can be organized in six horizontal layers (layer I, closest to the outer surface of the cortex, to layer VI, preceding the white matter) (Figure 1.1).

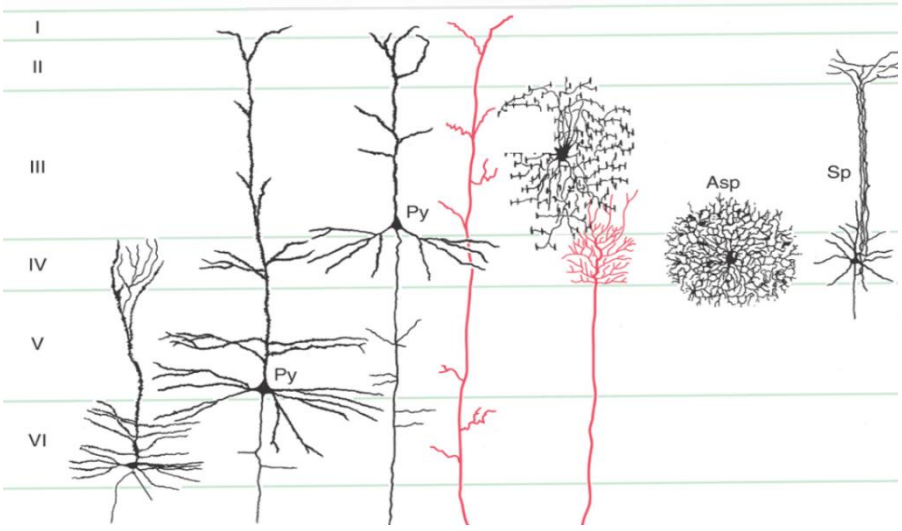

Figure 1. 1 - Cerebral cortex layers and representative cells. Pyramidal cells (Py) project globally across the 6 layers. Interneurons can be subdivided in aspiny and spiny stellate neurons (Asp, Sp) are mainly located in layer IV, although their processes extend into other layers. Adapted from [5].

Each layer is mainly characterized by the presence or absence of two main cell types: interneurons and pyramidal cells. Although interneurons only project locally [4], dendrites of pyramidal cells extend both horizontally and vertically, and form extensive networks in layers II to IV [6], which are thought to allow the flexible synaptic organization of the motor cortex [7].

1.1.1. Motor cortex

All aspects of movement control are ensured by the motor cortex, which is located in the precentral gyrus of the frontal cortex, anterior to the somatosensory cortex. Its composition includes namely primary motor cortex (located in Brodmann's area 4; M1), non-primary motor cortices of supplementary motor area (medial aspect of Brodmann's area 6), premotor cortex (medial aspect of Brodmann's area 6), and cingulate motor areas (Brodmann's areas 6c, 23c, 24c) [4].

Circuits in M1 work as an active local network, as they receive and integrate convergent inputs from sensory and motor systems, and their collective and coordinated output carries the corticofugal signals consequently generating movement [8]. Multiple long-range excitatory input pathways including corticortical projections, thalamocortical projections and neuromodulatory projections converge on M1. Major outputs from M1 include projections to spinal motor centers, striatum, thalamus, subthalamus, red nucleus and pons [8, 9]. Therefore, M1, common to all mammalian species, is a central area in the motor cortex and several research studies have revealed its crucial role in motor control [10, 11, 12].

The primary motor cortex contains giant pyramidal cells which originate in layer V and terminate directly on motor neurons in the ventral horn of the spinal cord. These cells, also referred to as Betz cells (Vladimir Betz, 1834-1894), provide the most direct pathway for movement execution [13].

Although it was thought only neurons originated from M1 could form a direct pathway with spinal motor neurons, additional studies suggest that other motor cortices besides M1, including premotor cortex, have a significant number of direct projections to spinal motor neurons [14]. However, the necessary threshold to evoke movements from

premotor areas is much higher than in primary motor cortex [15]. Therefore, voluntary movement is predominantly executed by signals from the primary motor cortex.

Ever since 1870, the discovery that electrical stimulation of the cortical surface was able to generate contralateral single muscle spasms and limb movements [16], several studies have proved the causal role of M1 in voluntary movements. The growing interest in this cortical area allowed Penfield several years later to characterize it with a functional mapping representation of the human body – the *Homunculus* (Figure 1.2) [17].

This topographical organization revealed by electrical stimulation and showed ordered representation of areas controlling the foot, leg, trunk, arm, hand, digits and face arranged from medial-to-lateral along the surface of the cerebral hemisphere [18]. However, not all body parts are represented equally; specifically, lips and tongue, thumb and hands which are used in tasks requiring precision and fine control have greater representations in the primary motor cortex.

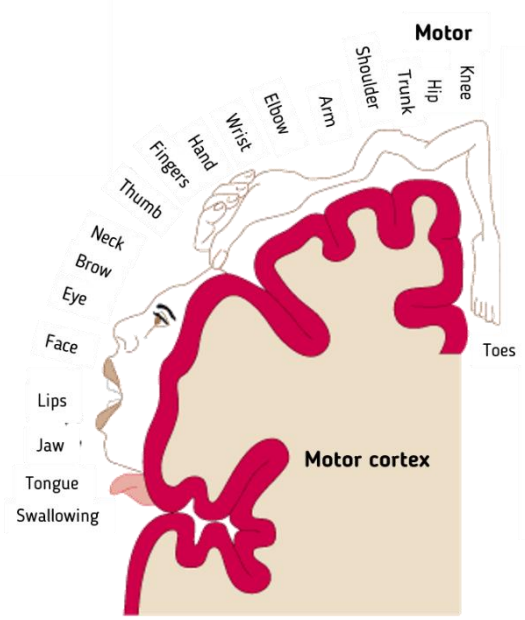

Figure 1.2 - The *Homunculus* - is a functional mapping representation of the human body in the primary motor cortex. The word homunculus comes from the Latin word which translates as "little man". Adapted from [19].

Although this topographical characterization has been confirmed by neuroimaging techniques [20, 21], *Homunculus* has been subject to discussion since

intracortical stimulation in motor cortex sites evokes movements of more than one muscle, and individual muscles can be activated by multiple, distributed sites in motor cortex [22]. Studies with positron emission tomography (PET) found overlapping activation patterns for distal and proximal arm movements within the M1 arm area [23], whereas higher-resolution functional magnetic resonance imaging (MRI) method has also revealed overlapping distributed activation in M1 for distinctive movements of the fingers, wrists, and elbows [24].

1.1.2. Motor cortex excitability

Motor behavior relies on the precision of the primary motor cortex circuits, which in turn is critically dependent on healthy inhibitory and excitatory systems (Figure 1.3).

Inhibitory states are mainly mediated by the activation of γ-Aminobutyric acid (GABA) A and B receptors [25]. Binding of GABA neurotransmitter to $GABA_A$ receptors, which are primarily post-synaptic, can allow chloride (Cl^-) entry through their pores. This Cl^- influx increases the negative charge inside the postsynaptic neuron, leading to hyperpolarization and therefore an inhibitory effect on neurotransmission. $GABA_B$ is a metabotropic receptor coupled to G-protein and is located both pre and postsynaptically. Postsynaptic $GABA_B$ receptor, when in active state, allows the opening of potassium (K^+) channels leading to a neuronal hyperpolarization. This change in membrane potential called an inhibitory postsynaptic potential (IPSP) reduces the firing rate of the neuron by temporarily keeping the membrane potential away from firing threshold. Plus, activation of $GABA_B$ receptors localized pre-synaptically will inhibit the release of neurotransmitters through a decrease in adenylyl cyclase activity, an enzyme that catalyzes the formation of cyclic adenosine monophosphate (cAMP), and membrane calcium (Ca^{2+}) conductance [26].

Excitatory processes are mainly facilitated by the action of cationic channels N-methyl-D-aspartate receptors (NMDARs) [27]. NMDARs activation requires the binding of the excitatory amino acid glutamate (Glu) to the receptor and also a sufficient postsynaptic depolarization (excitatory postsynaptic potential (EPSP)) to remove the

magnesium (Mg^{2+}) blocker ion from the channel, which results in intracellular Ca^{2+} and sodium (Na^{+}) increase [28] and the postsynaptic neuron depolarization giving rise to an NMDA-mediated prolonged EPSP. Ca^{2+} acts as a second messenger inside the neuron leading to the subsequent activation of a number of signaling cascades and several pathways as the phosphoinositide-3-kinase – protein kinase B (PI3K-Akt) pathway, modulating long-term potentiation (LTP), a persistent strengthening of synapses [29].

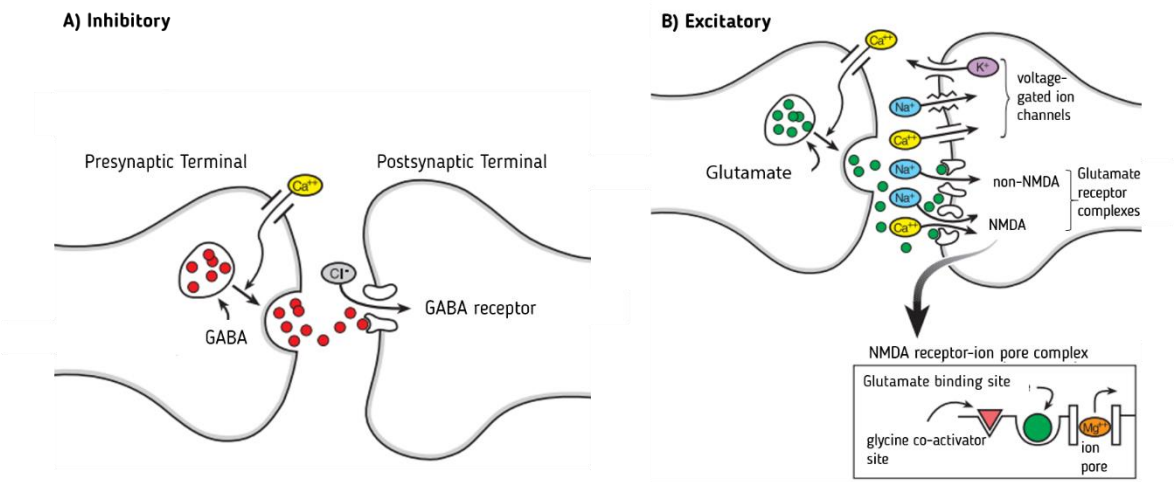

Figure 1. 3 - Inhibitory and excitatory synapses in the CNS. A) Inhibitory synapse. GABA binding to its postsynaptic $GABA_A$ receptors allows chloride anions entry, ultimately leading to hyperpolarization. B) Excitatory synapse. Glu can exert its functions by binding to several types of receptors: ionic receptors and metabotropic glutamate receptors (mGluRs), which are coupled to G-proteins. However, excitatory transmissions in the human brain are mainly facilitated by Glu coupling to NMDARs. This binding results in an increase of intracellular Ca^{2+} levels, which ultimately facilitates the excitatory synaptic transmission. The opening of the NMDA-ion pore requires not only Glu but also glycine binding, leading to the removal of remove the Mg^{2+} blocker. Adapted from [30].

Abnormal reorganization of these brain circuits can result in disturbed function and manifest as several neurological disorders, such as increased circuits excitability in patients who suffered from stroke, or patients with Huntington disease (HD); or impairment in intracortical inhibition observed in patients suffering from dystonia, Parkinsonian disorders, Tourette syndrome or attention-deficit/hyperactivity disorder (ADHD) [31].

Thus, the assessment and monitor of the state of excitability of the corticospinal pathway has gained interest not only in the research field but also for the diagnostic and therapeutic strategies in pathophysiological conditions affecting the

motor system. The currently most used technique to probe corticospinal (CS) excitability is transcranial magnetic stimulation (TMS).

1.2. Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) is a non-invasive, painless, neuromodulatory technique for cortical stimulation, first introduced by Anthony Barker [32].

A TMS stimulator is composed of a capacitor, which is capable of charging up to 2 kV and can produce a pulse current of 5-10 kA when discharged, and a magnetic coil made of copper wire, working as an inducer [33].

Stimulation is based on Faraday's law of Induction, which states that a time-varying magnetic field can be used to induce an electric current [34]. The phenomenon, known as electromagnetic induction, is the result of a relative motion between a conductor and a magnetic flux, ΦB, resulting in an induced electromotive force (voltage), ε, across the conductor:

$$\varepsilon = -\frac{d\Phi B}{dt} \qquad (1)$$

The magnetic field is typically 1-4 T and is produced with lines of flux passing perpendicularly to the plane of the coil (Figure 1.4). The resulting electric current is induced perpendicularly to the magnetic flux and flows in loops parallel to the plane of the TMS coil, allowing a more likely modulation of nerve cells that are distributed horizontally relative to the brain surface [35].

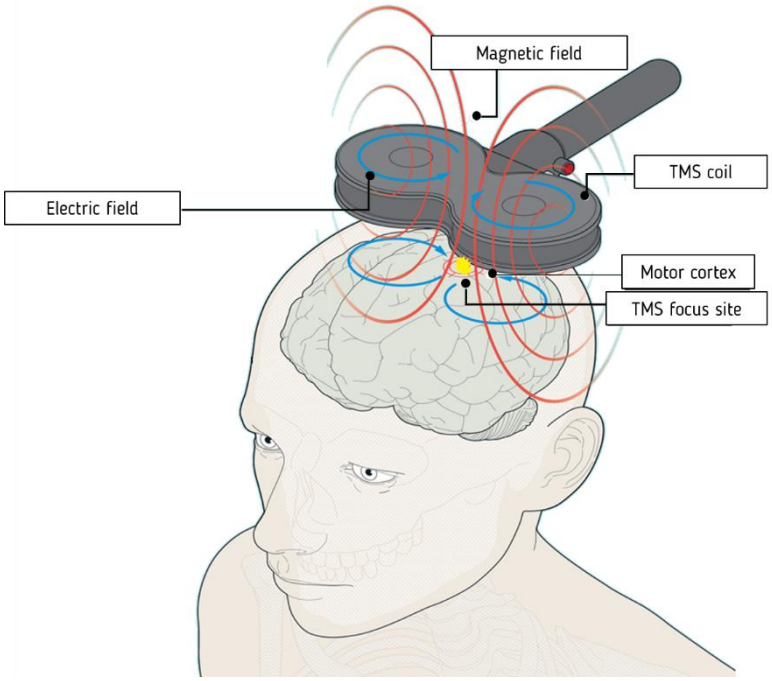

Figure 1. 4 - The current flowing in the magnetic TMS coil generates a perpendicular magnetic field that consequently induces an electric current in specific cortical regions below the coil position. Adapted from [36].

When TMS is applied, an electric field is induced in the brain region beneath the coil and a force $\vec{F}$ is exerted on circulating ions which, consequently, start to move at a constant velocity in the direction of the induced electric field and contrary to the magnetic flux variation that produces it, according to Lenz law. This oriented flow of ions is an electrical current. Thus, TMS is capable of modulating neural activity, since the induced electrical currents will alter the transmembrane potential of neural cells inducing action potentials.

Although there are different types of coils, figure-of-eight shaped coils are the most currently used when the purpose is to produce a more focal stimulus, however, weaker.

When placed over the primary motor cortex, TMS coil is able to activate CS neurons via the intracortical circuit's stimulation. This stimulation elicits descending volleys (D-wave and I-waves) in the corticospinal fibers which activate motor neurons and produce a short-latency motor response (contraction) of peripheral muscles contralateral to the stimulation region. This motor evoked response (MEP) can be recorded using

electromyography (EMG) and its amplitude provides an assay of corticospinal and motor neurons excitability responsible for the targeted motor response [37]. This brief, short (~250μs) electrical current is also painless being, therefore, a breakthrough for previous stimulation techniques, as electrical stimulation.

The MEP measured is a signal resulting from a number of waves that descend the CS tract. The initial waves are the result of direct activation of CS neurons – D-waves, preferentially recruited at higher intensities of TMS; whereas the later waves are the result of indirect excitatory synaptic activation of interneurons in M1– I-waves, mainly recruited at low TMS intensities [38]. I-waves can be classified as I1 and I2 – if appear early, or I3 (and so on) if are late I-waves [39]. The order of I-waves recruitment is well-established to depend on TMS coil induced-current direction [40, 41]. Posterior-anterior (PA) induced-currents predominantly recruits early I-waves, whereas anterior-posterior (AP) induced-currents preferentially recruits late I-waves, and lateral-medial (LM) currents majorly recruit D-waves (Figure 1.5). These findings suggest that the recruited waves might be a consequence of different excitatory inputs [42].

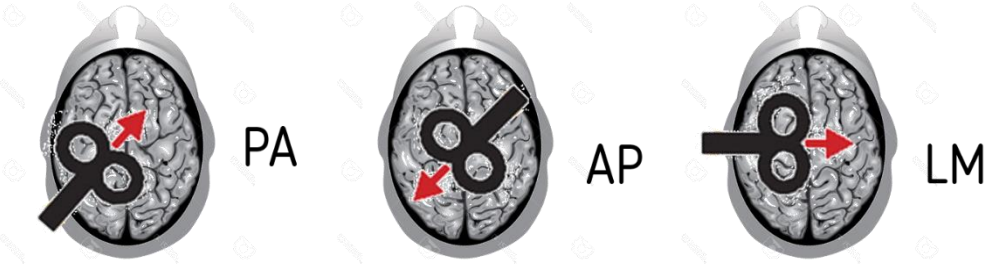

Figure 1. 5 -TMS coil orientation can give rise to 3 induced-currents orientations. PA induced-currents are produced when the handle of the coil points backward at ~45° from the midsagittal line, and AP induced-currents are elicited by placing the coil 180° from the PA direction. LM induced-current direction is achieved by pointing the coil handle laterally. Adapted from [43].

Several evidences have recently showed that the evoked descending volleys depend on the configuration of the TMS pulse [44, 45, 46]. Two types of stimulators are available for TMS: single-pulse and repetitive TMS (rTMS). Contrary to single-pulse, rTMS devices are able to generate trains of stimuli, operating at 10-60 Hz [45]. However, various manufacturers have add-ons modules to single-pulse devices allowing them to be used

with two (paired-pulse stimulator) to four (quadruple-pulse stimulator) pulses with intervals of 1 ms between them.

Coil heating limits the duration of sustained operation, but with proper coil cooling or using another material rather than copper, this duration of the stimulus train can be made unlimited [46].

There are three pulse waveforms for TMS: monophasic, biphasic and multiple-cycle damped sine pulse. Monophasic wave, characterized by a rapid rise to peak (less than 100 μs), a slow decrease to baseline and a low energy efficacy since only the first phase of the stimulus produces a current flow, is preferentially used to assess cortical excitability with single or paired pulses [46]. Biphasic wave, characterized for being a full-sine pulse, changes the direction of the current twice, is mainly used for rTMS, since part of the energy allows it to be re-used [47]. The differences between these two types of pulses are the reason why different pulse configuration may excite different groups of cortical neurons, despite the same coil orientation [44].

Finally, a multiple-cycle damped sine wave, also referred to as a half-sine TMS, only induces current flow in one direction, and therefore is appropriate for studies of direction-specific effects of magnetic stimulation [48].

1.2.1. Safety and side-effects of TMS

Although broadly used and well-described as a safe technique, TMS is based on the exposure of a high intensity magnetic field. Therefore, some precautions have to be considered and limits respected. Besides interacting with subcortical regions and inducing milliampère currents in neuronal circuits, the magnetic field generated in the TMS coil may be able to induce significant voltage changes in any metal and/or electrical device [49]. This interaction may cause damage or demagnetization in such devices and the induced-currents can lead to brain tissue overheating.

Therefore, subjects carrying implanted electronic or metal medical devices should not be submitted to the procedure. However when TMS protocols ensure that the magnetic coil is not activated near the respective devices, TMS practice is considered a minimal risk activity in subjects carrying cardiac pacemakers or spinal cord stimulators.

Another contraindication is the subject's history of epileptic seizures. Although, to date there is no cases reported on seizures in healthy subjects, the delivery of TMS single-pulse has been earlier reported to induce a low risk of seizures in epilepsy patients [50]. Regarding TMS side effects, occasionally subjects report nausea, neck pain and headache after prolonged sessions. Transitory hearing loss is also frequently reported since the coil is often placed proximal to the ear (more recurrent when using rTMS technique).

In order to avoid these risks, TMS protocols should include safety parameters limits concerning the frequency of the stimulation, intensity of the threshold used, session duration, total number of pulses and inter-pulse time [49].

Also, prior to any research or therapeutic session, subjects suitable to the procedure give written informed consent. However, in every case, the balance between the potential benefit and the risks associated should always be considered.

Several parameters can be measured with TMS that allow an indirect assessment of cortical excitability, which will be described in the next section with a particular emphasis on the ones used in the present study.

1.2.2. Motor Threshold

The lowest TMS intensity able to elicit a recordable evoked motor response in the target muscle defines motor threshold (MT) [51]. This value, variable among individuals [52, 53], reflects the excitability of CS and inter- neurons [54], and is of primordial importance for TMS procedures since allows calibration and TMS intensity normalization for every subject. Although between-subject variance in MT values is still unclear, skull cortex distance and age have shown to increase MT values linearly [55, 56]. Therefore, TMS studies usually normalize stimulus intensities to individual excitability of each subject.

Furthermore, threshold values are also directly linked with the muscle or group muscles being stimulated and their primary motor cortex topographic representation. For example, recent studies have already reported lower threshold values for finger extensors and hand muscles, which is consistent with their disproportionately large M1 representation [57]. Also, blockers of Na^+ channels as lamotrigine, lacosamide

and carbamazepine have been reported to increase MT values, suggesting that MT may be a good indicator of membrane excitability [58].

Motor threshold can be measured in relaxed (resting motor threshold, RMT) or during voluntary muscular contraction (active motor threshold, AMT). The RMT is defined as the minimum stimulus intensity that is able to evoke a MEP of 50-100 μV in at least half of 10 consecutive trials given at rest [51]. AMT is similarly determined as RMT but in voluntarily activated target muscles. Since cortical excitability is intrinsically related to voluntary contraction intensity and has higher values when active [59], a lower stimulus intensity is required to evoke a motor response in contracted muscles and, therefore, AMT values are typically lower when compared to RMT [60].

Therefore, motor threshold has been widely used as a standard parameter to determine TMS intensity in research studies, accordingly to dosage and safety limits [61]. In therapeutics, defining MT is also of great importance for calculating the patient-specific therapeutic dose [62].

1.2.3. MEP Amplitude

MEP peak-to-peak (p-t-p) amplitude is the most common measure to assess and analyze the recorded MEP (Figure 1.6). The obtained value is an indirect indicator of cortical and pyramidal tract activation and, when combined with MT value, becomes a reliable and useful parameter to assess cortical excitability [63].

Multiple factors can influence the MEP p-t-p amplitude, causing intra-subject variability [64]. Among these factors, the number of excited motor neurons recruited in the spinal cord [65] can influence the MEP p-t-p amplitude, as well as the intensity of the given stimulus [66]. In the early 90's, Kiers and colleagues suggested that this variability could be due to rapid, spontaneous fluctuations in corticospinal excitability [66], implying that an adequate number of MEP values is necessary in TMS comparative studies.

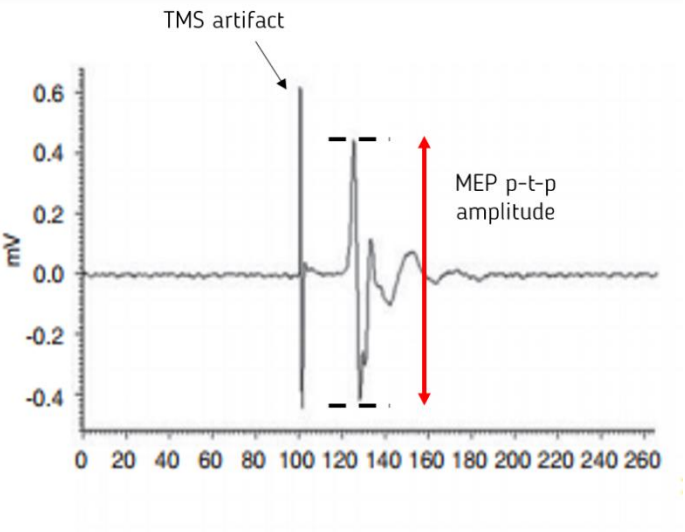

Figure 1. 6 - Standard motor evoked potential response to a suprathreshold single TMS pulse registered using EMG recordings. Adapted from [67].

The MEP p-t-p amplitude is, therefore, mostly used to assess motocortical excitability changes following specific events [68] and can be considered a marker for corticomotor impairment [69, 70, 71].

1.2.4. MEP latency

MEP latency is defined as the interval time between the MEP recording and the TMS pulse given [72] (Figure 1.7).

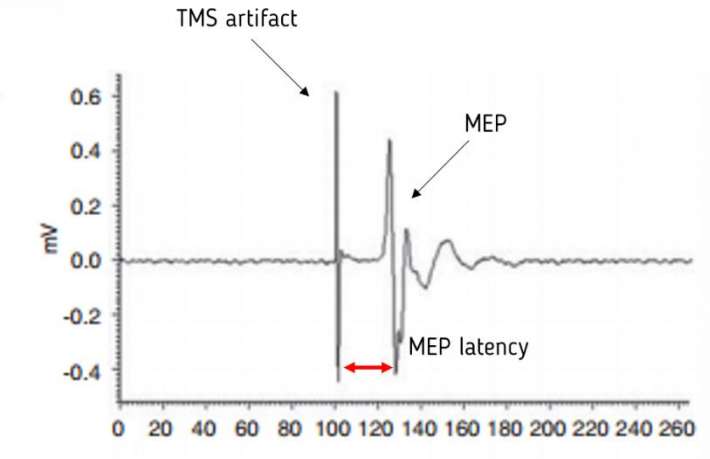

Figure 1. 7 - The effect of the magnetic pulse on the targeted muscle is not instantaneous and a latency period is observed, which duration depends on physiological-related parameters as stress level. Adapted from [67].

Contrary to the MEP p-t-p amplitude, the MEP latency remains stable along responses [73], which makes it the most reliable parameter measured with TMS. A short

latency value gives evidence of a most direct pathway, while a longer one is typical of pathways under stress [74]. Thus, a prolonged latency could provide evidence about damaged neuronal pathways, possibly pathologically affected and have valuable application in diagnosing pathologic conditions as multiple sclerosis [75].

1.2.5. Recruitment (I/O) curve

The recruitment curve represents the MEP amplitude as a function of stimulus intensity [72]. The relation between the two parameters is typically non-linear, with a sigmoidal shape [76]. In order for it to be obtained, a wide range of stimuli intensities must be delivered over the specific motocortical region of interest in M1 in order to evoke a response in the target muscle.

The slope of the obtained curve reflects the relation between inhibitory and excitatory corticospinal pathway inputs and their spatial distribution in the motor cortex [77], providing insights of intrinsically less excitable neurons function or spatially farther from the center of the TMS stimulation.

Consequently, variance in slope or gain in the recruitment curve can be a reliable indicator of corticospinal track abnormalities. Indeed, several studies have reported that a decrease in the recruitment curve parameters indicates a functional impairment, higher damage of CS track [78]. Also, more recently, a study has provided evidence that a recruitment curve in patients who suffered from stroke is characterized by a diminished gain and, thus, can be used for monitoring therapeutics and recovery [79].

1.2.6. Cortical silent period

The cortical silent period (SP) represents an inhibitory phenomenon and is obtained by delivering a single TMS pulse in the tonically activated target muscle resulting in a delayed electromyography response [72] (Figure 1.8).

This delay in EMG response is actually an inactivity period that follows the evoked response.

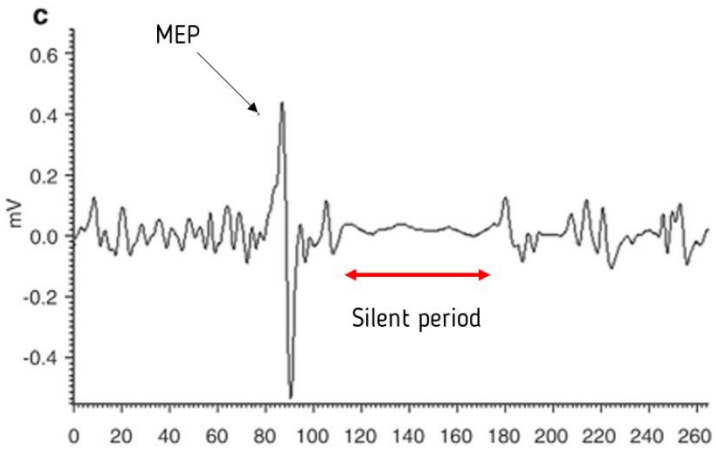

Figure 1. 8 - When TMS is delivered to the targeted muscle during tonic contraction, an electromyography silent is recorded following the motor evoked response, which is thought to be related to a cortical inhibition process. Adapted from [67].

Although the early part of the SP (nearly 50 ms) is likely due to spinal cord refractoriness, the latter is entirely mediated by cortical inhibition [80]. This inhibition process is mainly mediated by $GABA_B$ receptors [81] and, therefore, dopaminergic drugs are able to modulate it, resulting in a shorter SP, as well-documented in Parkinson's disease (PD) [82, 83].

In fact, several authors have used TMS to assess SP duration in patients with neurological or psychiatric disorders. A shortening in SP duration has been reported in schizophrenia [84], depression [85], obsessive compulsive disorder [86], Alzheimer's disease [87], Parkinson's disease [82, 83]. SP prolongation has been related to patients who suffered from stroke [88], Huntington's disease [89] and ADHD [90].

Hence, the SP duration is able to provide an indirect measure of cortical inhibition. Evidences have shown a high intra-subject constancy in healthy subjects, while longer and more variable SPs are reported immediately after a stroke [88], which makes of SP a good parameter for monitoring therapeutic and recovery.

Overall, the ability to stimulate both excitatory and inhibitory connections in M1, makes TMS a measurement technique of cortical excitability, useful in allowing a better understanding of motor behavior.

1.3. Motor preparation

Every aspect of motor behavior relies on the dynamics of excitability of the motor cortex [68].

Motor behavior can be understood as a constant competition between potential actions [91]. Action selection is one fundamental issue faced by every motor response, taking into account multiple factors in order to build an internal descriptive representation of objects in the external world and prepare an appropriate response [92].

When a goal-directed response is required, movement preparation requires several control mechanisms allowing selection, specificity and actions execution in a dynamic manner [93]. Among the processes required to do so, *competition resolution*, *impulse control* and *conflict* have been the processes frequently investigated.

Whereas *impulse control* prevents actions from being unleashed prematurely and *conflict* deals with irrelevant information or unexpected changes in the surrounding environment, preventing us from behaving in an automatic manner, *competition resolution*, related to decision making processes, resolves which action to choose among possible alternatives [94].

According to the 'affordance competition hypothesis' proposed by Cisek [91], the dorsal visual system specifies actions which compete against each other within the fronto-parietal cortex, while a variety of biasing influences are provided by prefrontal regions and the basal ganglia.

The action that wins the competitive process among alternatives in a goal-directed and context-dependent way is finally executed. In fact, studies in nonhuman primate's recordings from the dorsal premotor cortex (PMd) support this idea, revealing an increased firing in PMd neurons coding the 'winning' action, whereas firing rates of neurons responding preferentially for the alternative action are suppressed [95]. The winning action is thereby facilitated by an increase in excitability in the corresponding corticomotor region, while the remaining potential actions are withhold by a diminished corticomotor excitability in the respective cortical regions.

TMS studies have provided a better understanding of how a process of motor preparation affects state-changes in corticomotor system [68, 96, 97, 98]. Humans and

nonhuman primates are able to use bias collected information to prepare their motor system for a later goal-directed response.

Inhibition of the motor system has been widely studied in terms of different aspects of motor behavior. It is now well-established that the process of stopping an on-going action involves the rapid and global CS inhibition. Surprisingly, several studies have shown that the motor inhibition processes are also present way earlier than the actual movement execution: during movement preparation [68, 94, 96, 99]. This motor inhibition, generally referred to as preparatory inhibition, seems to have a role withholding automatic or inappropriate responses.

Reaction-time (RT) tasks have been proved useful for studying the CS excitability during motor preparation. Following an imperative signal, subjects are instructed to respond as quickly as possible and the time needed to do so, called the reaction time or response time, is recorded [100]. By applying TMS pulses at different time points between the imperative signal and the movement onset, it is possible to assess MEP amplitudes in different stages of the motor system preparation process allowing, ultimately, a better understanding of movement preparation[101, 102].

There are several types of RT paradigms depending on the purpose of the study. Regarding CS excitability during voluntary movement preparation, three main versions are popular: the simple reaction time (SRT), choice reaction time (CRT) and instructed-delay RT (Figure 1.9).

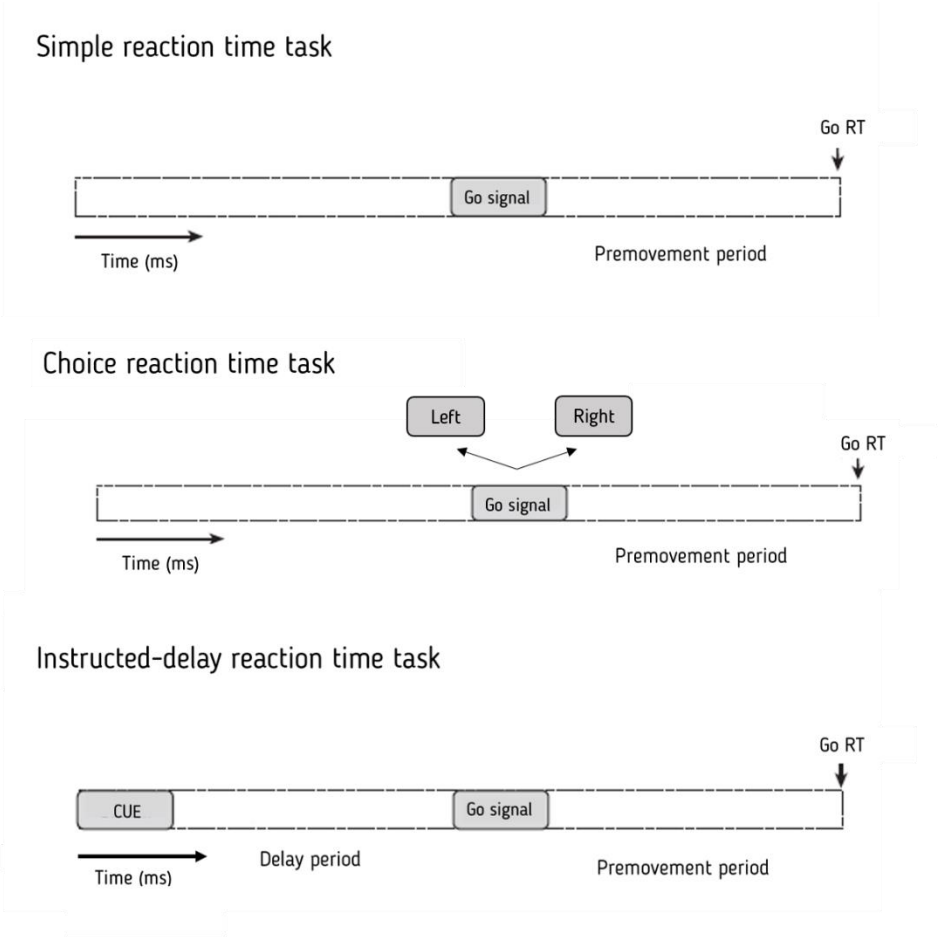

Figure 1. 9 - Reaction time paradigm tasks. The time interval between the imperative (or go) signal and the actual motor response, the premovement period, makes this paradigm ideal for studying motocortical processes preceding movement execution. The instructed-delay version of the task, where a preparatory cue is presented before the go signal, affords to investigate delay-related processes involved in motor preparation, without being confound by functions related to movement execution. Adapted from [68].

In the SRT task, only one motor response is possible and the imperative or go-signal always specifies it. In the CRT task, usually, two or more responses are possible, typically a right or left hand motor response, and therefore the go-signal requires choosing the accurate movement option [103]. In the instructed-delay RT task, a preparatory cue (usually cueing which hand to use in the task) is initially presented and after a delay period, in which the subject should withhold the further response movement, the go-signal pops up [68].

Various studies using the SRT task have reported a gradual increase in MEP amplitude recorded from the task-relevant muscle in the 100-120 ms before EMG onset of the motor response [101], which is consistent with the reported results of several other intracortical recording studies designed for animals [68, 104]. This increase is interpreted

as the reflection of the CS excitation level of the M1 region that codes the task motor response that will be executed [105].

In the more complex version of the paradigm (CRT), MEPs amplitudes recordings have shown an initial reduction below baseline value and, although greater in the task-relevant effector, this suppression effect is also seen in the homologous task-relevant and even task-irrelevant muscles [98, 101]. Closer to the go-signal onset, MEPs amplitudes appear to increase continuously for the task-relevant effector and remained diminished for the task-irrelevant muscles [98]. These observations are in agreement with the assumption that the action selection process involves a facilitation of the task-relevant muscle M1 cortical region but also an initial global suppression of both task-relevant and task-irrelevant muscles [68].

Instructed-delay RT task studies have also revealed that during the delay period, before the imperative signal onset, MEPs amplitudes are diminished for both the selected effector (cued) and the non-selected effectors muscles (not cued) for the upcoming response [106]. These evidences indicate a global preparatory inhibition; however, suppression is often stronger for the selected muscles, suggesting a greater focal inhibition for the effector that codes the forthcoming response [68].

Although several hypotheses have been emerged recently in order to understand the motor inhibition process during movement preparation in the delay period, the 'affordance competition hypothesis', proposed by Cisek [91], is yet the most accepted. According to Cisek, several potential actions compete with each other and the winning action is the one executed. Accordingly, the non-selected responses are suppressed, which is reflected by a decreased MEP amplitude, in order to favor the wining selected-response [91, 106]. This inhibitory process is frequently referred to as 'inhibition for competition resolution'. The hypothesis reliance is, however, questionable considering that the inhibitory process during the delay or pre-movement period is global, suppressing not only non-selected effector but also task-irrelevant muscles, contrary to the plausible assumption that inhibition would only be addressed to non-winning (non-selected) responses [106, 107].

Overall, the preparation of a further goal-directed movement appears to allow a better and finer execution of the wanted response. The motor preparation

excitability modulation has been widely suggested to reflect the preparatory activity processes occurring in cortical and subcortical structures [68]. Therefore, the assessment of the excitability levels of specified cortical regions responsible for the desired movement during the movement preparation period may allow a clearer understanding of the corticospinal contribution to the upcoming response.

1.4. The Locus Coeruleus-Norepinephrine (LC-NE) system

One of the major systems responsible for regulating cortical function is the locus coeruleus-norepinephrine (LC-NE) system. Besides its association with relatively simple and basic functions such as arousal and the sleep-wake cycle [108]; more recent studies have attributed a perhaps more important function in cortex regarding neuromodulation [109].

The locus coeruleus (LC) is present in all mammalian species and represents a small, homogeneous nuclear complex located bilaterally in the dorsal wall of the rostral pons in the lateral floor of the fourth ventricle [110]. It is comprised of a densely population of cells with a common embryonic region, all of which produce norepinephrine (NE). The LC nucleus receives its main afferent glutamatergic inputs from the orbitofrontal and anterior cingulate cortices [111], and projects to diverse brain regions including the spinal cord, brainstem, cerebellum, hypothalamus, thalamus and the entire isocortex via highly collateralized projections [112] (Figure 1.10). This extensive innervation makes of LC the major noradrenergic nucleus of the brain. Via its widespread projections, LC is able to modulate cortical, subcortical, and brainstem circuits.

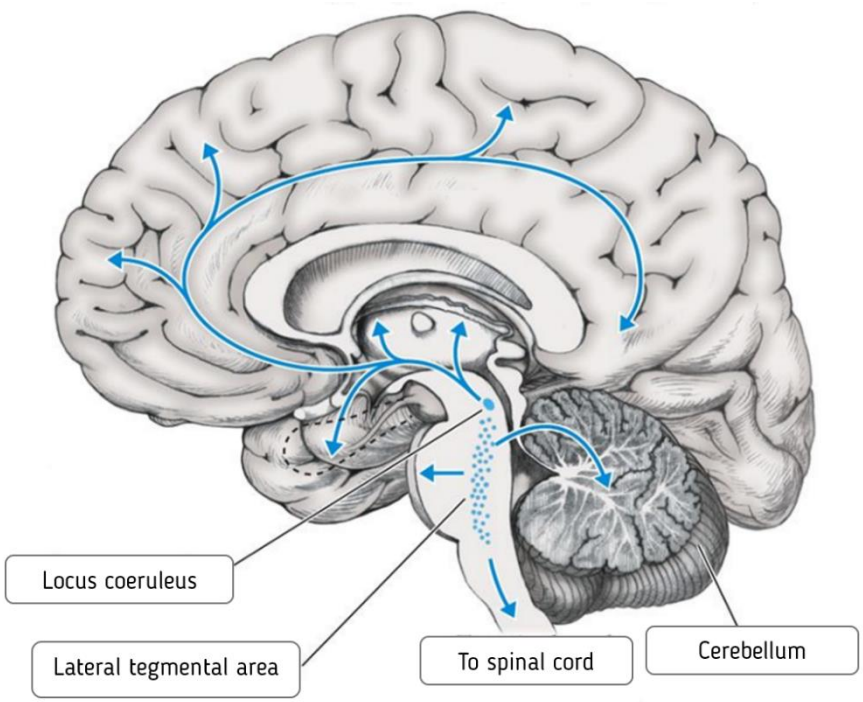

Figure 1. 10 - NE primarily derives from neurons whose cell bodies reside in the locus coeruleus. These pontine collections of neurons project to several brain regions including the spinal cord, brainstem, cerebellum, hypothalamus, thalamus and the entire isocortex via highly collateralized projections. Adapted from [113].

The LC activation drives a broad NE release over the cerebral cortex.

Norepinephrine was one of the first identified neurotransmitters (Ulf Svante von Euler, 1905–1983) and it is frequently associated with arousal states [114] and the rapid behavioral response to stress [115]. Besides being a neurotransmitter and a hormone secreted by the adrenal medulla, its effects in neuroplasticity have led to its recognition as a neuromodulator in the CNS [116]. Rather than conveying sensory or motor signals, neuromodulators are able to modulate effects produced by other neurotransmitters as Glu and GABA.

In terms of its chemical structure, NE is a catecholamine and is synthesized from the amino acid tyrosine by a series of enzymatic steps, resulting from the direct oxidation of dopamine by the action of the dopamine β-hydroxylase enzyme [115]. Ultimately, NE is transported into synaptic vesicles and its release is mainly mediated by the increase of intracellular Ca^{2+} levels.

LC-NE release is coupled with two distinct LC modes of activity – tonic and phasic [117].

Tonic firing activity is characterized by highly regular pattern of discharge that is highest during waking and lowest during slow-wave sleep. Through this firing mode, NE released exerts effects on sleep, vigilance states, stress and inflammation. Consequently, firing rates alterations in the LC appear to have a potential causal role in the regulation of arousal states [117]. However, during focused attention and accurate task performance, LC neurons change its firing mode and respond to task-relevant stimuli phasically. Therefore, while tonic rates are widely associated to control of arousal levels and behavioral states, phasic bursts have been described to be elicited by higher cognitive mechanisms involving novel sensory stimuli and decision making processes [118].

1.4.1. LC-NE system and motor cortex excitability

NE activation through adrenoreceptors is able to control and trigger modifications in ionic channels properties, culminating in adaptive alterations of postsynaptic excitability levels. These alterations induced by NE-binding to adrenoreceptors is known as neuromodulation and it can be understand as the alteration of cellular and synaptic properties .[119].This modulation effect is mediated by G-proteins coupled to the adrenoreceptors [120], and which activation leads to intracellular signaling pathways regulation.

The different types of receptors expressed by the target cell ultimately determines the cellular effect and, thus, NE has different actions on different cell types. The noradrenergic receptors are a class of G-protein-coupled receptors and can be classified as β-, α_1- and α_2-adrenoreceptors [121] (Table 1.1).

Table 1. 1| Properties of noradrenergic receptors subtypes. Adapted from [159].

Subtype	**Type of G-protein coupled receptor**	**Effector pathways**
α_1	G_q	Increase in phospholipase Cβ Increase in intracellular Ca^{2+} levels
α_2	$G_{i/o}$	Decrease in adenylate cyclase activation Decrease in cAMP production
B	G_S	Increase in adenylate cyclase activation Increase in cAMP production

Although its existence in small proportion in presynaptic neurons in specific regions as the dentate gyrus and the prefrontal cortex (PFC) [122], β-receptors are mainly located in postsynaptic neurons. Three types of β-receptors are described in the brain, i.e. β_1-, β_2- and β_3-adrenoreceptors, although, to date, they remain not well distinguished. In mammals, their primary function is to trigger glycogen breakdown during increased local neuronal activity [116]. This trigger is made through the activation of G_S protein (a heterotrimeric G protein subunit) which consequently activates adenylate cyclase and produces cyclic adenosine monophosphate (cAMP), further associated with cAMP response element-binding (CREB) protein activation [122]. Therefore, NE-binding to β-receptors ultimately facilitates transmission of action potentials. Moreover, the non-selective activation of these receptors also appear to strengthen synaptic contacts by increasing postsynaptic *α*-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) insertion.

α_1-adrenoreceptors are mainly located postsynaptically and composed of three subtypes (α_{1A}-, α_{1B}- and α_{1D}-adrenoreceptor). Although equally expressed in the hippocampus, cerebral cortex and brainstem, in the thalamus and deep layers of PFC, α_{1A}-adrenoreceptors are preferentially expressed [123]. All three subtypes of α_1-adrenoreceptors are able to increase Ca^{2+} entry *via* voltage-gated calcium channels. Their

stimulation leads to G_q protein activation which in turn activates phospholipase Cβ (PLC) and ultimately to increased calcium release from intracellular stores, thereby increasing cytosolic calcium concentrations [116]. Thus, NE-mediated α_1-adrenoreceptors activation generally modulates the excitability levels of the follower cells [124].

α_2-adrenoreceptors are located on both pre and postsynaptic sites, with their presynaptic location associated with their functions as autoreceptors, involved in NE control release [123]. These receptors are widely distributed in the brain, including the hippocampus and the cerebral cortex. Three subtypes of α_2-adrenoreceptors are described, known as α_{2A}-, α_{2B}-, and α_{2C}-adrenoreceptors. Contrary to the remaining adrenoreceptors, α_2-adrenoreceptors are linked to $G_{i/o}$ protein and its stimulation decreases calcium entry and limits neurotransmitter release [116]. This effect, independent of intracellular cAMP concentrations, allows NE to play an essential role in decreasing excitatory transmission.

Therefore, NE appears to have a crucial, though complex, role in cortical excitability modulation in the human brain. In fact, studies using a drug selectively blocking norepinephrine reuptake, reboxetine, have showed an enhancement of cortical excitability primarily driven by excitatory effects of β-adrenoreceptors [125]. Also, studies using a presynaptic α_2-adrenoreceptor antagonist, yohimbine, that increases brain extracellular NE levels, have shown a corticomotor enhanced excitability in humans [126]. Thus, greater NE levels appear to be related with higher corticospinal excitability.

Moreover, studies conducted in the hippocampus and sensory cortical areas have proved that NE is able to conduct modifications at the level of the synapse [127], therefore, having a critical role in synaptic plasticity.

Two well-described enduring forms of synaptic plasticity are long-term potentiation (LTP) and long-term depression (LTD). LTP, as earlier described, is a strengthening and increase of the synapse efficacy and has been observed often in glutamatergic synapses in various brain regions such as the hippocampus and the PFC [128]. Contrarily, long-term depression (LTD) is a decrease of the synaptic efficacy, weakening specific sets of synapses and complementing LTP.

The direction of LTP and LTD depends on the activation of α- and β-adrenoreceptors. Several studies in the hippocampus have revealed that LTP is enhanced

by NE-induced β-adrenoceptors activation [127]. The activation of β-adrenoreceptors through the activation of G_S protein results in an increase in cAMP production which leads to the protein kinase A (PKA) activation. PKA activation results in its translocation to the nucleus through the nucleus pore, where it phosphorylates (and consequently activates) CREB, a transcription factor, crucial for the late stage of LTP [129] (Figure 1.11).

Thus, LC-NE system exerts a crucial role in modulating cortical excitability and, more importantly, these changes occur on both immediate and long-term timescales, allowing behaviorally relevant events to transform into permanent changes in brain function and behavior.

1.4.2. LC-NE system and cognitive functions

Due to its important role in synaptic plasticity, the LC-NE system has been related to cognitive functions as learning and memory [130]. Importantly, strong evidence have emerged linking many cognitive-related conditions and LC-NE system, as schizophrenia [131], ADHD [132], Alzheimer's disease and Parkinson's disease [133]. The fact that LC neurons show enhanced discharge in response to novel stimuli but rapidly habituate after repeated encounters, suggests that NE may participate in the information acquisition process [130], possibly by the induction of synaptic plasticity through LTP and LTD.

In fact, in prefrontal cortex neurons, which preferentially respond to task-relevant stimuli, methylphenidate administration, a NE/dopamine reuptake inhibitor, has shown to improve cognitive functions such as attention and working memory as well as prefrontal neuronal responsiveness [134].

Through β-adrenoreceptors activation, NE is capable of facilitating LTP and this induction may be the basis for the consolidation of long-term memory [130] (Fig 1.11). In fact, β-adrenergic antagonists have been shown to cause amnesia in spatial memory paradigms [135]. Also, NE levels deficit in mutant mice has shown to impair memory retrieval [136]. Furthermore, NE has been shown to play a crucial, α-adrenoreceptors-dependent, role in tasks involving changes of strategy [137]. Several

other cognitive effects have also been correlated with LC-NE system, including effects on motor learning, response inhibition, working memory and emotional memory [138].

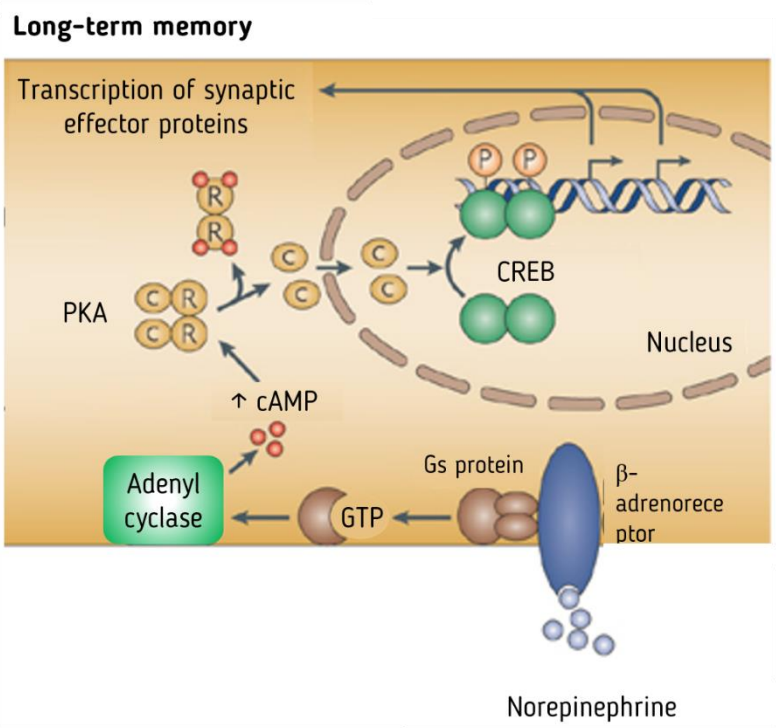

Figure 1. 11 - NE induces potentiation (LTP), the basis for the consolidation of long-term memory. NE binding to β-adrenoreceptors leads to activation of the cyclic AMP cascade by adenyl cyclase and, ultimately, to the activation of the transcription factor CREB, crucial in gene expression. This activation defines the important role of NE in neuromodulation, promoting long-term plasticity underlying memory formation. Adapted from [139].

Studies carried out in non-humans primates [140] and computational modelling studies [141] have revealed that NE modulation of cognitive processes also appears to be involved in decision making processes. This involvement has been reported to depend on the LC's phasic firing rate. Extracellular recordings have revealed that phasic bursts typically precede a behavioral response to a target stimulus and are tightly locked to the timing of the behavioral response rather than to the stimulus onset [142], which is consistent with the LC' role in task-relevant decision making process. Authors hypothesize that this decision-driven LC activation may facilitate behavioral responses once subjects are engaged in the task.

Interestingly, these modulations of cognitive processes are closely related to stress and anxiety [143]. When NE concentration increases due to elevated LC discharge during acute stressful episodes, the NE lower affinity with α_1-adrenoreceptor becomes engaged, impairing functions in PFC and affecting attention and working memory [144].

In fact, large cognitive impairments have been described following chronic stress [145], suggesting down-regulation of NE transmission after chronic stress exposure. These impairments have been suggested to involve dysfunctional response inhibition, resulting in impulsive behavior. Accordingly, blockade of α_2-adrenoreceptors, which increases synaptic NE, have been shown to increase impulsivity levels in rats [146], and in preliminary studies in humans [147].

Overall, considering the wide expression of NE's receptors subtypes in human brain have made of LC-NE system subject of several research studies as a target for therapeutic approaches in cognitive-related conditions as Alzheimer's disease, ADHD and schizophrenia [148].

1.4.3. Pupil size and the LC-NE system

The locus coeruleus-norepinephrine system is functionally involved in the control process of pupil size changes [149].

Although pupil main function in humans is to adjust the amount of light entering the eye in response to luminance changes: contracting in response to a transient luminance increase, a reflex known as the 'pupil light response' [150]; pupil size is not only determined by luminance.

The wide LC projections to several brain regions, particularly regions related to cognitive processing, such as PFC and the parietal cortex, have suggested that pupil size is influenced by high order cognitive processing [151]. Accordingly, several pupillometry studies have established a well described correlation between pupil diameter spontaneous fluctuations and changes in alertness, decision making [152] and arousal level [153], under constant luminance. Relatedly, recent evidence have often associated pupil size fluctuations with several task-related parameters as engagement [149] and task difficulty [154, 155], with greater pupil size increase related to subjects task engagement and high demanding tasks.

These fluctuations have been interpreted in terms of NE-containing neurons activation in the LC nucleus [156], through several amount of evidence including pharmacological, fMRI and EEG studies [158]. Studies carried out in primates have also

related event-driven changes in LC firing activity and pupil diameter [157]. Pupil diameter also appears to covaries with neuronal activity in cortex, which is thought to reflect cortical modulation mechanisms of the LC-NE system [149].

Although the exact mechanism by which pupil size fluctuations are linked to the activity of LC remains undefined, the idea that pupil size may provide a direct index of LC activity and thus, NE release, has received considerable attention. Joshi and colleagues [158] have provided compelling evidence of this correlation in primates. By recording activity in LC and assessing simultaneously pupil size during an auditory task performance, the authors were able to see that fluctuations in the firing rate of LC neurons were consistent with the oscillations in pupil size. Moreover, they observed that stimulation of LC evoked transient increases in pupil size, with maximal pupil change occurring 250–700 ms following the onset of stimulation.

1.5. Objectives

Every aspect of motor behavior relies on the dynamics of excitability of motor cortex [68]. When this dynamics is unbalanced, several motor system impairments may emerge as a consequence, further resulting in physiological disorders as depression [85], Alzheimer's disease [87], Parkinson's disease [83] and ADHD [90].

Pupillary fluctuations have been established to reflect NE-release through LC activation [149], which has shown to be dependent on a number of factors as arousal [114] and stress [115] levels, but also task-dependent factors as decision making [152]. Furthermore, cortical excitability has been closely linked to NE-induced neuromodulation of neuronal plasticity and CS excitability in the human brain [127]. These evidences have allowed to raise crucial questions regarding the crosstalk between cortical excitability and pupil dilation. In particular, the specific role of norepinephrine in CS excitability and, more importantly, how parameters known to have an effect on NE levels are able to differently modulate CS states.

Therefore, the present study aimed at investigating if NE played a role in the modulation of motor cortical excitability during motor preparation. For that purpose, two main objectives were pursued:

I. Investigate whether decision complexity is able to modulate arousal as indexed by task-related pupil dilation.
Taking into account that pupil dilation is a reliable indicator of NE release through locus coeruleus activation [149], and considering that this release is increased in arousal states [148], we hypothesized that task engagement and decision complexity would differently modulate task-related pupillary response as a consequence of the different arousal-induced states.

II. Evaluate if task-related pupillary responses are an indirect indicator of task-related modulation of motor cortex excitability.
Considering the well-established role of NE in motor cortical excitability [127] and, since pupil size fluctuation has been widely described as a reliable indirect indicator of NE release, we hypothesized that pupillary response to conditions involving different decision complexities would allow an indirect assessment of task-related modulation of CS excitability.

2. CHAPTER II. MATERIALS AND METHODS

2.1. Study division: Part I and Part II

The present study was divided in two parts in accordance with its two main goals.

Therefore, Part I mainly focused on the investigation of whether pupil size varied during visuomotor task performance and if increased decision complexity affected these variations; and Part II principally focused on the assessment of CS excitability and its correlation with pupil size changes.

For these purposes, Part I involved pupillometry data acquisition using an eye tracker system. In Part II, in addition to pupil recordings as an indirect measure of arousal modulation, transcranial magnetic stimulation was used to elicit motor evoked potentials and EMG recordings allowed a direct corticomotor excitability assessment. All the previously mentioned measures, both in Part I and Part II, were acquired while subjects performed a cued choice reaction time task.

2.2. Participants

Ten healthy young adults were recruited for the entire study. For Part I, five participants (4 female), aged between 18 and 25 years old (mean age 22±2 years), were tested. For Part II, five participants (5 female), aged between 18 and 35 years old (mean age 30±8 years), were investigated. All subjects were neurologically healthy, with no history of mental illness. Subjects tested in Part II also did not report any history of epilepsy and no contraindication for TMS [159]. All participants were right-handed and had a normal or corrected to normal vision. Participants signed an informed consent prior to data collection, following protocols approved by the Ethics Committee of the Faculty of Medicine of the University of Coimbra, in accordance with the Declaration of Helsinki.

2.3. Procedure

The experiment was carried out in a darkened room where the only source of light was the stimulus presentation screen. Participants sat in front of a computer screen with both hands placed in the response apparatus, palms down and the arms semi-flexed. Since pupillometry data would be acquired in both Part I and Part II, subjects also had to place their heads on a forehead and chin rest. Participants were required to produce a speeded response pressing the correspondent key when a visual stimulus appeared on the screen.

Task was composed of 4 runs, between which participants were asked to relax.

In Part I, a standard keyboard was used as response box and subjects should respond pressing the accurate arrow key according with the ongoing block Cue and target stimuli. In Part II, two numeric keypads were vertically positioned and subjects should respond by abducting the correspondent left or right index finger according with the visual target presented.

Stimuli were presented on a 61,21 cm display monitor (Color Edge CG243W, EIZO) with a refresh rate of 86 Hz and a resolution of 1280x1024 pixels. All participants were positioned at a distance of 68,5-70 cm from the display screen.

Before the experiment started, participants practiced one test run (~5min) to become familiar with the task.

2.4. Task design

The main goal of the present study was to investigate the modulation effect of decision complexity on motor cortex excitability, during the motor response preparation period. Since reaction-time paradigms have proved useful for studying the CS excitability during response preparation [68, 101], a cued choice reaction time (cued-CRT) task was chosen, allowing to investigate motor processes directly involved in the motor preparation period.

The task was designed using Matlab R2017a (MathWorks, Natick, MA), the Psychophysics toolbox (Version 3) and converted also to Presentation (Neurobehavioral Systems, Inc., Albany CA). Although several parameters were adjusted according with the goals of each Part of the study, the main structure of the paradigm was equal for both Part I and Part II. For each block, one indicative cue was presented at the beginning. After a short interval, the motor preparation interval, 4 stimuli were presented sequentially. The indicative cue provided information about the forthcoming stimuli and participants should withhold their responses until the stimuli presentation, which here are equivalent to the imperative signals. The time interval between the cue offset and the 1st stimulus presentation corresponded to the motor preparation or delay period. The study of the neural mechanisms occurring during this delay period would allow a better understanding of the preparation processes underlying a motor response. The imperative stimuli were arrows pointed to the left or pointed to the right, or a sphere. The participants were instructed to respond by pressing a left or right button according to the direction of the arrow as quickly as possible after the stimuli emerged on the screen and refrain from responding when presented with the sphere. After every response, a feedback was provided informing the participant of his correct or incorrect response. A response was considered correct when, besides being accurate, it was given during the period of stimulus presentation (<600 ms). An incorrect response was either an inaccurate or slow response. At the end of each block, a fixation cross was presented during 4000, 6000 or 8000 ms.

In order to investigate whether active motor task engagement had an effect on motocortical excitability, a Passive-viewing condition was included, where participants were instructed not to respond. In order to investigate the effect of decision complexity, 2 other conditions were included: Condition 1 (C1) and Condition 2 (C2). The passive-viewing condition, as the denomination suggests, did not require any motor response and, thus, subjects should not give any response during stimuli presentation. In C1, the less complex active condition, the preparatory cue specified the forthcoming target stimuli. In C2, however, the cue presented indicated that two types of target stimuli might occur and that subjects should prepare the two possible motor responses and decide which response should be delivered according to the target stimulus that

emerged on the screen. This condition corresponded, therefore, to the more complex condition.

The cue was either two spheres (Passive-viewing condition), two left or right arrows (Condition 1), informing the subject which arrow would appear as target stimuli, or an ambiguous cue composed of one of each arrows (Condition 2) and, therefore, the subject should be prepared for each one of the target stimuli that could appear with equal probability, which could be accordingly either one left or right arrow (Figure 2.1).

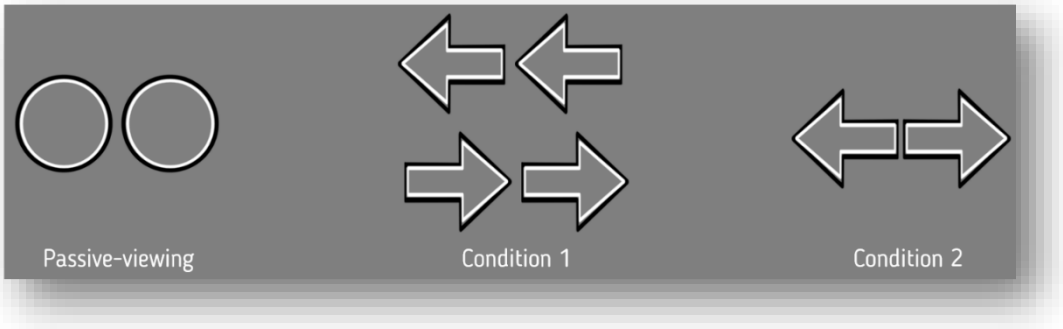

Figure 2. 1 - Types of cues composing the designed task according to the respective condition: two spheres (Passive-viewing condition), two left or right arrows (Condition 1) and two arrows (one of each direction) (Condition 2).

In order to maintain constant the luminance values (see Appendices for luminance measures of the different stimuli used in the task) of the visual task, cues were designed with one black and one white contour lines, and target stimuli were designed with a chess pattern. In order to participants could easily distinguish a cue from a stimulus, stimuli were composed of only one symbol: accordingly, either one sphere (Passive-viewing condition) or one arrow (C1 or C2 conditions) (Figure 2.2).

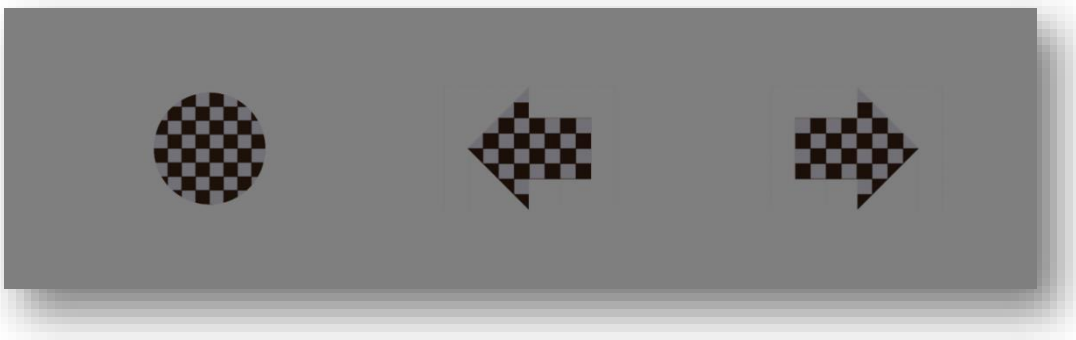

Figure 2. 2 - Types of stimuli: a chess pattern was chosen in order to keep luminance levels constant in the entire visual task.

2.4.1. Part I

The experiment was composed by 4 runs with an approximate duration of 10 minutes each. Every run contained 36 blocks, each one composed by one cue, 4 stimuli and a rest period (fixation cross).

After the presentation of the preparatory cue during 1500 ms, an interval between cue offset and 1st stimulus presentation (which here corresponds to the imperative signal) had a fixed duration of 500 ms. This fixed interval corresponded to the motor preparation or delay period. The 1st stimulus remained visible for 600 ms and participants should respond during this period, with a response feedback being presented after this time (Figure 2.3). Afterwards, a blank screen of duration randomly distributed between 100 and 500 ms was presented before the next stimulus onset. The block sequence would continue as described until 4 total stimuli were presented. Subsequently, a fixation cross was presented during 4000, 6000 or 8000 ms, thus marking the end of the presented block.

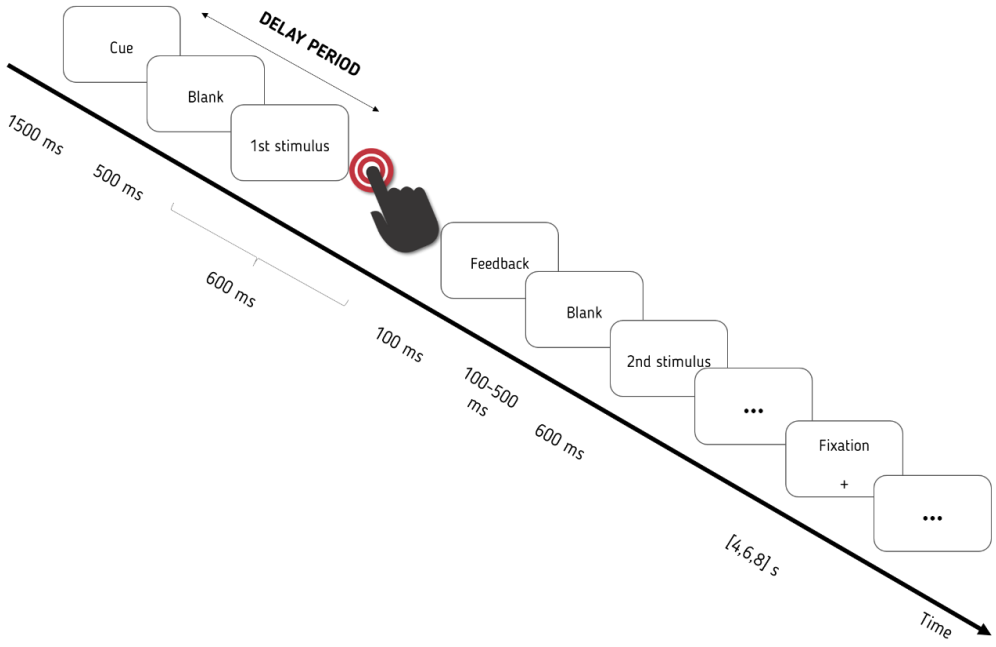

Figure 2. 3 - Time course of the computed visual task in Part I: a cue (1500 ms) was followed 500 ms later by the 1st stimulus (imperative signal) that lasted for a fixed time of 600 ms. The imperative signal arise indicated that the response should be initiated. Correct responses, besides accurate, should be given during the stimulus presentation time. Every response would be followed by a respective feedback informing participant whether it was correct or incorrect. A blank screen with variable delay (100-500 ms) appeared before the next stimulus was presented. At the end of the 4th stimulus and its respective response feedback, a fixation cross lasted for 4000, 6000 or 8000 ms, sequentially, indicating the end of the ongoing block.

Participants should respond by pressing the key in a standard keyboard, using the information provided by the preparatory Cue, when the visual stimuli appeared on the screen. No restrictions were made and subjects could respond using either one or both hands.

Every run was composed by 10 blocks of each condition, comprising a total of 40 blocks per condition in the entire experiment. Additionally, another type of blocks were added to the experiment. Similar to the other blocks, an initial cue was presented; however, no target stimuli appeared, contrary to what happened in the other blocks. Consequently, in these blocks, referred to as 'Cue-only' blocks, no response was required.

These Cue-only blocks comprised 20% of the entire number of blocks in the task and contained the three conditions in equal proportion (1/3). Therefore, there were 6 'Cue-only' blocks in every run (2 per condition), comprising a total of 8 'Cue-only' blocks per condition in the entire experiment.

Although participants were previously informed of the existence of Cue-only blocks, the two types of blocks were intermingled in every run, so participants could not predict the next block's nature. Also, the cues used were equal to the ones used for the remaining blocks and the timings were also the same, ensuring that the only difference between the Cue-only blocks and the remaining blocks was the absence of target stimuli.

Cue-only blocks were added in order to allow a better understanding of the motor processes regarding the motor preparation interval when subjects were cued for the response but no actual response was given. Since blocks were randomly distributed, participants would only perceive the type of blocks they were being presented after the cue presentation, after realizing no target stimulus was being presented. Therefore, a motor response was, indeed, prepared and the existence of these blocks would allow a clearer understanding of pupil diameter fluctuations in the delay period during motor preparation.

2.4.2. Part II

In the second part of the study, the computed visuomotor task comprised 4 runs with an approximate duration of 12 minutes and containing 50 blocks each. Every block was composed by one cue, 4 stimuli and a rest period (fixation cross).

Contrary to the task designed for Part I, the cue presentation duration was reduced to 200 ms and a greater interval between the cue offset and the 1st stimulus presentation was defined (randomly distributed between 1400 and 1800 ms). The longer delay period allowed for a longer period of response preparation. Furthermore, this increased variable motor preparation period was chosen in order to avoid the appearance of the imperative signal of becoming predictable. Thus, allowing to additionally explore changes in motor response preparation when the preparatory period was of uncertain duration, contrarily to what happened in Part I task (Δt(delay period)= 500 ms). After the delay period, the 1st target stimulus was presented during 600 ms, during which subjects should respond (Figure 2.4). A response feedback was consequently presented informing participants if the response was correct or not. Also in here, a response was considered correct when, besides accurate, was given during the stimulus presentation period (600 ms). Finally, a blank screen of variable duration (100-500 ms) was presented before the next stimulus onset. This sequence of events would continue until a total of 4 target stimuli per block and, afterwards, a fixation cross remained visible for 4000, 6000 or 8000 ms, marking the end of the current block.

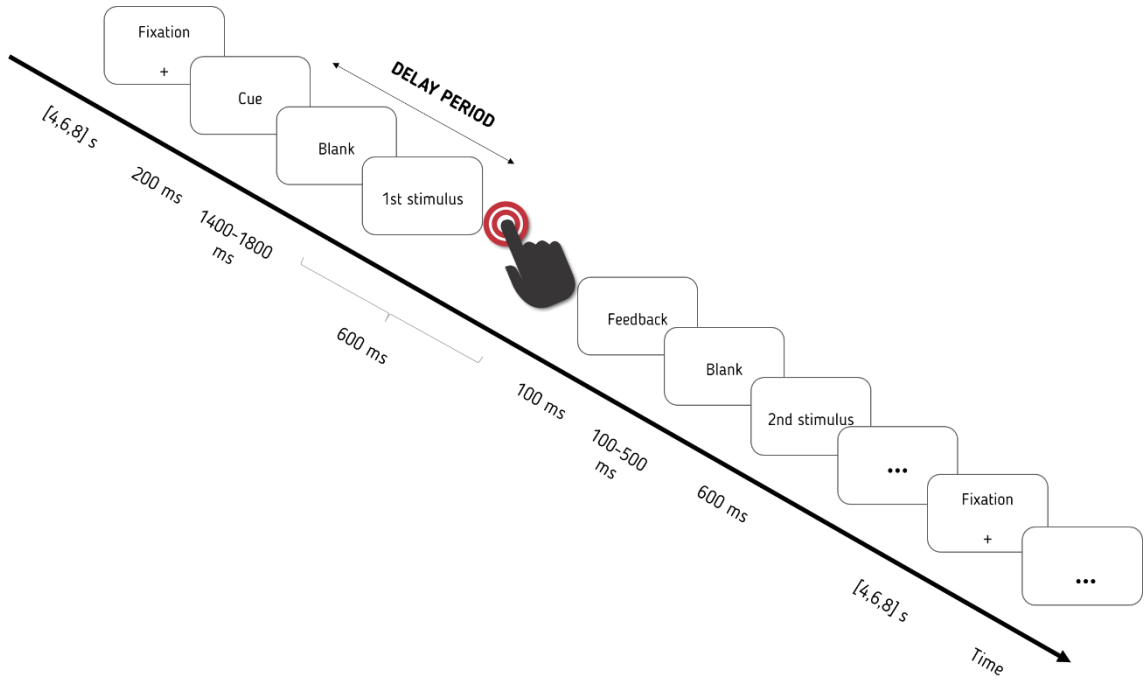

Figure 2. 4 - Time course of the computed visual task in Part II. A preparatory cue was presented during 200 ms and, after a variable time between 1400 ms and 1800 ms, was followed by the 1st stimulus (imperative signal) that lasted for a fixed time of 600 ms. A visual feedback was posteriorly presented during 100ms, indicating a correct (checked icon) or incorrect (cross mark icon) response. A blank screen with variable delay (100-500 ms) appeared before the next stimulus was presented. At the end of the 4th stimulus and its respective response feedback, a fixation cross lasted for 4000, 6000 or 8000 ms, sequentially, indicating the end of the ongoing block.

In this Part, 'Cue-only' blocks were discarded in order to increase the number of blocks of the other conditions. Every run was, therefore, composed by 10 blocks per each condition, comprising a total of 40 blocks/condition in the complete experiment.

The main purposes of Part II were to assess CS excitability using TMS technique and investigate the association between pupil dilation responses and modulation of motor corticospinal excitability. Two different timings were chosen for the TMS pulse delivery (Figure 2.5). To establish a motocortical excitability baseline, the magnetic pulse was delivered before the start of the block. Therefore, in order to ensure statistical significance of this value, 10 additional blocks per run were added (comprising the 3 conditions in equal proportion for the entire task). Thus, in these blocks TMS pulse was delivered during the fixation cross presentation, 100 ms before the preparatory cue onset ($TMS_{BASELINE}$). For the second timing and in order to assess CS excitability during the

motor preparation interval, TMS was applied 1300 ms after the cue offset, i.e., during the delay period (TMS_{DELAY}).

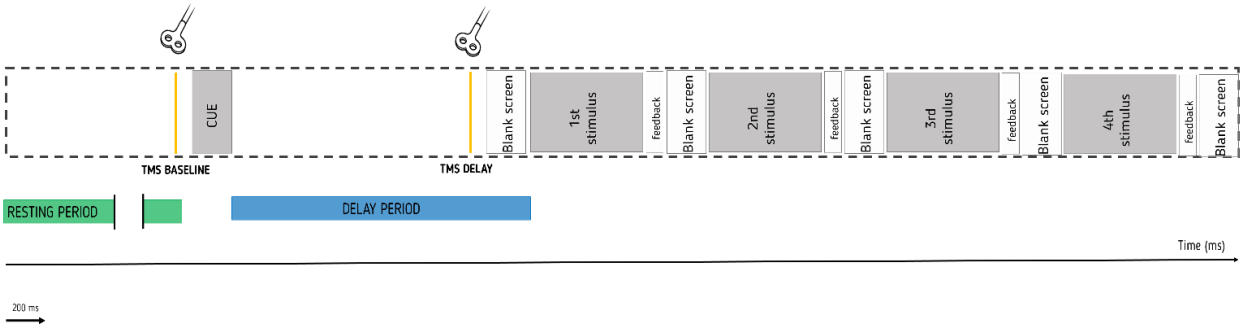

Figure 2. 5 - Task sequence and TMS stimulation. Two different timings were chosen for the TMS pulse delivery: during the fixation cross presentation, 100 ms before the preparatory cue onset ($TMS_{BASELINE}$); and during the delay period, 1300 ms after the cue offsett (TMS_{DELAY}).

Furthermore, in order to investigate the task-related pupil response without the interference of the TMS pulses, the TMS pulse was not given in every block. In the blocks with TMS, only one pulse was delivered per block in order to ensure high reliability in the evoked response amplitude measures [161]. Thus and to obtain statistically significant CS excitability measures, in 30 out of 40 blocks per condition a TMS pulse was delivered. In total, per participant, were acquired 30 $TMS_{BASELINE}$ MEPs values (10/condition) and 30 TMS_{DELAY} MEPs values per condition. The remaining 10 out of 40 blocks per condition had no TMS pulse delivery.

Contrary to Part I, in Part II participants were required to use both hands performing an abduction with either left or right index finger. Two numeric keypads were placed vertically with the key surfaces facing laterally. Subjects placed their index fingers against a key on each vertical keypad in order to respond with a key press by moving the index fingers of each hand inward in a lateral abduction (Figure 2.6). This movement has been described as optimal for measuring electromyography responses from the index muscle (first dorsal interosseous, FDI) [161]. Therefore, if the preparatory cue was two left arrows, only left arrow stimuli would be presented and the subject should respond using the left index finger to laterally press the button once he/she detected the target stimuli. Otherwise, if the cue was two right arrows, only right arrow stimuli would be presented and the subject should press the keypad button using the right index finger abduction

once he/she detected the target stimuli. Alternatively, if the preparatory cue was ambiguous (Condition 2), both arrow stimuli could be presented and the participant should be prepared for any of the two responses and wait for the stimuli presentation to respond accordingly to the target stimuli.

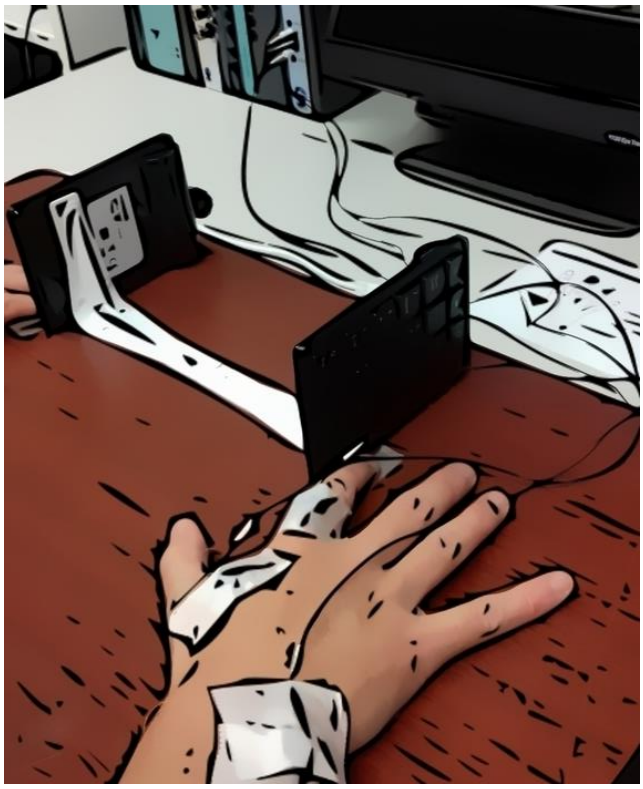

Figure 2. 6 - Response setup. Keypads were positioned vertically in order to allow the optimal FDI movement of the index fingers for the EMG recording. Participants responded by making a lateral abduction movement from the correspondent index finger, according to the preparatory cue information.

2.5. Pupillometry

In order to investigate whether pupil size fluctuations were affected by decision complexity, either in Part I and Part II, eye movements and pupil size of each participant were recorded during task performance using a Tobii X120 Eye Tracker. This remote, non-intrusive eye tracker system employs the pupil center corneal reflection

technique, in which a near-infrared light source used to illuminate the eye causes visible reflections which are recorded by a camera. The image captured is used to identify both cornea and pupil. Eye mode algorithms using the measured diameter of the pupil on the image and multiplying it by a scaling factor allow to obtain the real pupil size. The eye tracker output gives information of pupil size for each eye with each gaze point making it possible to record pupil size variation during task performance [162].

Tobii eye tracker recorded pupil size of both eyes (binocular tracking) with an inclination degree of 40˚ at a rate of 120 Hz. A chin rest allowed to preserve a constant distance of 62 cm between the eye tracker and the participant position.

In order to rule out pupil fluctuations due to pupillary light reflex, task was performed in a small room ensuring the lighting conditions were the same for all participants. Also, luminance measures were carried out for all stimuli presented (Mean luminance = 23,52±0,24 cd/m^2) ensuring all stimuli were isoluminant with the grey background (see Appendices).

All participants completed the standard 5 point calibration procedure for the Tobii system before task started.

2.6. Transcranial magnetic stimulation

For the assessment of the motor cortex excitability during task performance, a single-pulse TMS technique was used.

Magnetic stimulation was applied using a figure-of-eight-shaped magnetic coil (MCF-B65) with a 7 cm diameter connected to a MagPro X100 magnetic stimulator (Magstim, Farum, Denmark) with a peak induced magnetic field of 3,2 T at maximum machine output. The 'figure-8' coil consists of two adjacent round coils with opposite current direction and is widely used in TMS studies where a more focal stimulation is needed (Figure 2.7)

The current of the magnetic pulse was chosen to be biphasic, as frequently used in several TMS studies [62, 94, 96, 98]. Taking this into account, the coil positioning over the primary motor cortex was set in order to elicit an induced current in a posterior-anterior direction (Figure 2.7). This positioning was in accordance with findings reporting

the effectiveness of stimulation as a function of the waveform type and the induced current direction [163]. Accordingly, in the case of the biphasic waveform, the postero-anterior current direction is more effective than the antero-posterior current direction.

Figure 2. 7 - TMS coil positioning in the setup. A figure-eight-shaped coil was positioned tangentially over left M1 in order to elicit induced-currents with a posterior-anterior direction. This direction is ideal when using a biphasic waveform [163].

Although earlier studies have reported inconsistent findings regarding stimulation of the left or right hemispheres, recent approaches have not revealed differences between the two hemispheres when CS excitability is probed prior to the imperative signal, during the delay period [98]. Also, the use of a relaxed small muscle of the dominant hemisphere is a well-established method to determine interindividual differences in cortical excitability [163]. Therefore, the left hemisphere was chosen to probe CS excitability.

Thus, the magnetic coil was placed tangentially on the scalp over left M1 area. The handle of the coil was positioned backward and laterally at a 45˚ angle relative to the

midline, in order to be centered over the primary motor cortex of the right hand. Although its initial placement, an optimal coil position had to be defined for every subject. The optimal site for the coil positioning was defined as the skull location where stimulation elicited visibly greater motor responses in the FDI muscle. Once the position was found, a spot perpendicular to the coil center was drawn with a felt-tip pen to allow consistent coil placement during the experiment.

The resting motor threshold was determined for each participant. RMT was defined as the minimal TMS intensity required to evoke motor responses (MEPs) with a peak-to-peak amplitude greater than 200μV in the relaxed FDI in at least 5 out of 10 consecutive trials [68, 94, 96]. Across the five participants, the averaged RMT was 64% (SE=2,59%) of maximum stimulator output.

The TMS intensity for the experiment was set at the suprathreshold level, 115% of RMT, in order to allow a direct stimulation of M1 and elicit descending volleys of activity that could be recorded. The mean intensity used during the task among participants was 74% (SE=2,99%).

Finally, TMS pulses were delivered respecting a time interval of 5s between them in order to avoid persistence following the end of the stimulation and accumulation over time [164].

2.7. Electromyography Recordings

In order to record MEPs amplitudes, surface electromyography was recorded from the first dorsal interosseous of the right index finger using bipolar silver-chloride electrodes and Biopac MP-160 electromyograph (Biopac, U.S.A.). Abrasive gel (Nuprep Skin Prep Gel) and ethanol were used to prepare the hand skin and lower the impedance between the skin and electrodes. The active electrode was placed over FDI, the reference electrode over the metacarpophalangeal joint and the ground electrode over the ulnar styloid process (Figure 2.8).

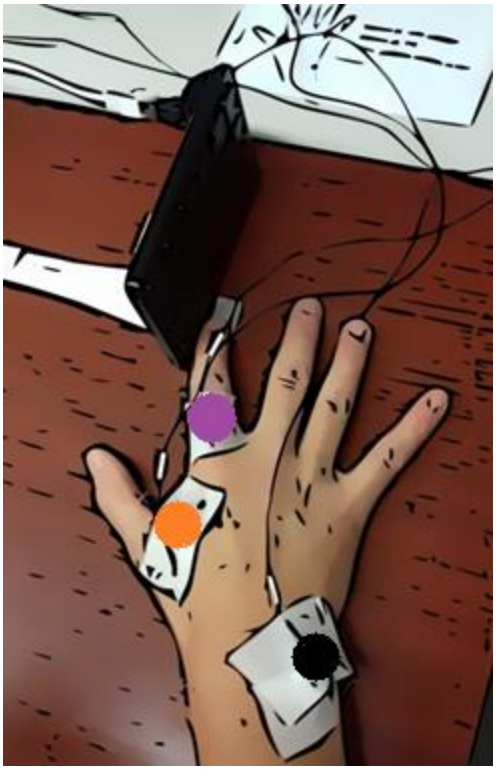

Figure 2. 8 - EMG electrode configuration. Active electrode was placed over the FDI muscle (purple dot), reference electrode was placed above the metacarpophalangeal joint (orange dot) and ground electrode was placed over the ulnar styloid process (black dot).

The EMG signals were amplified with EMG100C (1000 gain) (BIOPAC, Goletta, CA), bandpass-filtered between 1-500 Hz, digitized at 5 kHz by an A/D interface (Micro1401; Cambridge Electronics Design, Cambridge, UK) and recorded using AcqKnowledge 4.4 (BIOPAC, Goletta, CA).

2.8. Experimental setup

The experimental setup for Part I is represented in Figure 2.9. The eye tracking system external unit (1) was connected to the computer used for the stimulus presentation (2) via USB cable and was also connected to the acquisition desktop (3), where the Tobii software was installed, via a local area network (LAN) connection using an Ethernet cable. Finally, the stimulation computer (2) was able to send analog triggers to the acquisition computer, providing information of the task sequence presentation, via a TCP/IP communication protocol.

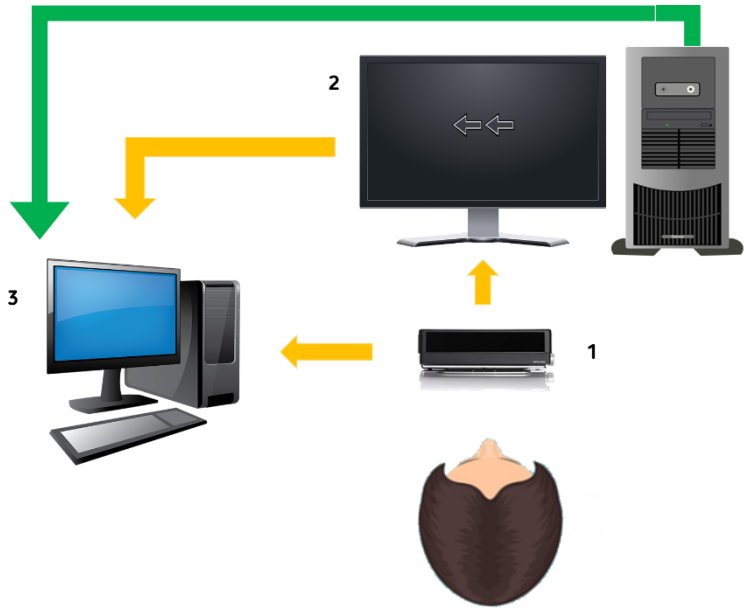

Figure 2. 9 - Setup in Part I. (1) Tobii eye tracker external unit, (2) stimulation computer, (3) pupillometry data acquisition computer. The computer running the visual task (2) was connected to the acquisition computer (3) allowing synchronization and trigger sending (green arrow).

In Part II, besides the pupillometry data acquisition during the task performance, TMS pulses were also delivered and EMG recordings were made. Therefore, a new setup was needed for this part (Figure 2.10). In addition to the connections already made regarding the eye tracker system (1) and the pupillometry data computer acquisition (2) connection to the stimulation computer (3), new connections had to be established ensuring the accurate synchronization and the triggers reception/sending between machines.

The magnetic stimulator (4) was connected to the stimulus computer via a parallel port connection (LPT1), allowing digital triggers to be sent to the TMS machine while task was running in Presentation software and, therefore, controlling magnetic pulses delivery time automatically.

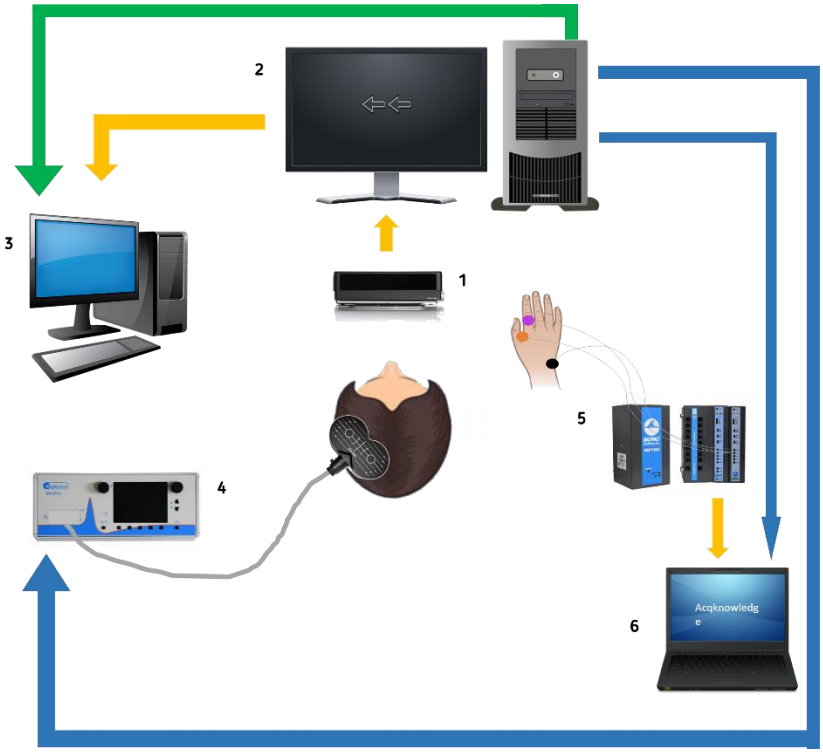

Figure 2. 10 - Setup in Part II. (1) Tobii eye tracker external unit, (2) stimulation computer, (3) pupillometry data acquisition computer, (4) magnetic stimulator, (5) electromyograph, (6) EMG responses acquisition computer. The computer running the visual task (2) was connected via a parallel port to the magnetic stimulator and via a serial port to the EMG acquisition computer (blue arrows).

The motor evoked responses elicited in the FDI muscle were recorded using an EMG BIOPAC recorder (5), which was connected to the EMG acquisition computer (where AcqKnowledge 4.4 software was recording the electromyography responses) (6), via an Ethernet connection.

The stimulation computer was also connected to the EMG recorder via a serial port connection (COM1). This connection allowed sending digital triggers via Presentation software facilitating the electromyography data analysis and interpretation.

2.9. Data analysis

All statistical tests were performed using the IBM SPSS Statistics 23 (IBM Corporation) and had an alpha level of 0.05.

2.9.1. Reaction Time

The software used for the task design and presentation (Presentation [Neurobehavioral Systems, Inc., Albany CA] allows the user to record every sequence of stimulus through a log file. The code generated for the task was developed in order to record the reaction time of every keyboard response given by the subject. Additionally, a script was developed using Matlab R2017a (MathWorks, Natick, MA) for the RTs analysis.

After extracting all the triggers and the recorded information from the log file, the entire task was divided by blocks and each block was identified according with the type of condition. For each block, four stimuli were presented. All the correct responses, i.e., accurate responses and given during the stimulus presentation, had a reaction time associated. Therefore, the number of responses given during the stimulus presentation was also extracted and an average RT value for correct responses was calculated for each block. Incorrect responses given in other moment of the block were not taken into account.

Finally, after collecting the reaction times for every block across the five participants, both in Part I and Part II, a mean RT value was calculated for each condition.

The RTs were subjected to paired-sample *t*-tests between conditions (C1 and C2). A Pearson product-moment correlation was run to determine the relation between RT and response accuracy. A two-way repeated measures ANOVA was also performed with Condition and Pulse as within-participant factors to investigate the influence of the TMS pulse in RTs between blocks with TMS and NO TMS.

2.9.2. Pupillometry data analyses

Pupillometry data were exported from Tobii Pro Studio and subsequent analysis was made using MATLAB scripts and EEGLAB toolbox [165]. The raw pupil data

for both right and left eye was smoothed using a 5-point average filter. Afterwards, data were corrected for blinks (which is correspondent to negative pupil size values in the eye tracking recordings) and linearly interpolated for missing values. Finally, outliers were removed using the interquartile range as a threshold (Tukey method).

However, the left eye, for all subjects, appeared to show a high amount of missing data due to failures of the eye tracker to detect the left eye. Therefore, considering the strong correlation between left and right pupil diameters (r>0,9) [166], the pupillometry data concerning the right eye was chosen for analysis.

The recordings were divided by blocks, according with the digital triggers sent by the stimulation computer, using MATLAB scripts. Next, using EEGLab, continuous data were epoched (Part I: -1500 to 6000 ms; Part II: -1000 to 8000 ms) surrounding the trigger informing the Cue offset. The epochs were visually inspected and remaining noisy epochs were marked for removal.

Baseline, defined as the interval from -200 ms to 0 ms, was removed from each data point in order to study cue evoked pupil changes and to reduce inter-subject variability in terms of pupil size, in accordance with earlier studies [166, 167].

The pupil size variations were averaged across participants for each recorded time frame, saved according with the respective condition and plotted as a function of time after the Cue offset (Figure 2.11).

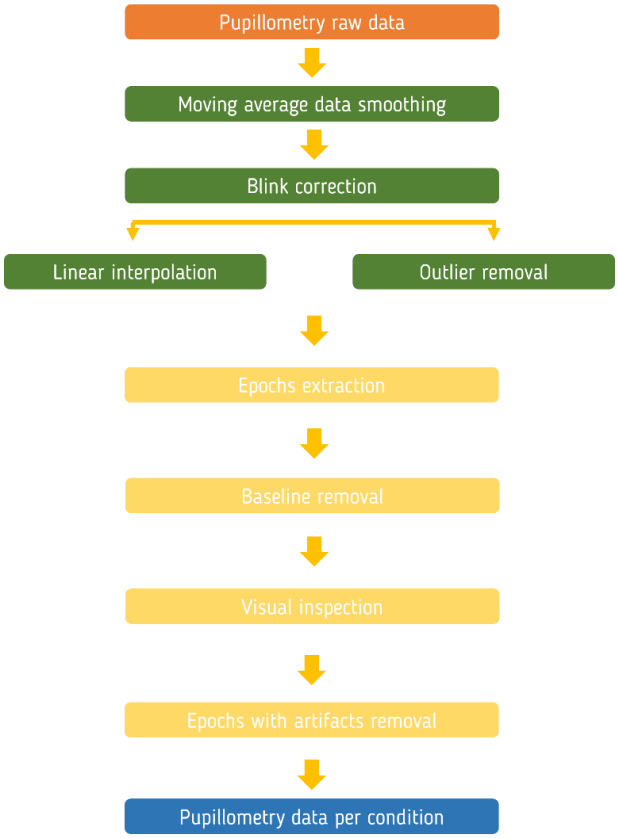

Figure 2. 11 - Scheme of the pupillometry data analysis workflow. Raw data was pre-processed with appropriately developed MATLAB scripts and epochs were extracted with the toolbox EEGLab. Pupillometry data organized per condition was the output of this analysis, allowing plotting of pupil response as function of time and group analysis.

The pupillometry data was subjected to a two-way repeated measures ANOVA with Condition and TMS pulse as within-participant factors to investigate their influence in pupil response in blocks with and without TMS pulse between the three conditions: C1, C2 and Passive-viewing. Further post-hoc paired *t*-tests were performed to allow a better understanding of the statistical differences between C1 and C2; and in order to investigate the influence of the magnetic pulse on the pupil response within each condition.

2.9.3. CS excitability analyses

To assess CS excitability during the task performance for the three conditions, motor evoked potentials peak-to-peak amplitudes were compared across participants. P-t-p amplitude was defined as the difference between the minimum and maximum elctromyographic signal 20 to 50 ms after the TMS artefact (Figure 2.12). The MEPs

amplitudes and background EMG activity were manually investigated using the AcqKnowledge 4.4 (BIOPAC, Goletta, CA) software.

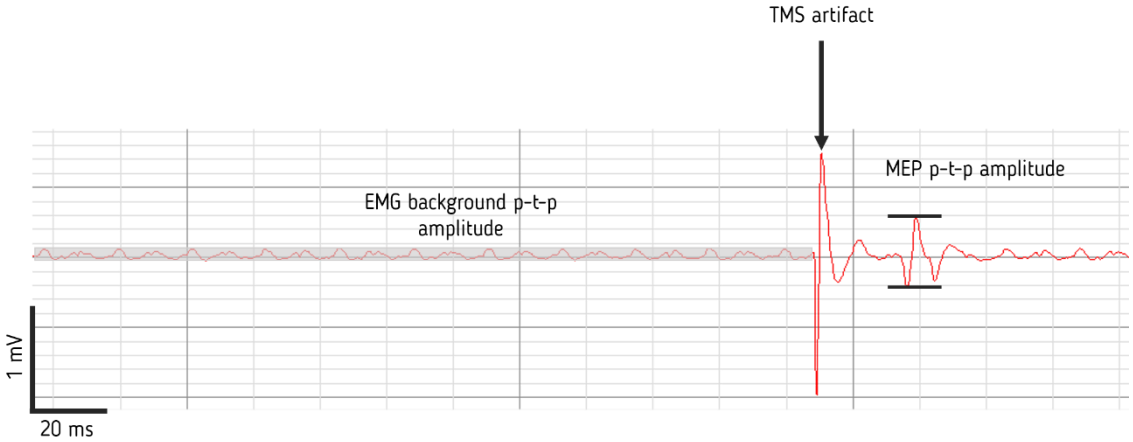

Figure 2. 12 - Example of a motor-evoked response (MEP) elicited in Part II and recorded using electromyography recording.

MATLAB scripts were developed for analysis of CS excitability data.

In order to prevent contamination of the MEP measurements, blocks with background EMG activity greater than 2.5 standard deviations around the mean of background noise in the 200 ms window preceding the TMS artifact were excluded (6,11 %). No other blocks where MEPs were registered were excluded and no other outlier exclusion method was performed to prevent data overprocessing.

The MEPs amplitudes were categorized by condition and an average across participants was calculated. The absolute amplitude values were analysed but a normalization of the MEPs p-t-p was also carried out. Since the amplitude value of the evoked responses during the resting period ($TMS_{BASELINE}$) did not depend on the condition, an average of the total MEPs amplitudes was made for each subject, which corresponded to the value of CS excitability baseline (MEP Baseline). MEPs recorded for every condition during the delay period were calculated relative to this value and an average across participants was calculated for each condition, allowing an interpretation of the amplitude percent variation for each condition.

To investigate the influence of decision complexity in CS excitability, two-way repeated measures ANOVA tests were performed to assess differences between the three conditions. Paired *t*-tests were also used to compare motocortical excitability between each pair of conditions, for both the resting and the delay periods.

3. CHAPTER III. RESULTS AND DISCUSSION

3.1. Part I – the effect of decision complexity on task-related pupil responses

In the present study, it was first investigated whether pupil size varied during visuomotor task performance and if increased decision complexity affected these variations. In this context, pupil size was recorded while subjects completed a cued-CRT task (see Methods).

Pupil diameter of the right and left eye was recorded at 120 Hz in 5 healthy young adult subjects while performing a computerized visual task. The blink-corrected pupil size recorded during task performance for one subject is shown in Figure 3.1.

Luminance values were measured (see Appendices) and all visual stimuli used were isoluminant with the background so that luminance levels were kept constant during all task. However, even without changes in luminance levels, it is possible to observe that pupil diameter fluctuated. In humans, pupil main function is to adjust the amount of light entering the eye in response to luminance changes: contracting in response to a transient luminance increase, a reflex known as the 'pupil light response' [150]. However, pupil size is not only determined by luminance but has also shown to vary with several others parameters regarding cognitive processing as stimulus awareness and arousal level [153], and decision making [152].

The designed task and its different block types allowed the study of the pupil size modulation related to decision complexity under conditions of constant luminance.

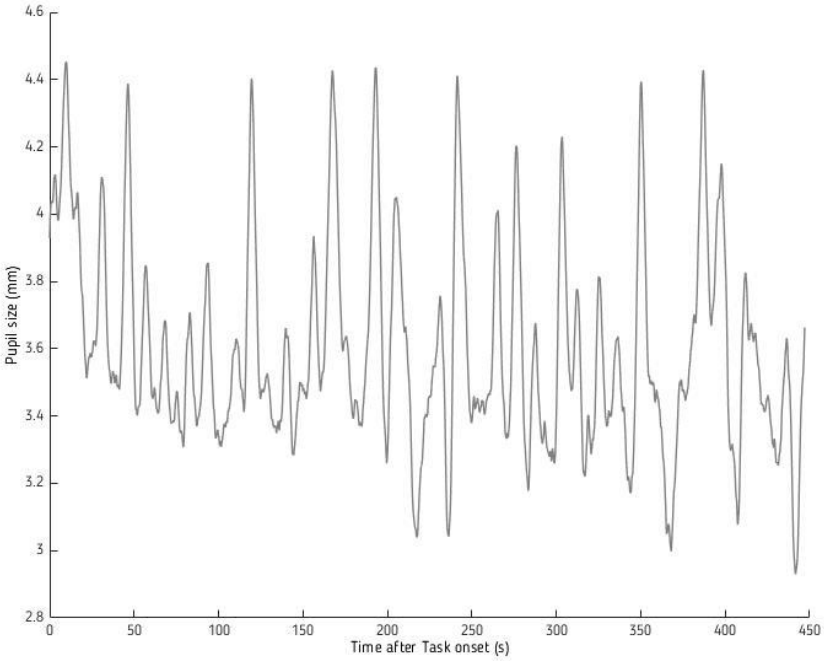

Figure 3. 1 - Time course of pupil response for one subject. Pupil diameter (mm) varied visibly along the CRT task performance.

3.1.1. Reaction Time Analysis

The reaction times for each condition (C1 and C2) were also recorded and are shown in Figure 3.2. For the most complex decision condition blocks (C2), the RT mean value was 434,54 ms (SE=16,06 ms); while for C1, the mean RT value was 362,11 ms (SE=20,53 ms). The RT values between the two conditions were significantly different ($t(4)$=-5,154, p<0.01 – paired t-test) and, as expected, high difficulty blocks (C2) were accompanied with longer reaction times.

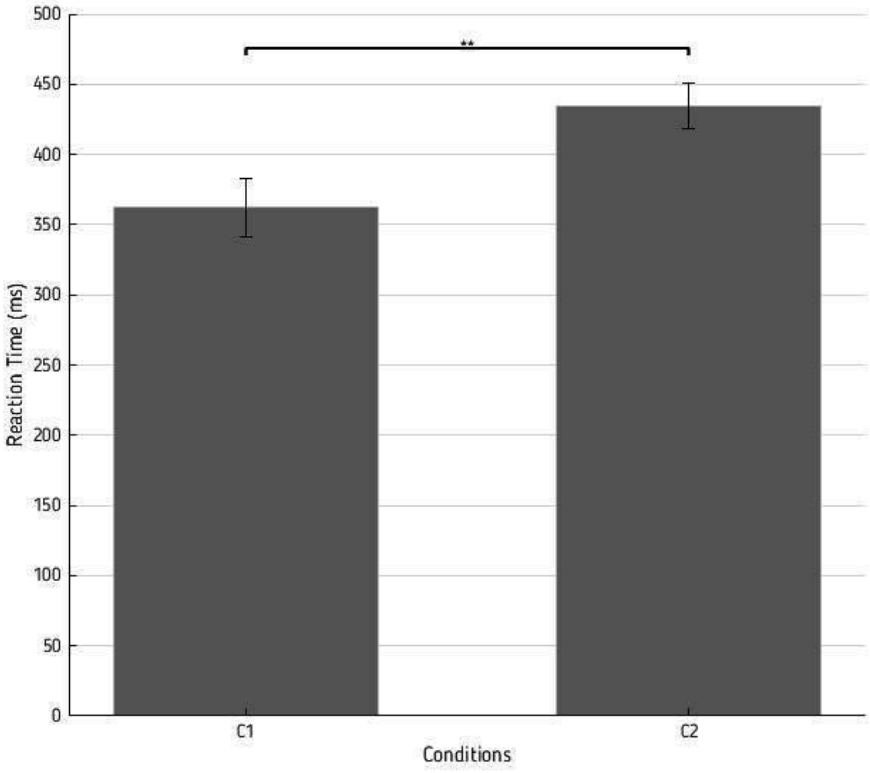

Figure 3. 2 - Reaction times for C1 and C2: less difficulty condition (C1) and more difficulty condition (C2). C1: M= 362,11 ms; SE=20,53 m; C2: M= 434,54 ms; SE=16,06 ms. Error bars represent standard errors. ** = significantly different (*p*-value<0.01).

Several studies have shown that RT reflects the workload in the brain [168] and, more specifically, several aspects of decision making [169] and response planning [170].

Even though the responses required in the task remained the same for both conditions C1 and C2, the type of decision was different, with C1 requiring a simple detection of a visual stimulus, while C2 required the discrimination between two different stimuli and a choice between two possible responses. The significant RT difference indicates a different workload and, consequently, a different cognitive processing time for each condition, reflecting the different decision complexity level between C1 and C2. Therefore, it seems fair to say that a slower RT, even when not specified, will belong to a higher decision difficulty condition. These results are in accordance with recent studies in which task difficulty had a significant effect in RT [171] and with the assumption that a more complex condition requires a longer time of response planning [172].

Furthermore, a correlation between the reaction time and response accuracy was observed either for C1 or C2 among subjects (Figure 3.3). For the less complex condition, C1, a low negative correlation was seen between RT and response accuracy (r=-0,497, p=0.395). On the other hand, for the more complex condition, C2, a low positive correlation was seen between the two parameters (r=0,274, p=0.656).

However, considering the p-value obtained given to the small number of participants included in this study and the variability in the data, conclusions should be made with caution.

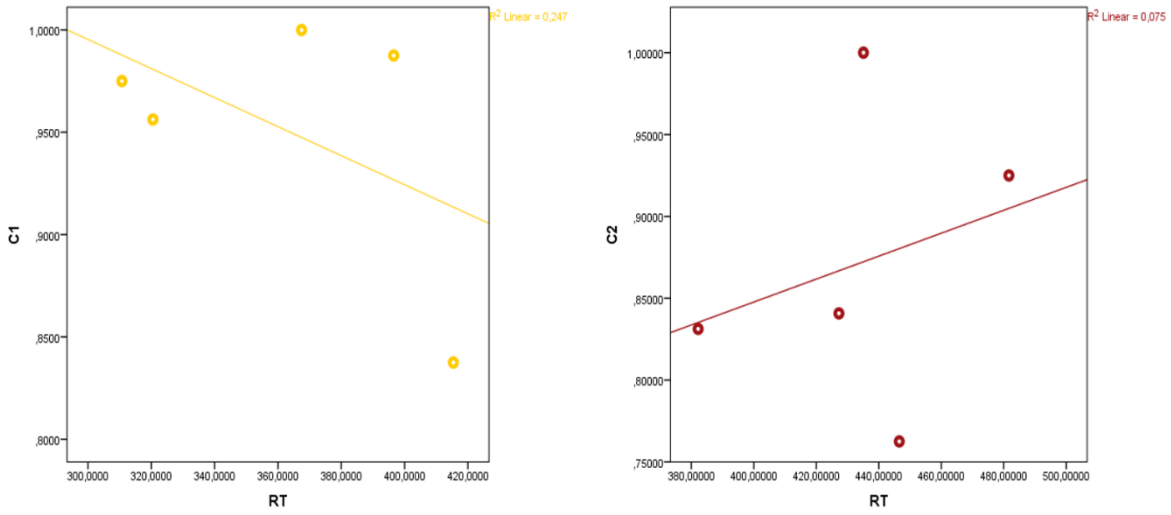

Figure 3. 3 - Pearson correlation between reaction time and response accuracy during task performance. Left: Negative correlation between the 5 subjects averaged RTs and their response accuracy in condition 1 blocks (r=-0,497, p=0.395). Right: For condition 2, a positive correlation was seen across the 5 subjects between their RTs and response accuracy (r=0,274, p=0.656), suggesting that slower responses may indicate greater response accuracy.

The negative correlation seen for C1 indicates that subjects who took longer to respond, in average, tended to err more. It must be considered that, in the present task, responses are incorrect not only when inaccurate but also when are not given during the stimulus presentation time (600 ms). Therefore, this negative correlation may also be due to the fact that subjects who responded slower were more likely to miss the response time.

However, for the more complex condition, C2, in which the preparatory cue was ambiguous and subjects should decide between the two possible stimuli that

emerged on the screen, these data suggests that a slower response was, on average, correlated with a better performance. Since reaction times are often used as an index of motor preparation and programming [173], a slower response in the higher difficulty condition may, therefore, reveal an underlying better response preparation, which ultimately results in a higher accuracy and a better task performance.

3.1.2. Pupillometry data Analysis

In the cued-CRT task used, the subject, after a cue was presented during 1500 ms, should withhold the response until the stimuli appeared on the screen. The cue was either two spheres (Passive-viewing condition), where subjects should not give any response, 2 left or right arrows (Condition 1), informing the subject which arrow would appear as target stimuli, or an ambiguous cue composed of one of each arrows (Condition 2) and, therefore, the subject should be prepared for the randomly distributed stimuli, which could be either one left or right arrow. The passive-viewing condition allowed the investigation of whether pupil size changes between a passive and an active task. For a more detailed description of the task, see Methods.

An overview of the task-related pupil dilation response, time locked to the offset of the cue, is showed in Figure 3.4. Average pupil diameter data between -1500 ms and 6000 ms for each block is displayed, allowing a complete observation of pupil size variation during the entire blocks' duration (on average, 6000 ms). The pupil diameter immediately before cue onset was subtracted from the data.

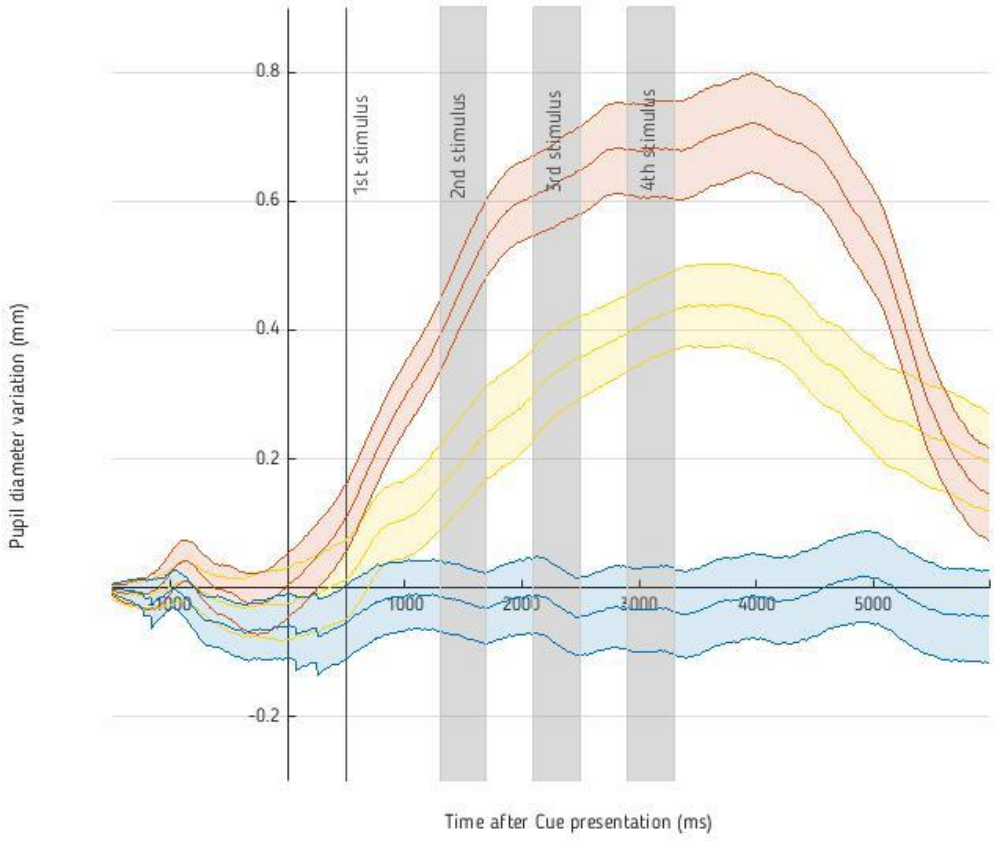

Figure 3.4 - Task-related pupil dilation for all three conditions: Passive-viewing condition (blue trace), C1 (yellow trace) and C2 (red trace). Averaged data for all 5 subjects (n=5) shown from [-1500, 6000] ms, where the origin point corresponds to the moment of the cue offset. Shaded regions represent the mean ± 1 SE.

A clear difference between pupil sizes' time course for the three conditions was observed during the whole block duration (from -1500 up to 6000 ms) and a repeated measures ANOVA comparing the average pupil dilation during the time window between -1500 (cue onset) and 6000 ms after cue offset revealed that this difference was statistically significant ($F_{2,8}$=18,503, p<0.005).

A further post-hoc paired *t*-test showed a significant difference between the less complex condition, C1, and the more complex condition, C2, during this period (t(4)=-3,234, p<0.05), with pupil response increased in average 0,2236±0,0660 mm in C2 blocks more than C1 blocks (Figure 3.5). This difference seen is in agreement with the reaction time previous results (Figure 3.2) and the different difficulty levels between the two conditions that, as explained in Methods, involved distinct decision complexities.

Pupil diameter has been shown to reflect cognitive task demands and to be a measure for mental activity and short-term memory load [154, 155]. Accordingly, a higher task complexity, which requires a greater mental effort and a more complex decision making process, is related to a greater increase in pupil diameter.

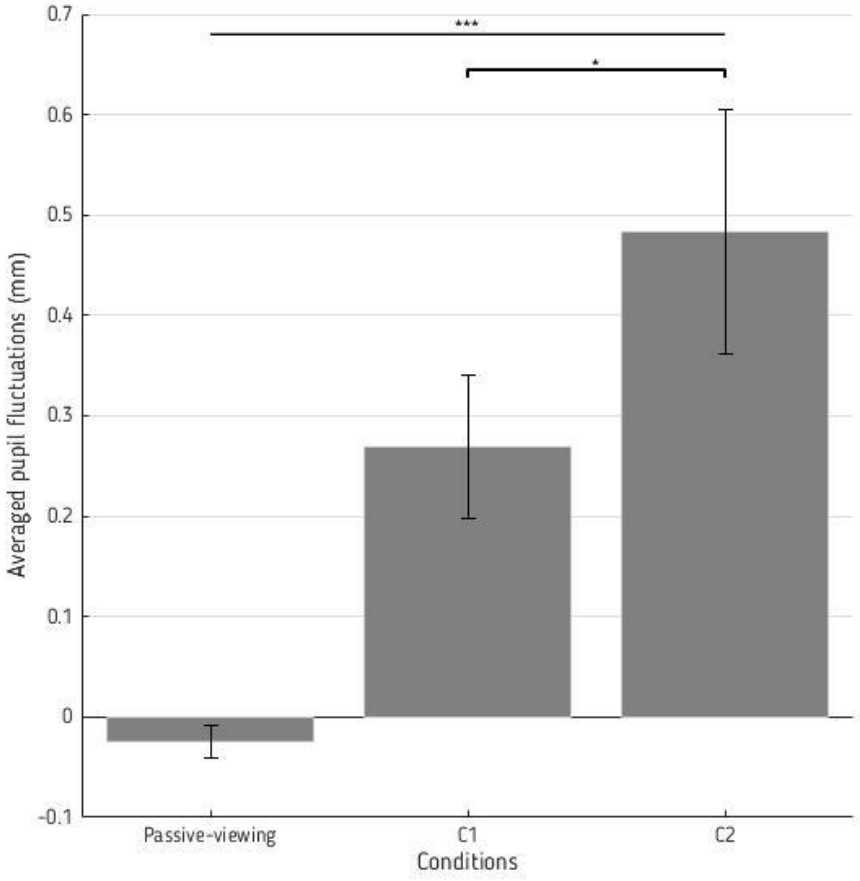

Figure 3. 5 - Averaged pupil dilation for all three conditions for [-1500, 6000] ms time window. C1: M=0,2694mm, SE=0,0709mm; C2: M=0,4830mm, SE=0,1219mm; Passive-viewing: M=-0,0251mm, SE=0,0160mm. Error bars represent standard errors. * = significantly different (*p*-value<0.05); *** = significantly different (*p*-value<0.005).

Interestingly, this difference is only seen after the cue offset. In fact, when testing time window [-1500, 0] ms, which is the time of the cue presentation, no statistically significant differences were seen in pupil response between the three conditions ($F_{1,040;4,159}$=0,837; p=0.468). Although an initial increase in pupil response is seen ~500ms after the preparatory cue onset, around -1000 ms, a posterior notable dip was also recorded in the later part of [-1500, 0] ms time window, closer to the cue offset.

A similar dip has been reported in other studies to be originated from the visual stimulation [154]. However, this dip in pupillary response may also be related to the attenuation of this response during the motor preparation interval, given the great time interval between the cue onset and the 1st target stimulus onset (Δt=2000 ms). Moreover, this dip may be a reflection of cortical inhibition, which typically characterizes the interval of motor preparation [68].

After the cue offset and before the 1st stimulus presentation (which here serves as the imperative signal), corresponding to the time window [0,500] ms, the pupil size started to increase markedly for both C1 and C2. When testing this interval duration, however, ANOVA repeated measures did not reveal significant differences between the three conditions ($F_{2,8}$=3,186; p=0.096).

However, in the time interval [400,500] ms, immediately before the 1st stimulus presentation, differences in pupil response between the three conditions were statistically significant ($F_{2,8}$=5,174, p<0.05). A further post-hoc paired t-test revealed that this significance was only seen when comparing C2 and the passive-viewing condition (t(4)=3,123, p<0.05) (Figure 3.6). This significant difference appears to confirm what was already seen by others that pupil size also reflects whether subjects are engaged in a task or not [149]. Furthermore, this result reveals that this modulation effect of task engagement in pupil response starts prior to the actual motor response, during the delay period.

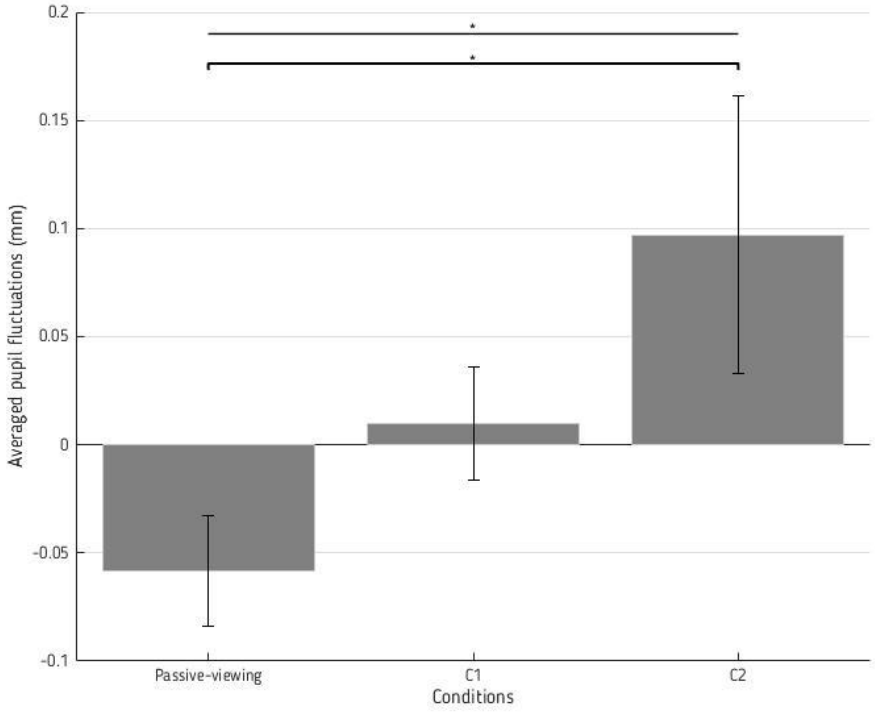

Figure 3. 6 - Averaged pupil dilation for all three conditions for [400,500] ms time window. C1: M=0,0096mm, SE=0,0261mm; C2: M=0,0971mm, SE=0,0642mm; Passive-viewing: M=-0,0583mm, SE=0,0254mm. Error bars represent standard errors. * = significantly different (*p*-value<0.05).

In this period, the mean difference between C1 and C2 was -0,0875±0,0563 mm, meaning that pupil fluctuations for the more complex condition, C2, were already greater than for condition C1. The fact that this difference is seen earlier in the task, during the motor preparation interval, means not only that subjects' pupil response is modulated by decision complexity, but also that the different conditions made subjects prepare differently.

Pupil activity is a reliable indicator of the activity in the locus coeruleus, which is the main source of NE to the cortex. Recent studies using NE reuptake inhibitors, as reboxetine, have shown an enhancement of cortical excitability in the human brain [125]. Therefore, a greater pupil size increase may imply a higher cortical excitability state.

In accordance with the earlier results, a condition involving a more complex decision process (C2) is related to a greater pupil dilation, which indicates greater NE release through LC in this condition. Therefore, decision complexity is able to modulate pupil response. Moreover, considering the crucial role of NE in cortical excitability

modulation, the previous results appear to show that a condition involving a more complex decision may be related to a more excitable CS tract, not only in the entire task duration but also during the motor preparation interval.

Several authors have reported a cortical inhibition prior to motor response, during the delay period, in order to favour and facilitate the upcoming response [68]. The proposed hypothesis in this study was based on these evidences. However, the present results showing positive pupil fluctuations in the delay period and suggesting CS excitability states during this period appear to be in contradiction with the proposed hypothesis of cortical inhibition prior to motor response.

Finally, the maximum peak in pupil size variation for C1 condition (max=0,4780 mm) was reached at 3775 ms after the cue offset and for C2 (max=0,7213 mm) was reached at 3958 ms. The two peaks take place ~1000 ms after the 4th stimulus presentation, which is probably due to the cessation of the task-related cognitive load [174]. The plotting of these responses shows a behaviour that accompanies the block duration with successively increased pupil size overtime and, after the last stimulus was presented, a slow decrease in pupil size was recorded. This behaviour is in accordance with findings that pupil response scales with attention workload during task performance [154].

In addition to the CRT blocks, other type of blocks were introduced in the initial task, cue-only blocks. Similar to the other blocks, an initial cue was presented (the cues were equal to the ones used for the remaining blocks and so was their duration), but no further stimuli were presented. Consequently, no response was required in these blocks and they will be referred to as 'Cue-only' blocks.

These Cue-only blocks comprised 20% of the entire number of blocks in the task, contained the three conditions in equal proportion (see Methods) and were pseudo-randomly distributed throughout the task. These blocks allowed a better understanding of pupil diameter fluctuations when the subject was cued for the response but no actual response was given (Figure 3.7).

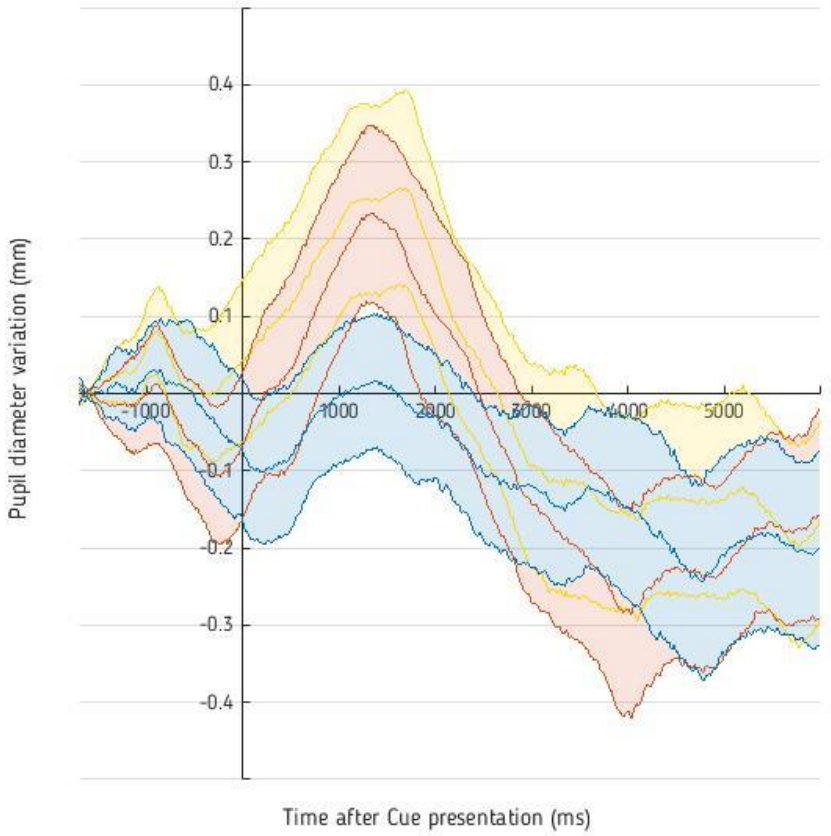

Figure 3. 7 - Pupil diameter changes during Cue-only blocks for the three conditions. Passive-viewing (blue trace), C1 (yellow trace) and C2 (red trace). Averaged data for all 5 subjects (n=5) shown from [-1500, 6000] ms, where the origin point corresponds to the moment of the cue offset. C1: M=-0,0013mm, SE=0,1086 mm; C2: M=-0,0611mm, SE=0,1069mm; Passive-viewing: M=-0,0905mm, SE=0,0943 mm. Shaded regions represent the mean ± 1 SE.

A statistical analysis allowed to see that, in these blocks, considering the entire task time window [-1500, 6000] ms, no significant differences were seen between the three conditions ($F_{2,8}$=1,058; p=0.391). This result appears to be contradictory with what was seen earlier in the remaining blocks (Figure 3.4). However, one must be reminded that, in these Cue-only blocks, the preparatory cue was not followed by any stimuli, thus, no response was required. Therefore, the previous result not showing significant differences between the three conditions appears to be in accordance with the fact that, after realizing the type of blocks they were being presented with, which should have occurred around 500 ms after the cue offset – the time of the 1st target stimulus onset in the remaining blocks- subjects attention waned. This non-engagement would explain

the non-differences reported between the three conditions and, particularly, between the less complex condition C1 and the more complex condition C2. Thus, this result reveals once more that pupil response may therefore be a good indicator of task engagement.

An analysis of pupil response before this moment was also carried out. For the time window [-1500, 0] ms, correspondent to the cue presentation, ANOVA repeated measures analysis did not show significant differences between the three conditions ($F_{2,8}$=3,487, p=0.081), which was also earlier seen for the remaining blocks.

During the delay period ([0,500] ms), a significant difference between the three conditions was seen ($F_{2,8}$=7,815, p<0.05) and post-hoc paired t-tests revealed that the pupil size fluctuations differences during this interval were significant between C1 and the passive-viewing condition (t(4)=3,307, p<0.05). Although only marginally significantly (t(4)=2,297, p=0.083), C1 and C2 differed in average -0,0855±0,0372 mm (C1-C2). However, the number of blocks considered for this analysis was much lower than the remained blocks (only 20%). Thus, a future confirmation of this result with a higher N (# of blocks) and number of participants is needed.

A further analysis of the pupil fluctuations for the three conditions using the time window [400, 500] ms was also carried out in order to investigate how subjects prepared immediately before the time of the supposing 1st stimulus presentation (Figure 3.8).

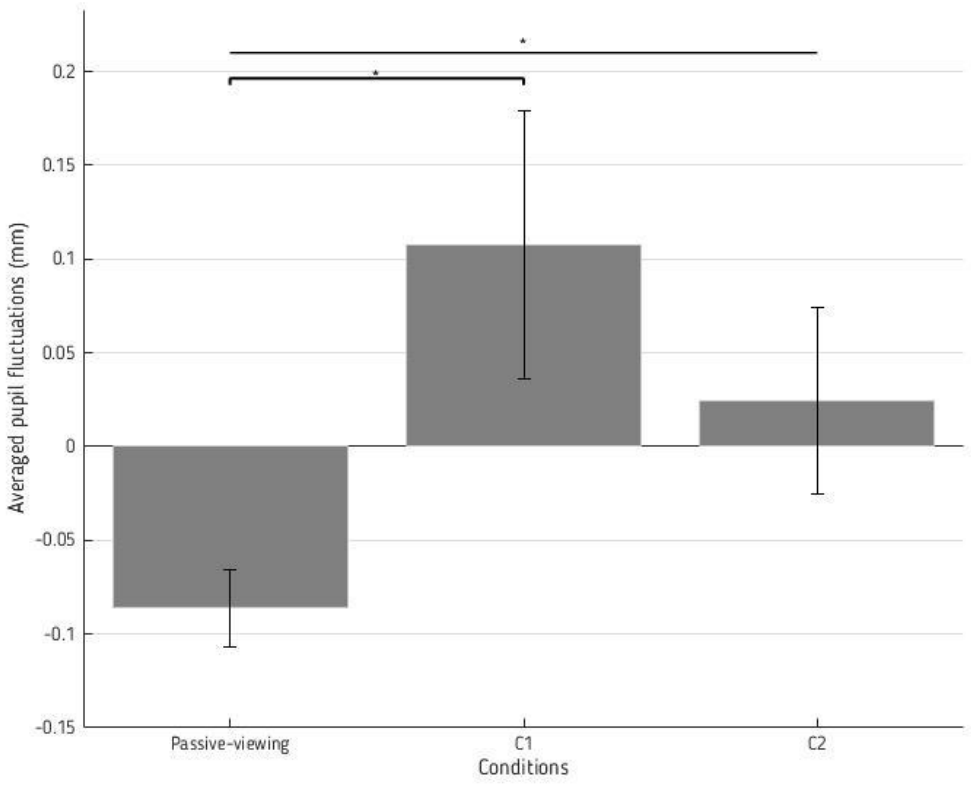

Figure 3. 8 - Averaged pupil dilation for all three conditions for [400,500] ms time window. C1: M=0,1075mm, SE=0,0713mm; C2: M=0,0246mm, SE=0,0497mm; Passive-viewing: M=-0,0862m, SE=0,0205mm. Error bars represent standard errors. * = significantly different (*p*-value<0.05).

Statistical analysis revealed significant differences between the three conditions in this period ($F_{2,8}$=6,893, p<0.05). A significant difference was only reported between C1 and the passive-viewing condition (t(4)=2,981, p=0.041).

In Cue-only blocks, immediately before the 1st stimulus onset, a significant difference is seen between the passive-condition and C1. However, in the remaining blocks and as discussed earlier, during this interval, significance was seen for pupil responses differences between the passive-viewing condition and C2. It must also be emphasized that the only difference between the two block types (Cue-only and remaining task blocks) is the non-appearance of imperative stimuli after cue offset in Cue-only blocks. Therefore, when it comes to pupil fluctuations occurring until the 1st stimulus supposing onset time (500 ms after cue offset), participants would prepare similarly for the two block types. Although a further greater sample size may be needed to allow a better understanding of this result, the differences between either C1 or C2

and the passive-viewing condition during this period in both block types strongly suggests pupillary response as a good task-engagement indicator.

The maximum peaks in Cue-only blocks for C1 and C2 were recorded at ~1683ms and ~1350ms, respectively, much earlier than what was recorded for the remaining blocks. These shorter times, concomitant with the appearance of the 2nd stimulus in the remaining blocks ([1300, 1700] ms) may be justified with the fact that all subjects, before starting the task, were instructed for the random presence of Cue-only blocks. Consequently, after perceiving what block type they were presented with (after 1st stimulus did not appear on the screen), pupil response diminished. This observation also shows what was seen earlier for the passive viewing condition: pupil diameter changes reflect whether subjects are engaged in a task or not during the motor preparation period.

3.2. Part II – the effect of decision complexity on CS excitability probed by TMS and pupillometry

As described in Methods, the visuomotor task used in Part II was slightly different than the visuomotor task used in Part I. The 'Cue-only' blocks were discarded in order to increase the number of trials for the other conditions and reduce time on task, avoiding participant tiredness, and, although it remained a cued-choice RT paradigm and the cues and stimuli used were the same, cue presentation duration was 200 ms and a higher interval was defined between cue offset and the 1st stimulus presentation (randomly distributed between 1400 and 1800 ms). By increasing the interval time between cue offset and 1st stimulus, the period of motor preparation was therefore longer.

CS excitability was assessed with transcranial magnetic stimulation (TMS), while subjects performed the task. TMS pulses were given in two different time points: during the preparatory period, 1300 ms after the cue offset (delay period) (100-500 ms before the 1st stimulus presentation), allowing to assess cortical excitability during the movement preparation period for the three conditions (TMS_{DELAY}); and during the

fixation cross interval, 100 ms before the cue onset, which corresponded to the baseline measure of CS excitability ($TMS_{BASELINE}$).

Motor evoked responses, pupil size fluctuations and reaction times were recorded from 5 healthy young subjects during the entire task performance. Before data acquisition, a preliminary session study was run in order to adjust task parameters (results are shown in Appendices).

3.2.1. Reaction Time Analysis

RT data showed a significant difference between Condition 1 (C1) and Condition 2 (C2) blocks independently of the TMS pulse (NO TMS: $t(4)=-5{,}609$, $p<0.05$; TMS_{DELAY} : $t(4)=-5{,}959$, $p<0.05$) (Figure 3.9).

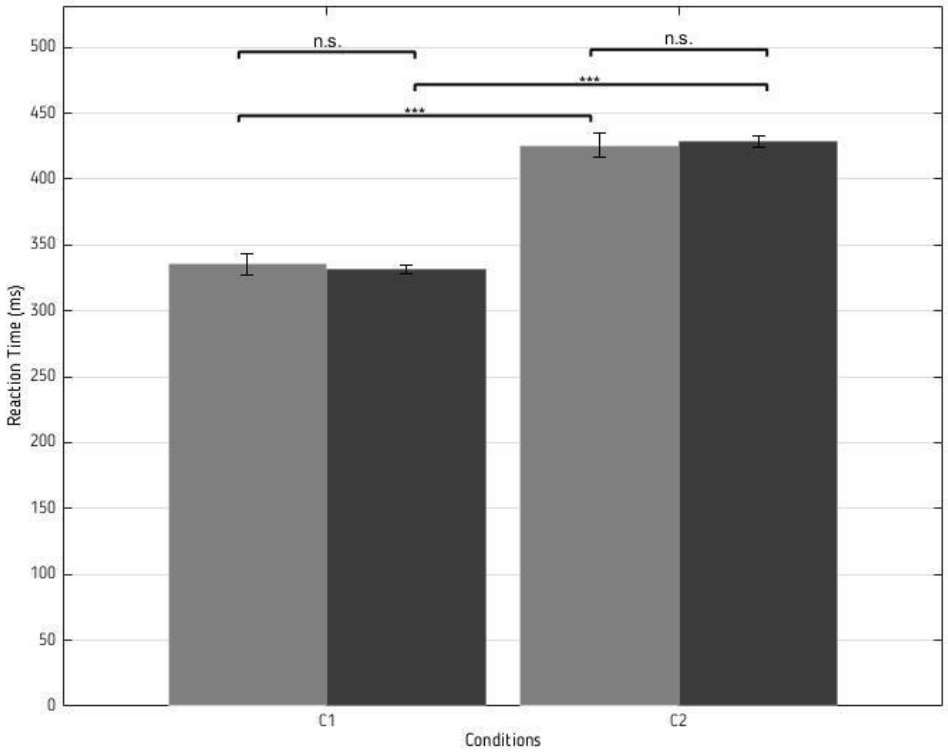

Figure 3. 9 - Reaction times for the two active conditions: less difficulty condition (C1) and more difficulty condition (C2) in TMS_{DELAY} (dark grey) and NO TMS (light grey) blocks. NO TMS blocks: C1: M=334,26ms, SE=6,71ms; C2: 423,59ms; SE=9,26 ms. TMS_{DELAY} blocks: C1: M=331,52ms, SE=3,48ms; C2: M=427,93ms, SE=4,57ms. Error bars represent standard errors. *** = significantly different (p-value<0.005).

As expected, RTs were shorter for C1 condition, which specified the forthcoming response. In blocks with no TMS pulse, the mean RT for C1 was 334 ms (SE=6,71 ms) whereas for C2 was 423ms (SE=9,26 ms). Shorter RTs in C1 blocks were also observed when TMS was applied during the action preparation period (C1: 331 ms [SE=3,48 ms] and C2: 427ms [SE=4,57 ms]). Accordingly, a two-way repeated measures ANOVA revealed a significant effect of Condition on RT ($F_{1,4}$=44,126, p<0.05) for the two types of blocks.

Shorter RTs in C1 in both no-TMS and TMS blocks indicate that participants used the preparatory cues to prepare the appropriate response which resulted in shorter reaction times. On the contrary, the ambiguous cue in the C2 blocks resulted in longer RT, meaning that subjects took longer to prepare the response. This result is in perfect accordance with the higher complexity decision involved in C2 condition, as explained earlier. Since cued-CRT paradigms have been well-established to allow the study of movement preparation and response programming, different reaction times reveal different cognitive processes and movement preparation timings between the conditions.

Earlier studies have shown that when a suprathreshold TMS pulse is delivered over M1 at an intensity high enough to induce motor-evoked potentials in muscles involved in the response, simple RTs are delayed [175]. On the contrary, subthreshold TMS pulse causes the opposite effect and is capable of reducing RT. Several authors have come to the conclusion that these facilitation or slowing effects on RT depend not only on the pulse intensity but a greater effect was seen when different timings of pulse delivery were tested [175]. For instance, Leocani and colleagues [176] showed a time interval of 40 ms period after the magnetic pulse (20 ms after the motor evoked response) of electromyography silence, where no motor response was recorded, which resulted in a delay reaction time.

In the present study, a suprathreshold level TMS pulse was applied (115% RMT) 1300 ms after the cue offset (thus falling 100-500 ms before the imperative signal). It is important to understand that the imperative signal here refers to the 1st stimulus presentation.

A two-way repeated measures ANOVA analysis of the present data, however, did not show any influence of TMS in reaction time ($F_{1,4}$=0,021, p=0.892). Furthermore, no interaction was seen between Condition and TMS ($F_{1,4}$=0,200, p=0.678), meaning that the effect of the TMS pulse did not depend on the type of condition.

According with Leocani [176], a 40 ms EMG silent period appears after the TMS pulse. This EMG silent period, described in previous studies, appears to correlate with the cortical silent period earlier described [177]. Silent period duration is related to the activity of the inhibitory neurotransmitter GABA and is consistent with the $GABA_B$ receptor activation peak which is reported to be 100-300 ms. Many other authors have also reported SP duration to be 100-300 ms [177] in healthy subjects.

However, in the current study the magnetic pulse was given 100-500 ms before the imperative signal, during the motor preparation period, when targeted muscles of the further response were relaxed. Also, considering the reported duration of the SP, TMS pulse would only be able to affect the RT relative to the 1st stimulus and the effect of the magnetic pulse may have been, therefore, diluted in the 3 further stimuli, which explains the non-existent RTxTMS interaction, when considering the averaged RT of each block.

A weak positive correlation between RT and the accuracy of the responses was seen for both C1 (r=0,185, p=0.766) and C2 (r=0,268, p=0.663) (Figure 3.10).

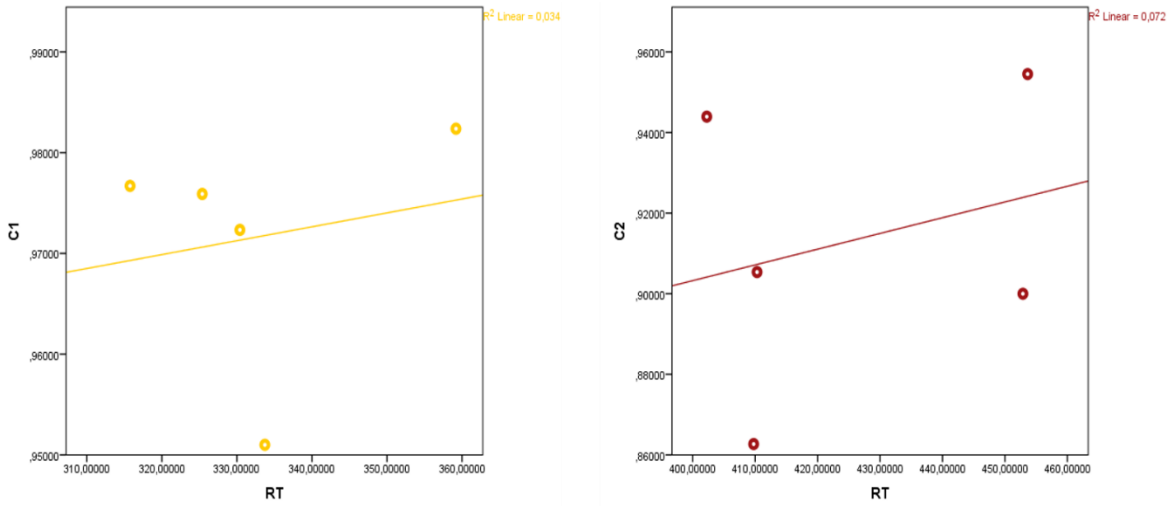

Figure 3. 10 - Pearson correlation between reaction time and response accuracy during task performance. Left: Positive correlation between the 5 subjects averaged RTs and their response accuracy in condition 1 blocks (r=0,185, p=0.766). Right: For condition 2, a positive correlation was seen across the 5 subjects between their RTs and response accuracy (*r*=0,268, *p*=0.663).

Even though, for condition 1, the negative correlation seen in Part I was not replicated here, the r value obtained is close to nullity. The correlation appears to be stronger for the more complex condition, C2. As seen earlier in Part I, a slower RT in C2 blocks appears to be correlated to a higher response accuracy. Although this correlation was not strong and needs further confirmation, the positive relation between RT and accuracy in the higher difficulty blocks seen in the present project may be an interesting preliminary result and boost future studies regarding the speed-accuracy trade-off.

3.2.2. CS Excitability Analysis

Motocortical excitability during the motor preparation period was assessed with single-pulse transcranial magnetic stimulation (TMS) over the primary motor cortex (M1). The magnetic pulse was applied at 2 different times. For CS excitability baseline measures, the pulse was delivered 100 ms before the Cue onset (during the fixation cross presentation) ($TMS_{BASELINE}$: 10 MEPs/condition). The second timing chosen for the pulse delivery was 1300 ms after the cue offset, corresponding to 100-500 ms before the 1st

stimulus presentation, during the delay interval which corresponds to the movement preparation time (TMS_{DELAY}: 30 MEPs/condition).

Before the experiment started, the resting motor threshold (RMT) was determined for each subject (see Methods). Across participants, the averaged RMT corresponded to 64% (SE=2,59%) of maximum stimulator output.

During the $TMS_{BASELINE}$ blocks, before the Cue was presented, the mean MEP±SE amplitudes were 1,2526±0,4642mV, 1,4945±0,4357mV and 1,2282±0,4503mV for the passive-viewing, C1 and C2 conditions, respectively (Figure 3.11). These amplitudes were not significantly different ($F_{2,8}$=0,943, p=0,429) in this period.

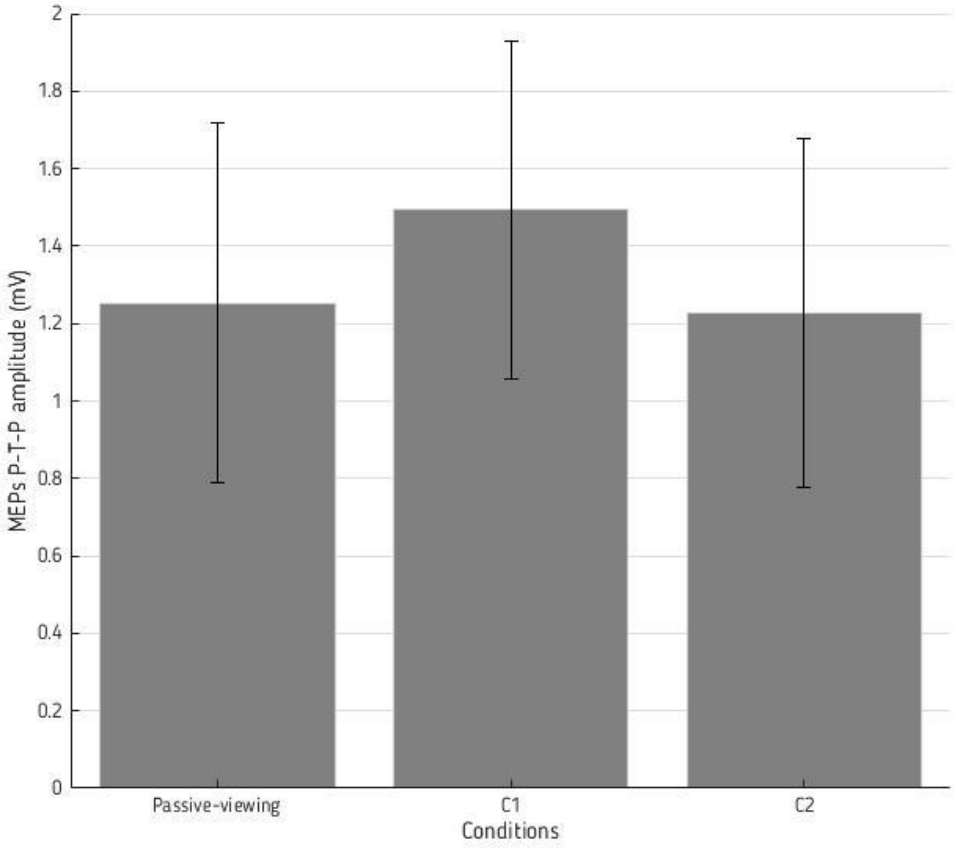

Figure 3. 11 - Group mean amplitudes for the three conditions (Passive-viewing, C1 and C2) during the resting period. Condition complexity had no significant effect on the MEPs amplitudes recorded during this period ($F_{2,8}$=0,943, p=0,429). C1: M=1,4945mV, SE= 0,4357mV; C2: M=1,2282mV, SE=0,4503mV; Passive-viewing: M=1,2526mV, SE=0,4642mV. Error bars represent standard errors.

The MEPs amplitude is a reliable indicator of the CS excitability of the cortical region stimulated in M1 [63]. Therefore, this non-significant difference between the three conditions during the resting period suggests that there are no differences in corticomotor excitability before the subject becomes aware of the decision complexity level of the further condition. Obviously, when subject has no foreknowledge of the forthcoming condition, pre-cue CS excitability is independent of decision complexity.

However, when looking to the delay period (TMS_{DELAY} blocks), the amplitudes between conditions were significantly different ($F_{2,8}$=4,910; p<0.05), showing a modulation effect by the type of condition. Paired t-tests revealed statistically significant differences between C1 and Passive-viewing (t(4)=3,698, p<0.05) and C2 and Passive-viewing (t(4)=2,138, p<0.05) conditions. However, this was not the case when comparing condition 1 and condition 2 (t(4)=-0,672, p=0.538) (Figure 3.12).

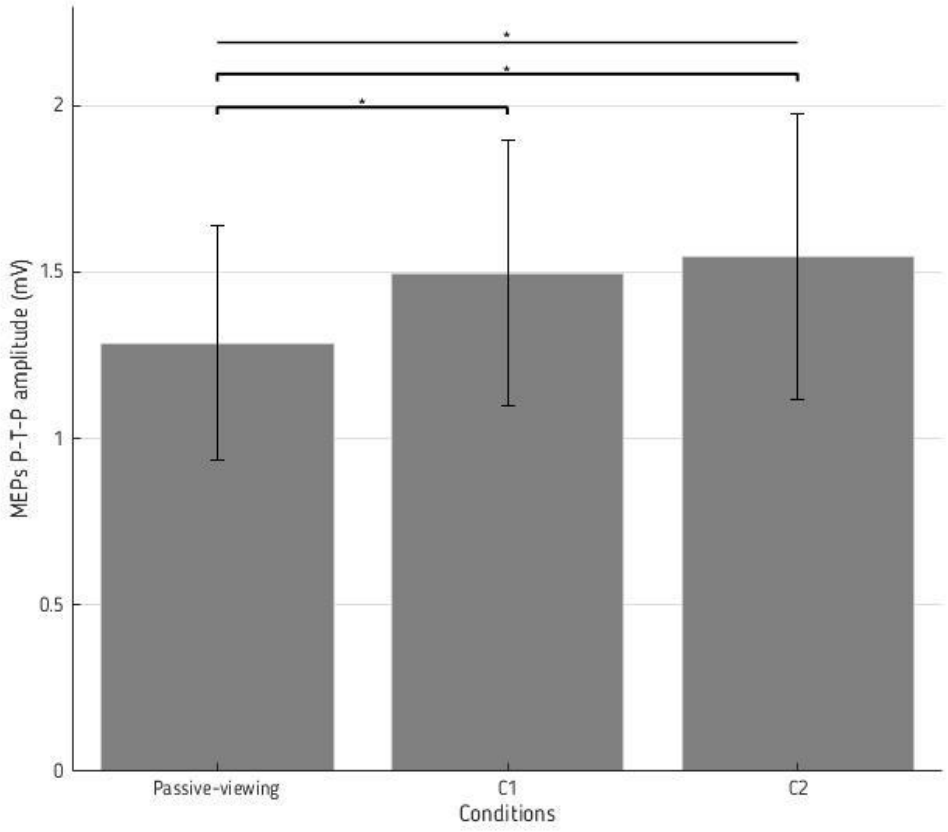

Figure 3. 12 - Group mean amplitudes for the three conditions (Passive-viewing, C1 and C2) during the delay period. C1: M=1,4967mV, SE= 0,3986mV; C2: M=1,5452mV, SE=0,4300mV; Passive-viewing: M=1,2874mV, SE=0,3528mV. Error bars represent standard errors. Error bars represent standard errors. * = significantly different (p-value<0.05).

The averaged amplitude of MEPs either for C1 or C2 were significantly increased by a factor of 0,2093±0,0566 mV and 0,2578±0,1206 mV relative to the passive-viewing condition values, respectively. Therefore, the CS excitability during the motor preparation interval was modulated by task engagement. The fact that the amplitude differences among conditions were not statistically different in the resting period but, when looking into the delay period, which corresponds to the motor preparation period, are considered to be significant, elucidates that the pre-cueing of the forthcoming condition has a direct effect on CS excitability already in the motor preparation interval. Thus, when the subject becomes aware of the condition type, CS excitability differences are registered. Taking into account the fact that in C1 and C2 participants had to produce a motor response while in the passive-viewing condition they did not, it becomes possible to say that task engagement is able to modulate corticomotor excitability during the motor preparation period.

In order to determine if CS excitability increased or decreased during the preparatory period, its amplitude was calculated relative to the baseline. Since the amplitude value of the evoked responses during the resting period was independent of the further condition, an average of the total MEPs amplitudes was made for each subject, which corresponded to the value of CS excitability baseline (MEP Baseline). MEPs recorded for every condition during the delay period were *a posteriori* calculated relative to the MEP Baseline and results are shown in Figure 3.13.

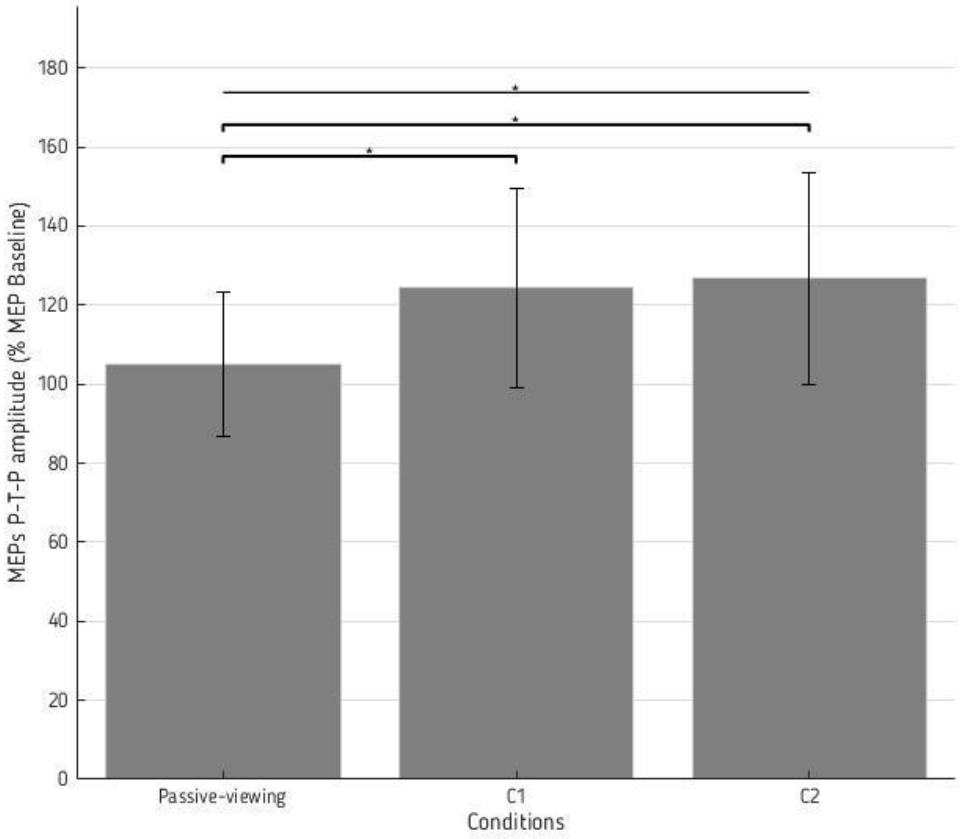

Figure 3. 13 - Averaged amplitudes percentage for the three conditions (C1, C2 and Passive-viewing) during the delay period relative to the averaged MEP Baseline value. C1: M=124,47%, SE= 25,20%; C2: M=126,70%, SE=26,63%; Passive-viewing: M=105,09%, SE=18,30%. Error bars represent standard errors. Error bars represent standard errors. * = significantly different (*p*-value<0.05).

In the delay period, which corresponds to the motor preparation period, the MEPs amplitude values relative to MEP baseline for each subject differed significantly across the three conditions ($F_{2,8}$=5,394, p<0.05). The amplitude of the evoked responses for the two active conditions C1 and C2 was facilitated and an increase of 24% was seen for condition 1 and an increase of 26% was recorded for the most complex condition, C2. The amplitude of MEPs for the passive-viewing condition was only increased, in average, by a factor of 5%, relative to the averaged MEP Baseline value.

This different modulation of the amplitude of the evoked responses suggests different excitability levels for each condition during the motor preparation interval. Thus, during the preparation of a motor response, the foreknowledge of the type of condition subjects would be further presented with had an effect on CS excitability baseline levels. A corticospinal facilitation was seen for the active conditions, whereas for

the passive condition CS excitability remained close to the level of excitability measured in the resting period, with an increase of only 5%.

In accordance, a statistical significant difference was seen when comparing MEPs values between either C1 ($t(4)= 2,754$, $p<0.05$) or C2 ($t(4)= 2,273$, $p<0.05$) and the passive-viewing condition. Also, even though not significant ($t(4)=-0,538$, $p=0,619$), an average differences of 2,23±4,15 % in MEPs amplitudes between C1 and C2 was recorded. This difference may, however, be further explored in future work using a greater sample size.

These results revealed facilitation of the CS excitability levels during the delay period, which are in contradiction with the initial proposed hypothesis, according to which would be expected to register a cortical inhibition during the period of motor preparation.

3.2.3. Pupillometry data Analysis

Pupil response was extracted using a time window of [-1000, 8000] ms, where the starting point plotted (x=0, y=0) corresponds to the moment of the cue offset. This time window allowed an overview of pupil response during the entire blocks duration (~7500ms).

TMS pulse was not given in every block of the task, in order to obtain pupil dilation measurements not contaminated by the TMS pulses. Also, as referred in Methods, in the blocks with TMS pulse, two different timings were tested.

For blocks with no TMS pulse (NO TMS blocks), an overview of pupil response during task performance is showed in Figure 3.14. The data showed is the averaged pupil response for the 5 subjects in each time point.

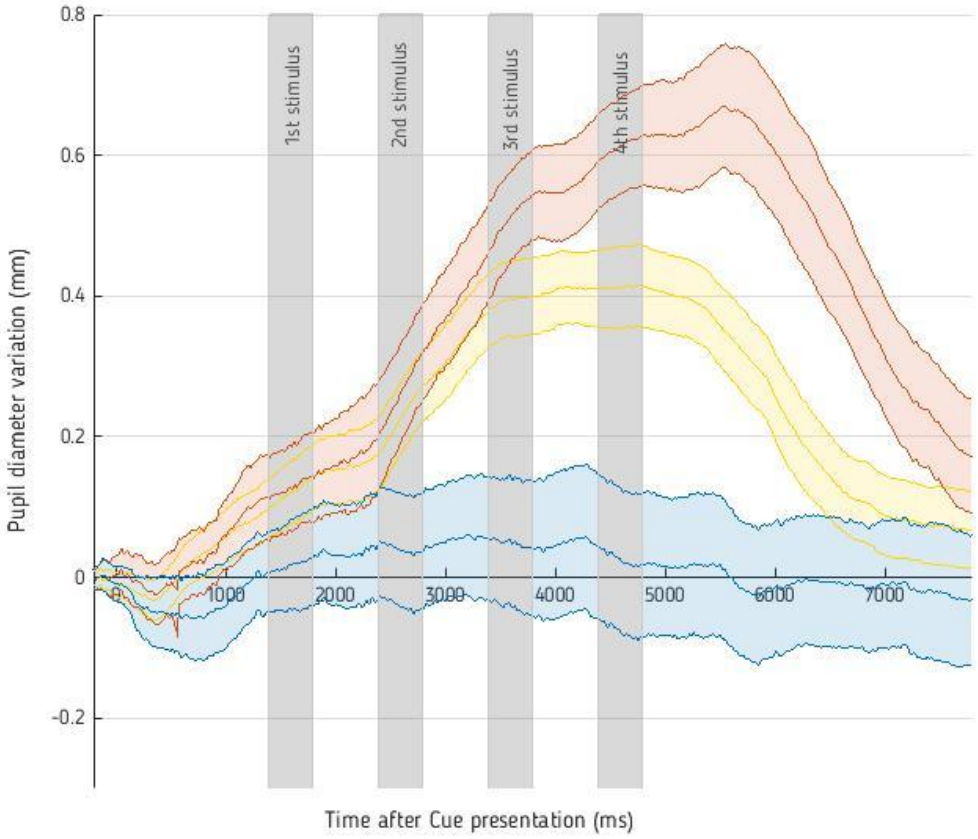

Figure 3. 14 - Pupil diameter fluctuations for all three conditions during NO TMS blocks. Passive-viewing (blue trace), C1 (yellow trace) and C2 (red trace). Averaged data for all 5 subjects (n=5) shown from [-200, 8000] ms, where the origin point corresponds to the moment of the cue offset. Time intervals in grey columns correspond to the interval time in which the according stimulus appeared. Shaded regions represent the mean ± 1 SE. C1: M=0,1863mm, SE=0,0461mm; C2: M=0,3037mm, SE=0,0654mm; BL: M=0,0074mm, SE=0,0754mm.

As also seen in Part I, after the cue onset, a brief constriction in pupil size was seen as a response to the visual stimulus presentation. After this constriction, for all three conditions an increase in pupil diameter was seen, which is in accordance with pupil dilation associated with cognitive processing [174]. However, the pupillary response during these blocks appeared to increase slower than what was seen in Part I after the cue offset.

As seen earlier, here too pupil changes differed depending on the condition. Considering the [-200, 8000] ms time window, an ANOVA analysis for repeated measures reported statistically significant differences between the means of C1, C2 and the passive-viewing conditions ($F_{2,8}$=17,337; p<0.05).

A further post-hoc paired *t*-test revealed a statistically significant difference between C1 and C2 ($t(4) = 3{,}242$; $p<0.05$) in pupil response fluctuations. A significant increase of (0,1332±0,0411 mm) was seen for the more complex condition (Figure 3.15). These results, therefore, are in accordance with what have been seen earlier in Part I (Figure 3.4) and are able to confirm that not only a higher decision complexity condition is related to a greater variance in pupil size during performance, but also that pupil response is a reliable and non-invasive indicator of task engagement.

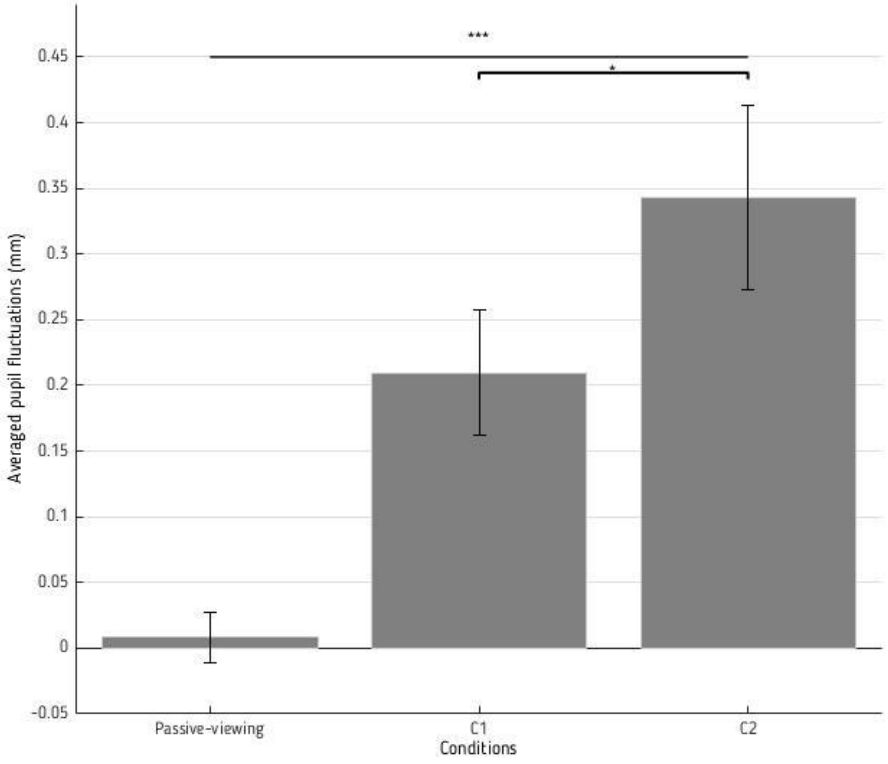

Figure 3. 15 - Averaged pupil dilation for all three conditions for [-200, 8000] ms time window. C1: M=0,2098mm, SE=0,0473mm; C2: M=0,3430mm, SE=0,0700mm; Passive-viewing: M=0,0082m, SE=0,0192mm. Error bars represent standard errors. * = significantly different (*p*-value<0.05); *** = significantly different (*p*-value<0.001).

After the presentation of the 1st stimulus, pupil variation started to ramp up for conditions C1 and C2, reaching its maximum peak (C1: max=0,4159 mm, C2: max=0,7040 mm) at 4950 ms and 5742 ms, respectively, which lie after the 4th stimulus presentation. Thus, pupil dilation accompanied the entire block duration and after the last stimulus returned to its baseline values.

Overall, the present pupillometry data have showed significant differences between the three conditions used during the entire block duration. A greater pupil dilation observed for the condition involving a more complex decision, C2, may indicate a higher motor cortical excitability in these condition blocks. Once more, these results reveal a modulation effect of decision complexity on pupillary response throughout task performance.

3.2.3.1. Correlation between pupil dilation responses and motor cortical excitability modulation

The previous pupillometry results showed a greater pupillary dilation for the active conditions (C1 and C2). Therefore, in order to evaluate whether or not pupillary response may be a reliable indicator of the motor cortex excitability, a correlation analysis was carried out between the average pupil response and MEPs p-t-p amplitudes relative to MEP baseline measured in TMS_{DELAY} blocks during the motor preparation period. Considering pupillary response to be slow, with pupil dilation following cognitive activation at about 300-500 ms [178] and in order to investigate inter-subjects variability, pupil response fluctuations in the time window comprising the entire block duration ([-200, 8000] ms) were analyzed for the three conditions.

Given that in this pilot study only a small number of participants was included, interpretation of correlational analyses should be done with caution. Nevertheless, this analysis was included as an example of the type of analyses that should be done in future studies where a higher number of participants would be included. For all three conditions, a positive correlation was seen between the MEPs amplitude percentages relative to MEP baseline and the average pupillary response in the considered interval (Figure 3.16) (C1: r=0,552, p=0.335; C2: r=0,276, p=0.653; Passive-viewing: r=0,141, p=0.821).

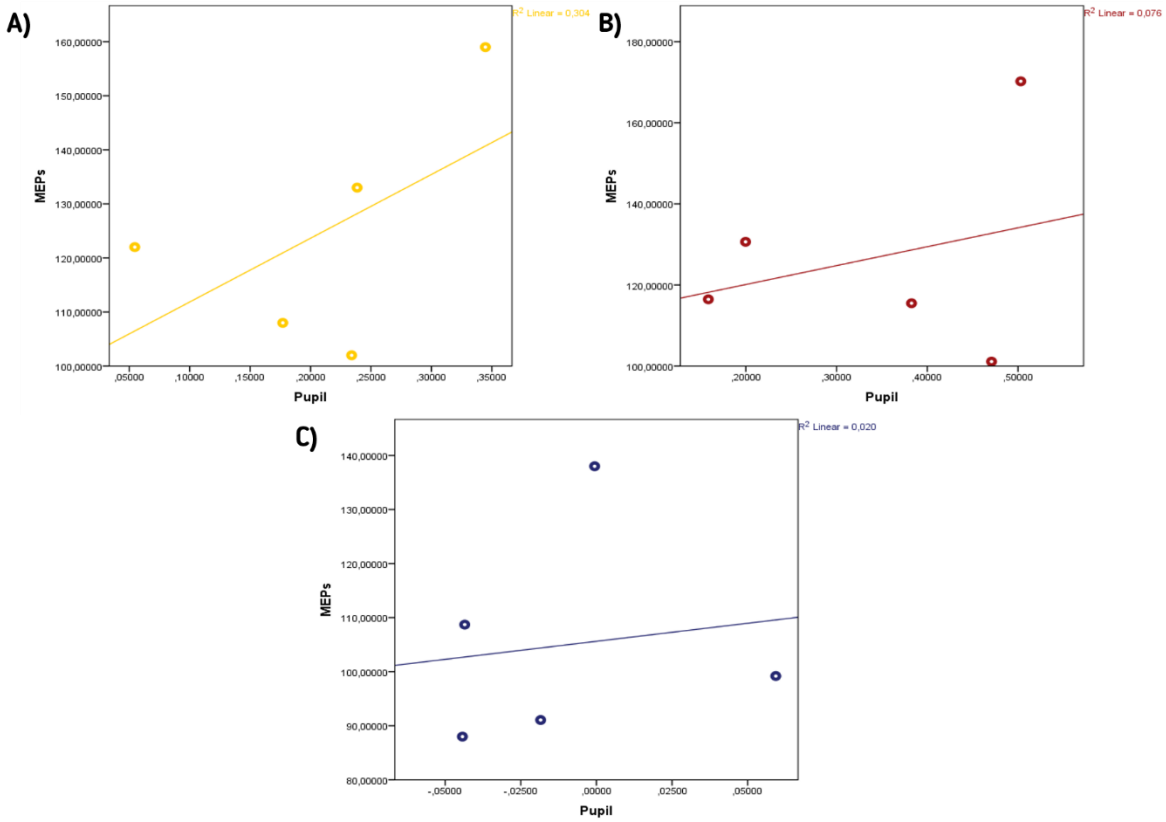

Figure 3. 16 - Pearson correlation between pupillary response and MEPs p-t-p absolute amplitudes during the delay period. A: Positive correlation between the 5 subjects averaged MEPs amplitudes and their pupil response fluctuations in condition 1 blocks (r=0,552, p=0.335). B: For condition 2, a positive correlation was also seen across the 5 subjects (r=0,276, p=0.653). C: Correlation between MEPs and pupil fluctuations during the motor preparation interval were seen even for the passive-viewing condition (r=0,141, p=0.821).

However, as seen in Figure 3.16, this correlation was only unmistakable in one participant for all three conditions. In fact, by plotting MEPs average p-t-p amplitudes across the 5 participants (Figure 3.17) in all conditions, it is clear that in one subject, the amplitudes relative to the MEP baseline value revealed a CS facilitation, greater than the ones recorded for the remaining subjects. Although furthest from the average, the MEPs amplitudes recorded from this individual were not significant outliers (p>0.05).

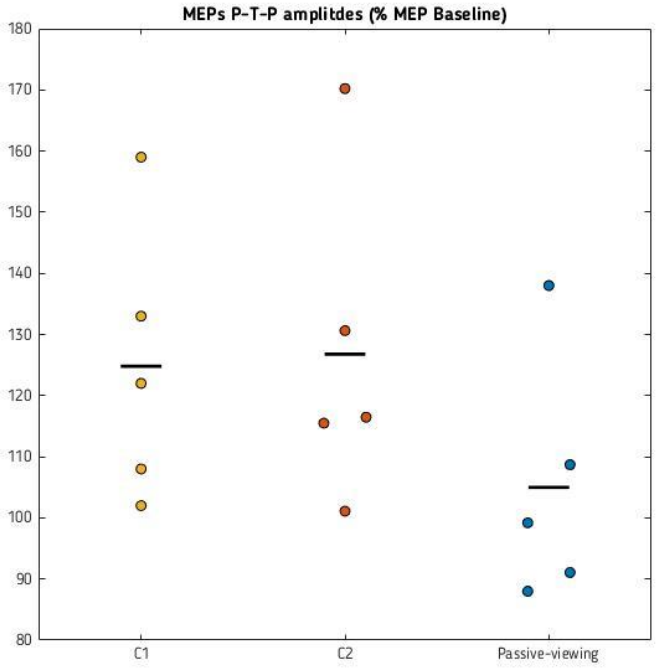

Figure 3. 17 - Box plot for all three conditions across the 5 subjects of MEPs P-T-P amplitudes relative to MEP Baseline value.

These analyses thereby show a correlation between the pupil response fluctuations and CS excitability measured during the motor preparation interval. Although these results contradict the initial proposed hypothesis, the timings used to assess CS excitability through TMS pulse delivery should be considered and will be further discussed. However, these results showing pupillary response as an indirect indicator of motor cortex excitability, not yet reported elsewhere, should be explored in future work, using a larger sample size.

3.2.3.2. Effect of TMS pulse on pupil dilation responses

Average pupil response for all subjects in blocks with TMS pulse given during the delay period (TMS_{DELAY} blocks) is shown in Figure 3.18.

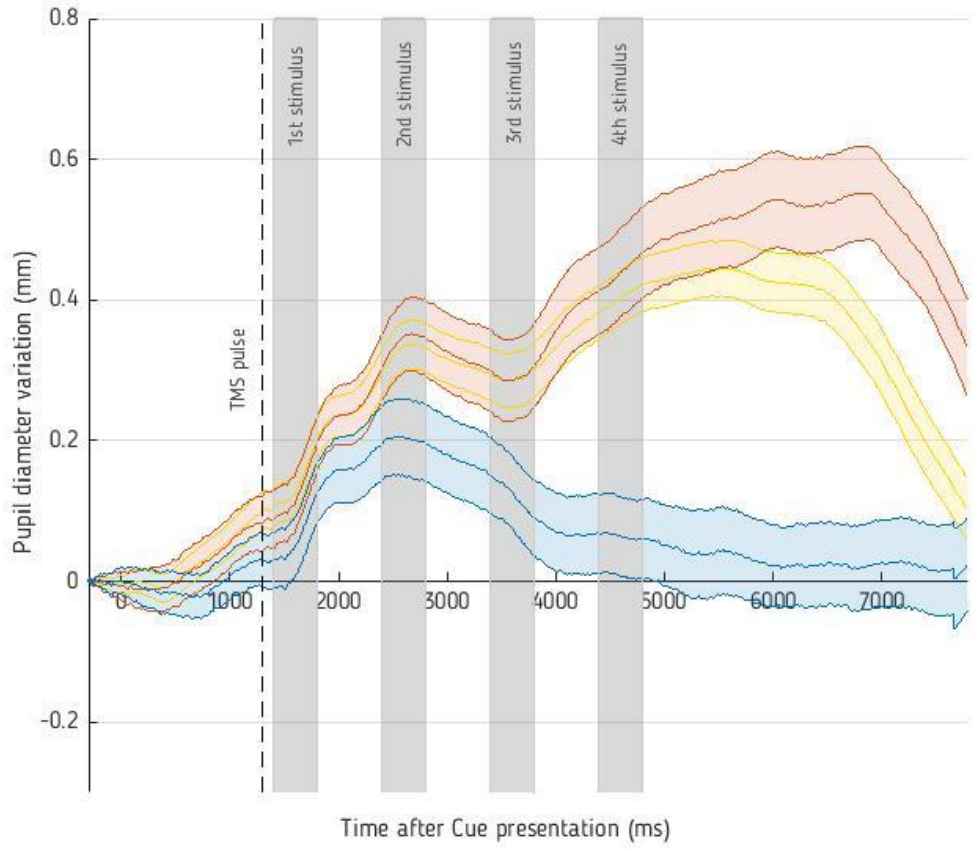

Figure 3. 18 - Pupil diameter fluctuations for all three conditions during TMS_{DELAY} blocks: passive-viewing (blue trace), C1 (yellow trace) and C2 (red trace) conditions. Averaged data for all 5 subjects (n=5) shown from [-200, 8000] ms, where the x origin point corresponds to the moment of the cue offset. Shaded regions represent the mean ± 1 SE.

In these blocks, pupil response was also modulated by decision complexity. Using a repeated measures ANOVA analysis, pupil fluctuations revealed significant changes between C1, C2 and passive-viewing condition ($F_{2,8}$=31,105; p<0.0005), considering the time window [-200, 8000] ms, i.e., the entire block duration. A paired *t*-test revealed that these differences were significantly different (Figure 3.19) between either C1 or C2 and the passive-viewing condition (t(4)=7,517, p<0.005; t(4)=5,983, p<0.005 respectively). However, no significant differences were seen between C1 and C2 (t(4)=-1,813, p=0.144).

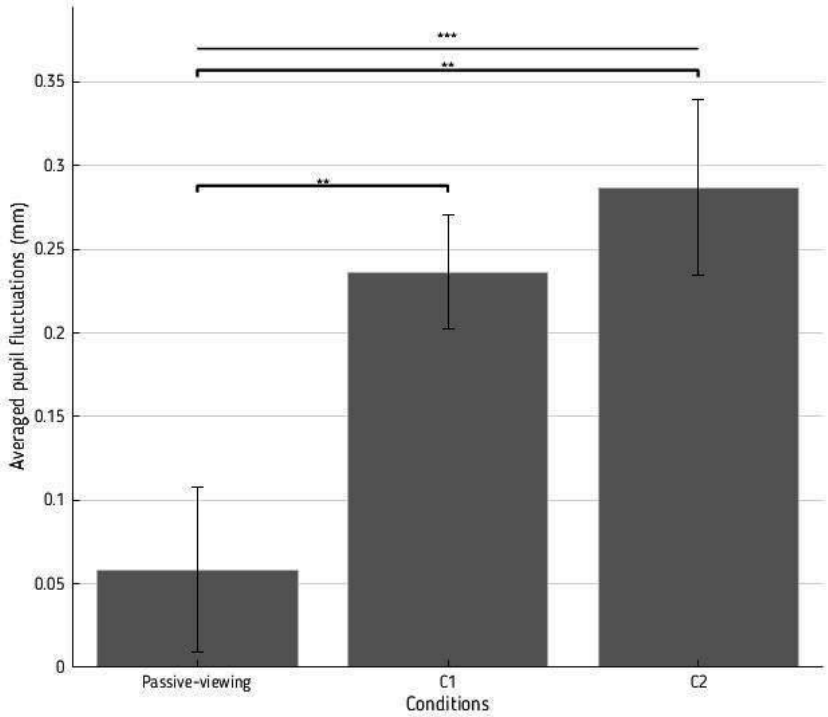

Figure 3. 19 - Averaged pupil dilation for all three conditions for [-200, 8000] ms time window. C1: M=0,2365mm, SE=0,0341mm; C2: M=0,2870mm, SE=0,0526mm; Passive-viewing: M=0,0583mm, SE=0,0494mm. Error bars represent standard errors. ** = significantly different (*p*-value<0.005); *** = significantly different (*p*-value<0.001).

It is evident that between 1500 ms and 4000 ms after the cue offset, an additional pupil dilation response appears in all task conditions, that was not present in the blocks with no TMS.

Contrary to what have been seen in blocks with no TMS pulse, pupil response in TMS_{DELAY} blocks did not accompany the stimuli presentation in the entire block duration. Although pupil size increased visibly after the 1st stimulus presentation (imperative signal) for conditions C1 and C2, a decrease in pupil response was seen for these conditions starting around the 2nd stimulus offset. Also, for the passive-viewing condition, a decrease is also seen around the same moment.

The timing for this decrease (~2700ms) is equivalent to an interval of ~1400 ms after the TMS pulse. After this moment, pupil response started to ramp up again until its maximum peak (C1: max=0,4449 mm, C2: max=0,5522 mm) which was reached at 5717 ms and 7058 ms, respectively, after the last stimulus was presented. Although its

ultimately response resembles to accompany the block duration; initially, comparing with what was seen earlier for the NO TMS blocks and even the pupillometry data in Part I, pupil response appeared to respond to the magnetic pulse.

Furthermore, in these blocks, pupil variation in the passive-viewing condition blocks also varied greatly from what have been previously showed for blocks with no pulse. In previous results showed, pupil response for this condition remained approximately stable during the entire block duration. However, in the present blocks, even though no differences besides the absence of the TMS pulse existed from the NO TMS blocks and, blocks with and without pulse were intermingled, after the magnetic pulse delivery, pupil fluctuations started to increase rapidly, behaving almost alike the responses recorded for C1 and C2 conditions, contrary to what have been earlier seen.

In order to understand these changes in pupil behaviour, a more careful analysis was carried out regarding the TMS influence in pupil response during performance for each condition.

To that end, pupillometry data for each condition from NO TMS blocks and TMS_{DELAY} blocks were compared and paired-samples *t*-tests were performed. Several time windows were tested for analysis. Since the main goal was to investigate whether TMS pulse had an effect on pupil response and once TMS pulse was delivered (in TMS_{DELAY} blocks) 1300 ms after cue offset, the first time window used was [1300,1600] ms.

Visual comparison of the pupillary waveforms obtained in blocks with TMS in comparison with blocks with no TMS (Figs. 3.20, 3.21 and 3.22) revealed that TMS produced a delay of the pupil dilation response, with the peak of the response occurring later and taking longer to return to baseline values.

For condition 1, no significant differences were seen in pupil fluctuations between NO TMS blocks and blocks with TMS pulse during the delay period ($t(4)$=-0,341, p=0.750) (Figure 3.20).

Taking into account that pupillary response for the three conditions in blocks in which magnetic pulse was delivered appear to suffer a decrease around the 3rd stimulus presentation, the time interval laying in [1600, 3600] ms was analyzed and a paired *t*-test also revealed no significance ($t(4)$=-0,901, p=0.419). The time window [3600, 5600] ms

also did not reveal an effect of the magnetic pulse in the pupil response ($t(4)=0{,}906$, $p=0.416$).

Finally, testing the time window correspondent to the final stage of block duration ([5600, 8000] ms), pupil variation changes between the two types of blocks revealed significance ($t(4)=-5{,}175$, $p<0.05$) with an averaged increment of 0,1712±0,0740 mm for blocks in which the magnetic pulse was delivered.

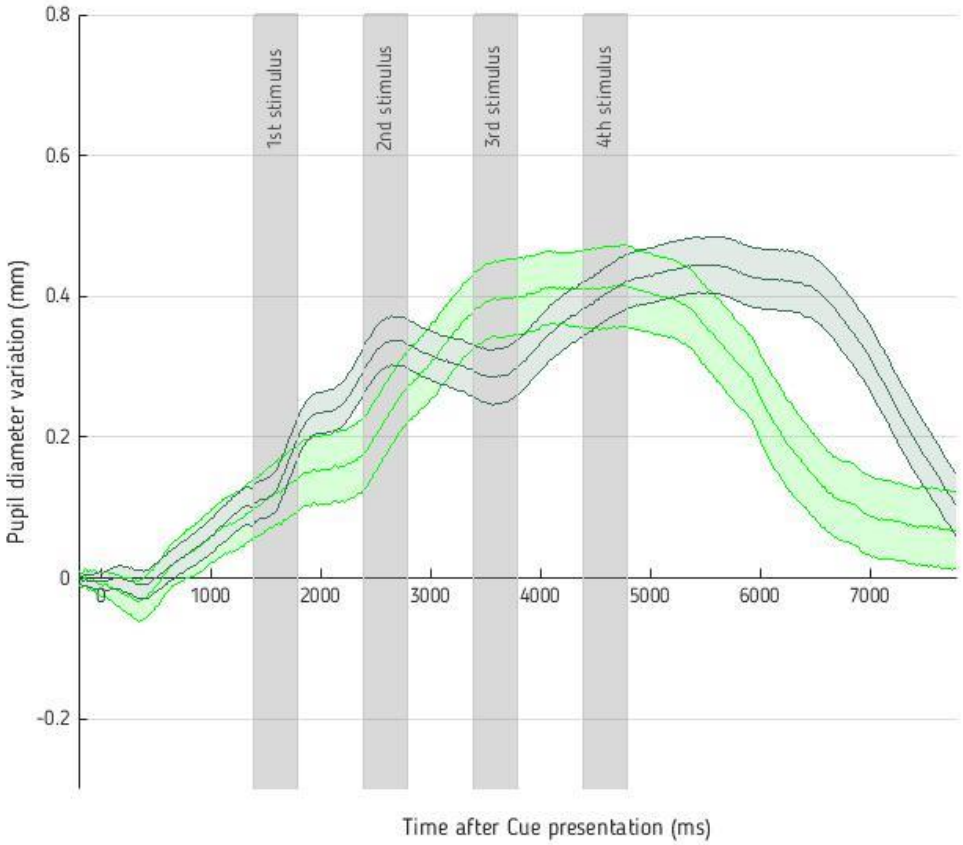

Figure 3. 20 - Pupil diameter fluctuations for Condition 1. Dark-green represents pupil size fluctuations recorded during TMS_{DELAY} blocks and light-green represents pupil size fluctuations recorded during NO TMS blocks. Averaged data for all 5 subjects (n=5) shown from [-200, 8000] ms, where the origin point corresponds to the moment of the cue offset. Shaded regions represent the mean ± 1 SE.

For condition 2 (Figure 3.21), no TMS effect was seen in pupil response during the delay period ([1300, 1600] ms) ($t(4)=0{,}633$, $p=0{,}561$). Also, when testing [1600, 3600] ms time interval, no significant differences were seen in pupil response between the two block types ($t(4)=-0{,}385$, $p=0{,}720$). However, a significant averaged decrease of

0,1774±0,0639 mm in pupil response was seen as an effect of the TMS pulse for the time laying in [3600, 5600] ms ($t(4)=2,778$, $p<0.05$).

Finally, testing the time window correspondent to the final stage of block duration ([5600, 8000] ms), pupil variation changes between the two types of blocks did not reveal an effect of the magnetic pulse in pupil response ($t(4)=-1,014$, $p=0.368$).

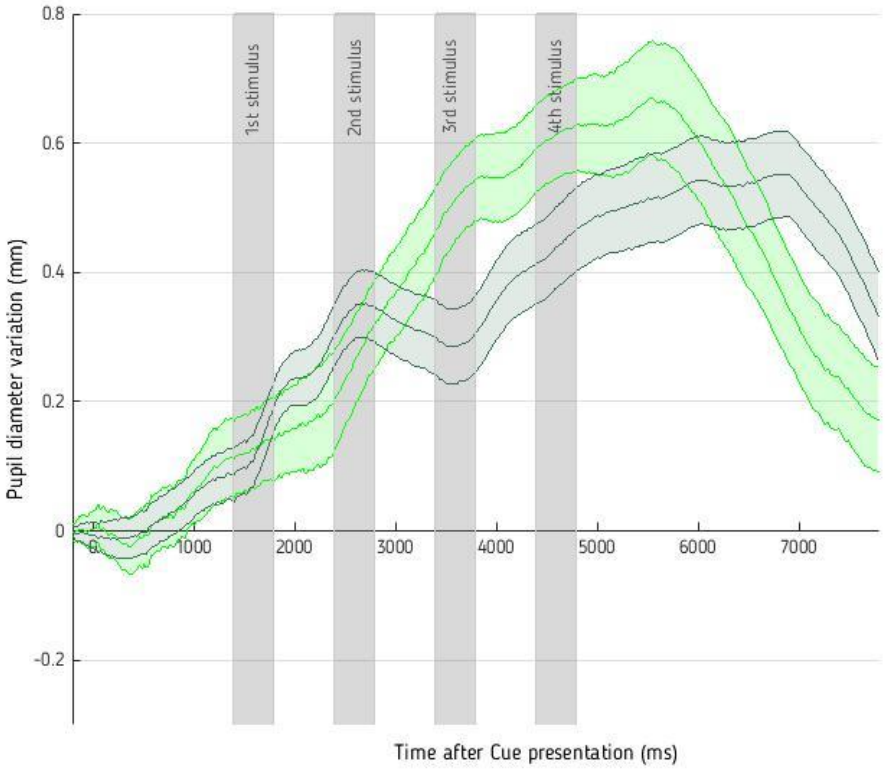

Figure 3. 21 - Pupil diameter fluctuations for Condition 2. Dark-green represents pupil size fluctuations recorded during TMS_{DELAY} blocks and light-green represents pupil size fluctuations recorded during NO TMS blocks. Averaged data for all 5 subjects (n=5) shown from [-200, 8000] ms, where the origin point corresponds to the moment of the cue offset. Shaded regions represent the mean ± 1 SE.

A more interesting result was found for the passive condition. Based on what the previous results showed, it would be expected an approximately stable pupil response in the entire block duration; however, pupil fluctuations during block performance were also altered by the magnetic pulse (Figure 3.22).

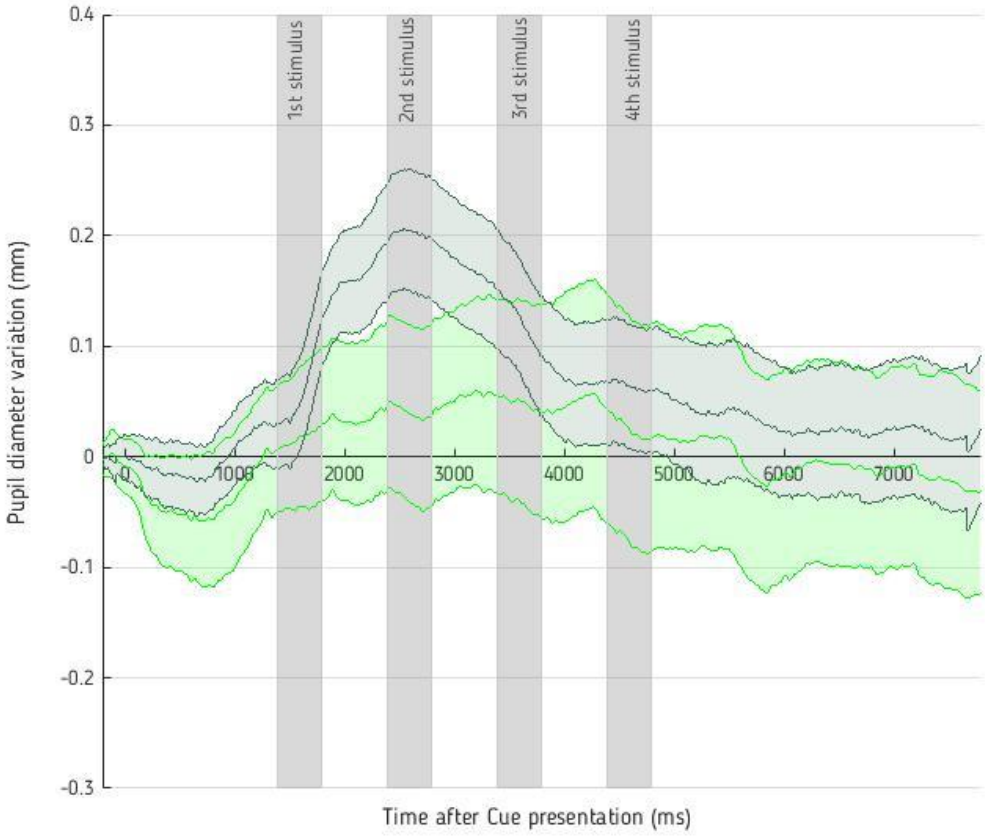

Figure 3. 22 - Pupil diameter fluctuations for the Passive-viewing condition. Dark-green represents pupil size fluctuations recorded during TMS_{DELAY} blocks and light-green represents pupil size fluctuations recorded during NO TMS blocks. Averaged data for all 5 subjects (n=5) shown from [-200, 8000] ms, where the origin point corresponds to the moment of the cue offset. Shaded regions represent the mean ± 1 SE.

No significant effect was seen during the time window [1300, 1600] ms ($t(4)$=-0,848, p=0.444), matching the moment in which the magnetic pulse was given. However, when considering [1600, 3600] ms time window, which lies in the period of time after the TMS pulse was delivered, a significant increase of 0,1153±0,0426 mm in pupil fluctuations was recorded ($t(4)$=-2,705, $p<0.05$). In fact, in TMS_{DELAY} blocks, pupillary response reaches its peak of 0,206 mm during the considered interval, around 2750 ms after the preparatory cue offset. Contrarily, as earlier referred, for blocks without TMS pulse, pupil response in the passive-viewing condition did not vary significantly during the entire block duration.

In the latter part of the block duration no significant differences were seen in the pupil response between the two block types ([3600, 5600] ms: $t(4)$=-0,792, p=0.473; [5600, 8000] ms: $t(4)$=-0,613, p=0.573).

Overall, pupil size variation revealed greater changes in pupil response magnitude in TMS_{DELAY} blocks. Interestingly, these effects of TMS were not in the same course for the three conditions: C1, C2 and passive-viewing.

For C1 and C2, an effect of the magnetic pulse in pupillary response was seen in the later part of the block duration; however, for the passive-viewing condition, this effect was seen earlier in the block. In these intervals in which the magnetic pulse had an effect, in the less complex active condition, C1, and in the passive-viewing condition, TMS pulse caused an increase in pupil response, which appears to be in accordance with earlier studies [179], where TMS pulse at suprathreshold level applied over the motor cortex resulted in higher pupillary dilation. This increase in pupil size is suspected to be a consequence of an arousal reaction caused by the acoustic artefact of the coil discharge.

However, for the more complex active condition, C2, the effect of the magnetic pulse was the opposite. In C2, after the TMS pulse was delivered, a significant decrease of pupil size was recorded.

These differences in effects caused by the TMS pulse may also be justified by the decision complexity. C2 is the more complex condition and, as showed previously, elicits greater variations in pupil size, when compared to less complex conditions.

Considering the correlation seen between CS excitability and pupillary response, a greater pupil dilation observed for the more complex condition, C2, may indicate a higher motor cortical excitability in this condition blocks. On the contrary, for the remaining conditions passive-viewing and C1, less complex, in which pupil size increases less than in C2 blocks, the motor cortex will be less excited. Therefore, when delivering the TMS pulse, it will be easier to cause an excitatory effect in less excitable cortical regions than in more excitable ones. The threshold to elicit an excitatory effect in less excitable cortical regions would consequently be lower than when cortical regions are more excited. This excitatory effect is reflected in larger pupil sizes.

In fact, the state of the stimulated cortex has a marked influence on the effect of TMS. An early model of the responsiveness of a single spinal motor neuron [180] showed that cortical areas having a highly active BOLD response during a particular task might actually be less responsive to TMS inputs. However, future work is required to explore this hypothesis.

Independently of the effect caused by the magnetic pulse, in all three conditions a pupillary response to the TMS pulse was observed overlapped with the task-response. This was evident as a peak (local maximum) around 2500 ms after the cue offset followed by a decrease in pupil fluctuations starting approximately 2700 ms after the cue offset. The timing of the peak was around 1000 ms after the TMS pulse delivery. After this moment, pupil response seemed to return to its baseline values, either by stabilizing (in passive-viewing condition) or continuing to increase until the block was ended (in C1 and C2 conditions). This sudden decrease and consequent return to baseline values appear to be a consequence of the end of the transient magnetic pulse effect in the stimulated cortical region.

Therefore, in order to investigate whether this effect was a consequence of the TMS pulse, and since two different timings for pulse delivery were tested, the pupil size along blocks with the TMS pulse delivered 100 ms before the Cue presentation ($TMS_{BASELINE}$ blocks) was also analyzed (Figure 3.23).

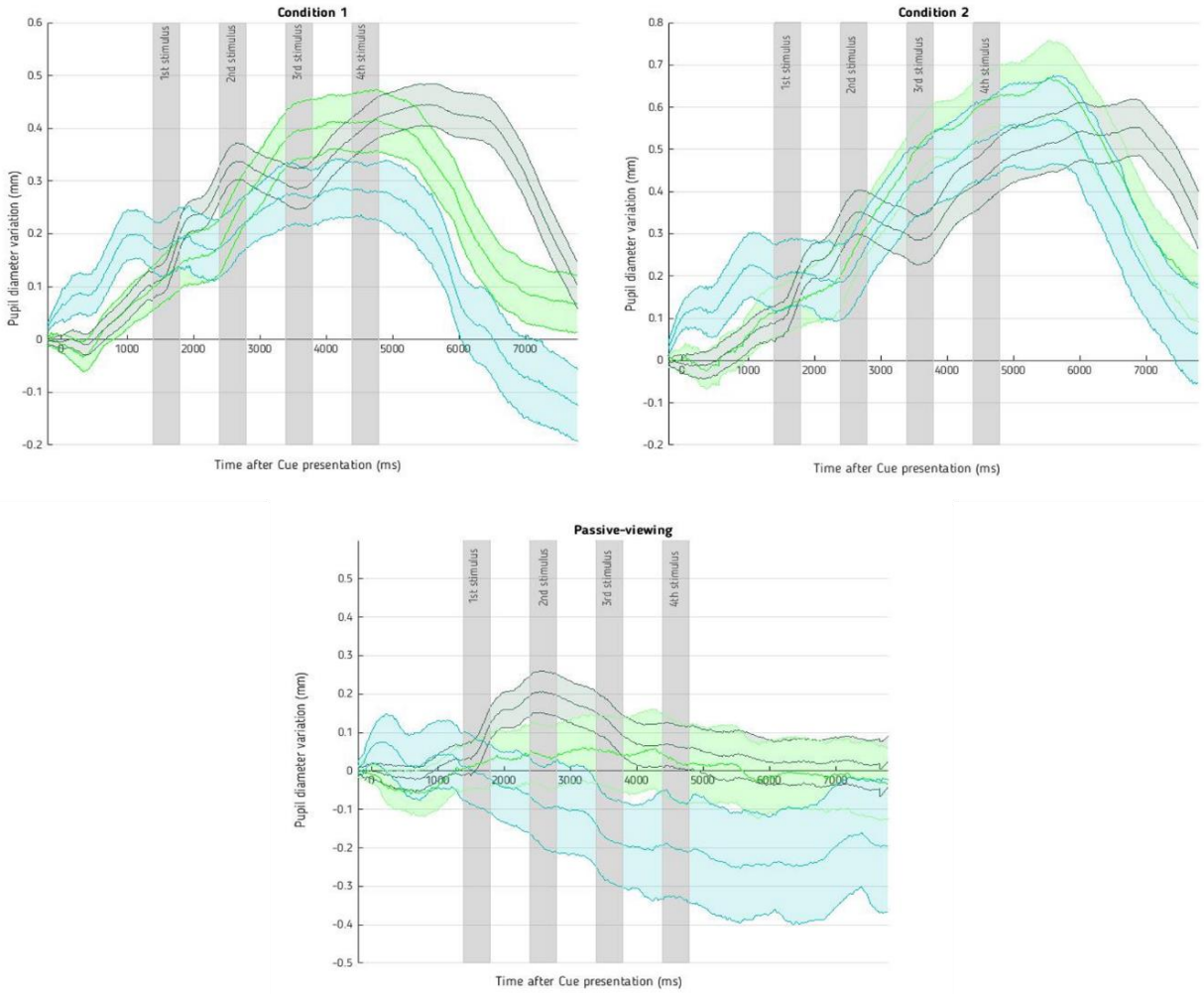

Figure 3. 23 - Pupil diameter fluctuations for condition 1 (A), condition 2 (B) and passive-viewing (C) in the three types of blocks. Dark-green represents pupil size fluctuations recorded during TMS_{DELAY} blocks, light-green represents pupil size fluctuations recorded during NO TMS blocks and light-blue represents pupil size fluctuations recorded during $TMS_{BASELINE}$ blocks. Averaged data for all 5 subjects (n=5) shown from [-200, 8000] ms, where the origin point corresponds to the moment of the cue offset. Shaded regions represent the mean ± 1 SE.

In accordance to the earlier results from TMS_{DELAY} blocks, also in $TMS_{BASELINE}$ blocks, a suddenly decrease in pupil response is seen for C1 and C2 starting around 1300 ms after cue offset, which is correspondent to an interval time of 1600 ms after the magnetic pulse delivery. After this decrease reached its lowest point, happening around the 2nd stimulus presentation, pupil size changes started to ramp up again until the end of the block. In fact, a previous study investigating the effect of TMS on pupil response reported that when stimulation was made over the motor cortex using a suprathreshold pulse, pupil dilation was significantly increased [180]. Furthermore, the authors reported

that the maximal dilation was observed approximately 1,5 s after the TMS pulse, which is in accordance with the present data.

Overall, in both $TMS_{BASELINE}$ and TMS_{DELAY} blocks, the pulse appears to have caused an increase in pupil dilation for all the three conditions in time intervals close to the pulse timing. However, this increase in pupil response appeared to be transient and this increased effect in pupillary response showed to be diluted.

Another interesting finding from the previous results is related to the pupil response for the passive-viewing condition in TMS_{DELAY} blocks. Although no motor response was required in this condition and, therefore, it would be expected a stable and approximate constancy in pupil size along the entire block, the pulse given in these blocks resulted in a different pupil response.

In TMS_{DELAY} blocks, the pupil variations for the passive condition increased rapidly after the magnetic pulse was given until around 2400ms after the pulse timing and then started to decrease until baseline values. The timing for the start of this decrease appears to be coincident with the time of the onset of the decrease in pupil fluctuations also observed for C1 and C2 conditions. This curve-shaped response for the passive condition seems to, in a certain way, mimic the effect of the induced-current generated by the TMS pulse in the stimulated cortical region. In fact, the duration of this response (~6000ms) is in accordance with the suggested inter-pulse interval when using TMS technique (5-6 s). This interval is proposed in order to allow cortical regions that are being stimulated to recover from the magnetic stimulation and prevent cumulative effects of the pulses given [181], therefore allowing the stimulated regions to return to basal values of excitability and preventing recorded values of being influenced by the induced-current elicited by the TMS pulse. Thus, the magnetic pulse applied on the passive-viewing condition, in which motor cortex excitability should remain approximately stable, appears to reflect the transient CS excitability in the stimulated motocortical region elicited by the TMS pulse.

Considering the pupil response in TMS_{DELAY} blocks in the passive condition a reflection of the magnetic pulse effect in pupillary variations, the pupil fluctuations recorded for this condition were subtracted from C1 and C2 for the entire block duration in order to obtain the real pupillary response minus the TMS pulse effect (Figure 3.24).

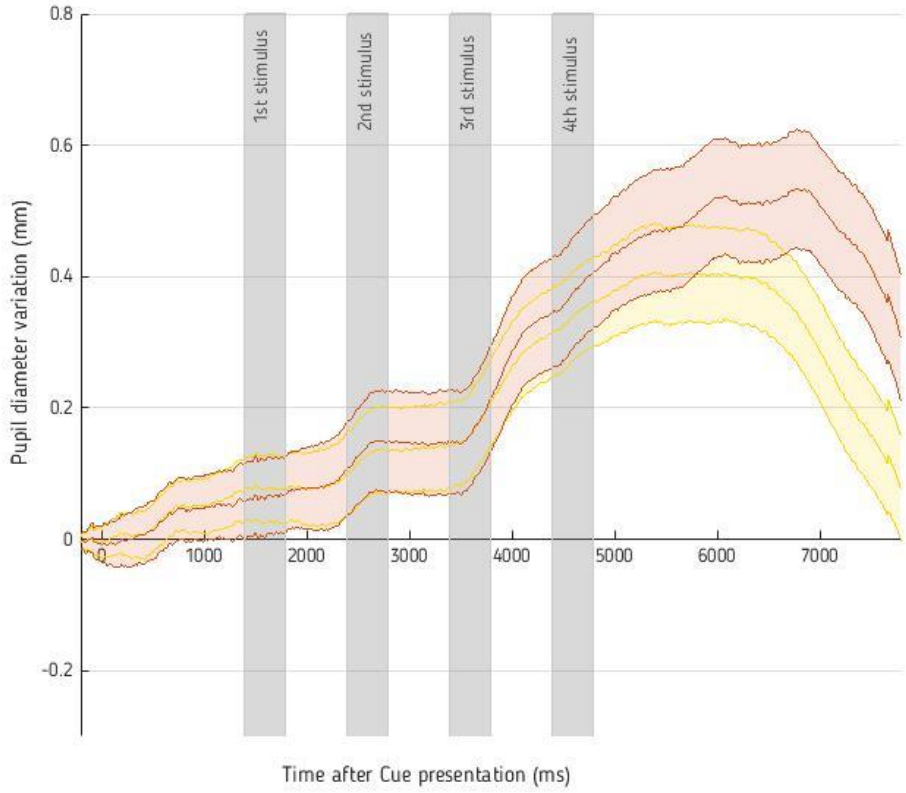

Figure 3. 24 - Pupil diameter fluctuations for the two active conditions during TMS_{DELAY} blocks, minus the TMS pulse effect: C1 (yellow trace) and C2 (red trace). Averaged data for all 5 subjects (n=5) shown from [-200, 8000] ms, where the origin point corresponds to the moment of the cue offset. C1: M=0,1781mm, SE=0,0569mm; C2: M=0,2287mm, SE=0,0685mm. Shaded regions represent the mean ± 1 SE.

In fact, when subtracting the pupil response for the passive condition in the TMS_{DELAY} blocks, pupil fluctuations for C1 and C2 were more resembling with the pupil response recorded for the NO TMS blocks, in which no TMS pulse was delivered.

In order to verify the resemblance between the response obtained from the subtraction of the pulse effect and the pupil fluctuations recorded in blocks in which no actually TMS pulse was delivered, a paired *t*-test was carried out. Although the pupil response appears to have suffered a slight delay, *t*-tests revealed no significant differences between the two responses for all the time windows tested ([1300, 1600] ms: $t(4)=0{,}261$, $p=0.807$; [1600, 3600]: $t(4)=1{,}396$, $p=0.235$; [3600, 5600] ms: $t(4)=1{,}729$, $p=0.159$; [5600, 8000] ms: $t(4)=-2{,}003$, $p=0.060$) (Figure 3.25).

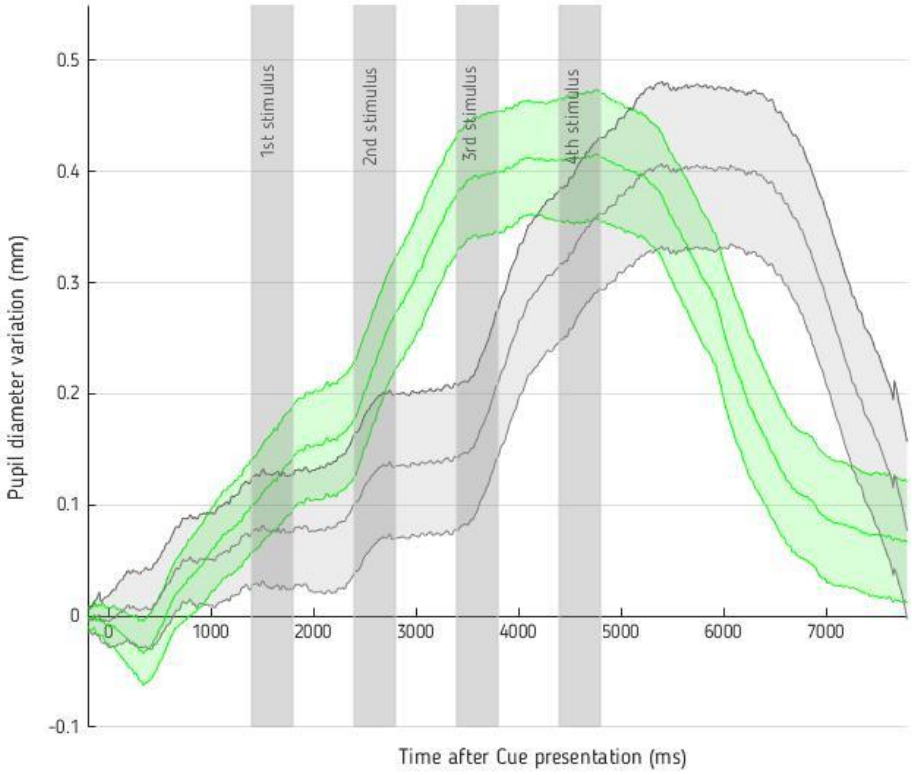

Figure 3. 25 - Pupil diameter fluctuations for condition 1 for the NO blocks (light green) and for TMS_{DELAY} blocks (grey) where the TMS pulse effect has been subtracted. Averaged data for all 5 subjects (n=5) shown from [-200, 8000] ms, where the origin point corresponds to the moment of the cue offset. Shaded regions represent the mean ± 1 SE. Green: M=0,1863mm, SE=0,0461mm; Grey: M=0,1781mm, SE=0,0569mm.

For condition 2, pupillary response behaved similarly and paired *t*-tests did not also reveal significance ([1300, 1600] ms: $t(4)=0{,}737$, $p=0.502$; [1600, 3600] ms: $t(4)=2{,}286$, $p=0.084$; [3600, 5600] ms: $t(4)=2{,}912$, $p=0.107$; [5600, 8000] ms: $t(4)=-0{,}733$, $p=0.504$) (Figure 3.26).

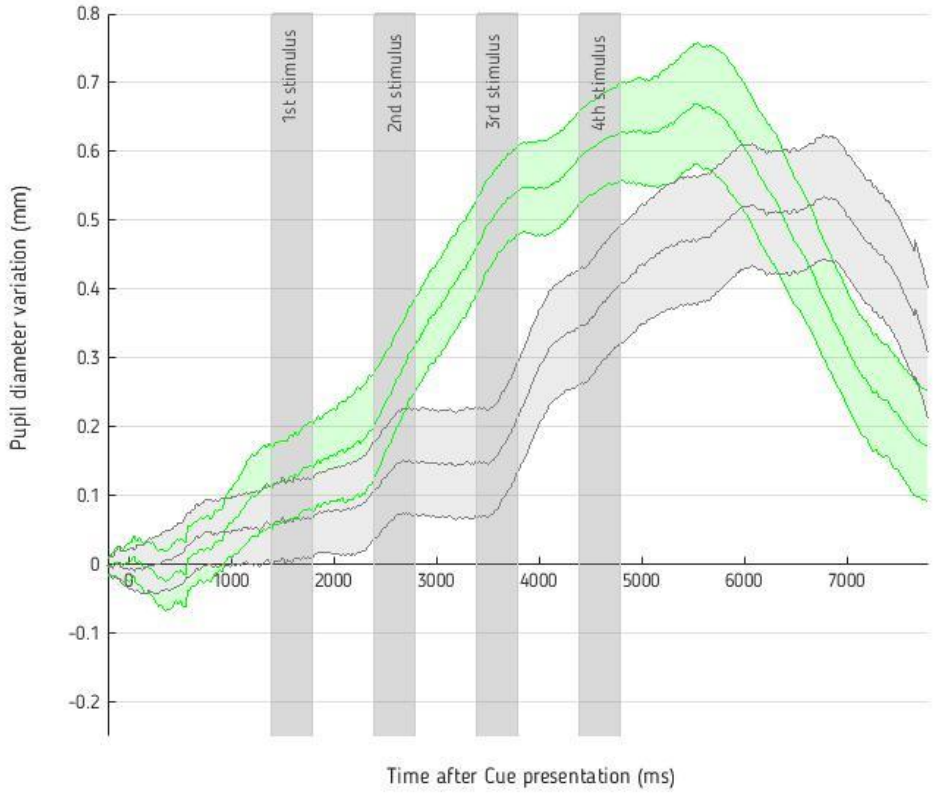

Figure 3. 26 - Pupil diameter fluctuations for condition 2 for the NO blocks (light green) and for TMS_{DELAY} blocks (grey) where the TMS pulse effect has been subtracted. Averaged data for all 5 subjects (n=5) shown from [-200, 8000] ms, where the origin point corresponds to the moment of the cue offset. Shaded regions represent the mean ± 1 SE. Green: M=0,3037mm, SE=0,0654mm; Grey: M=0,2287mm, SE=0,0685mm.

By subtracting the TMS effect in pupillary response, for both conditions it was still possible to see a brief delay in fluctuations, which implies other cause than the magnetic pulse. However, when subtracting this effect and comparing with blocks in which no pulse was present, no significant differences were seen for the complete block duration for both C1 and C2. This result may, therefore, imply that the response seen for the passive-viewing condition may indeed reflect the transient effect of the induced-current in the stimulated M1 region.

In summary, the previous results allowed confirming higher pupillary dilation when a condition involves a more complex decision, which, according to the correlation seen between MEPs amplitude and pupil response, appears to reflect higher CS excitability for this condition. Also, pupil response appears not only to be modulated by

decision complexity level but also by subject's engagement in the task. Finally, taking into account the overall delayed and increased effect on pupil size as a consequence of the magnetic pulse applied in TMS, in future studies where pupillometry data is recorded and TMS technique is used, pupil data of blocks in which TMS pulse is delivered may not be a reliable indicator of the effect of task parameters on pupil size since the TMS pulse itself affects pupil dilation.

3.3. Preliminary study – fMRI acquisition

Since the present study's goal was to investigate the role of NE in the modulation of motor cortical excitability states, a first, preliminary, functional magnetic resonance imaging (fMRI) acquisition was carried out during task performance. The use of fMRI would allow for a more direct measure of locus coeruleus activation in comparison with pupil dilation.

One healthy young subject (N=1) performed the original task while fMRI images were acquired. The keyboard was replaced by 2 response boxes.

Image data was processed using BrainVoyager QX (v. 2.8) and the results are shown in Figure 3.27. Locus coeruleus is the largest nucleus of norepinephrine neurons in the brain and it projects to all cortical regions which can result in both excitatory effects mainly via the activation of β-adrenoreceptors and inhibitory effects via the stimulation of α-adrenoreceptors [121]. Variations of cortical excitability are directly related to LC activity [125, 126, 127]. Therefore, LC was one of the cortical regions that was expected to be activated during task performance. However, this was not the case, which in part may be due to the reduced number of trials acquired and number of subjects (N=1). Nevertheless, other cortical regions related to motor behavior were activated.

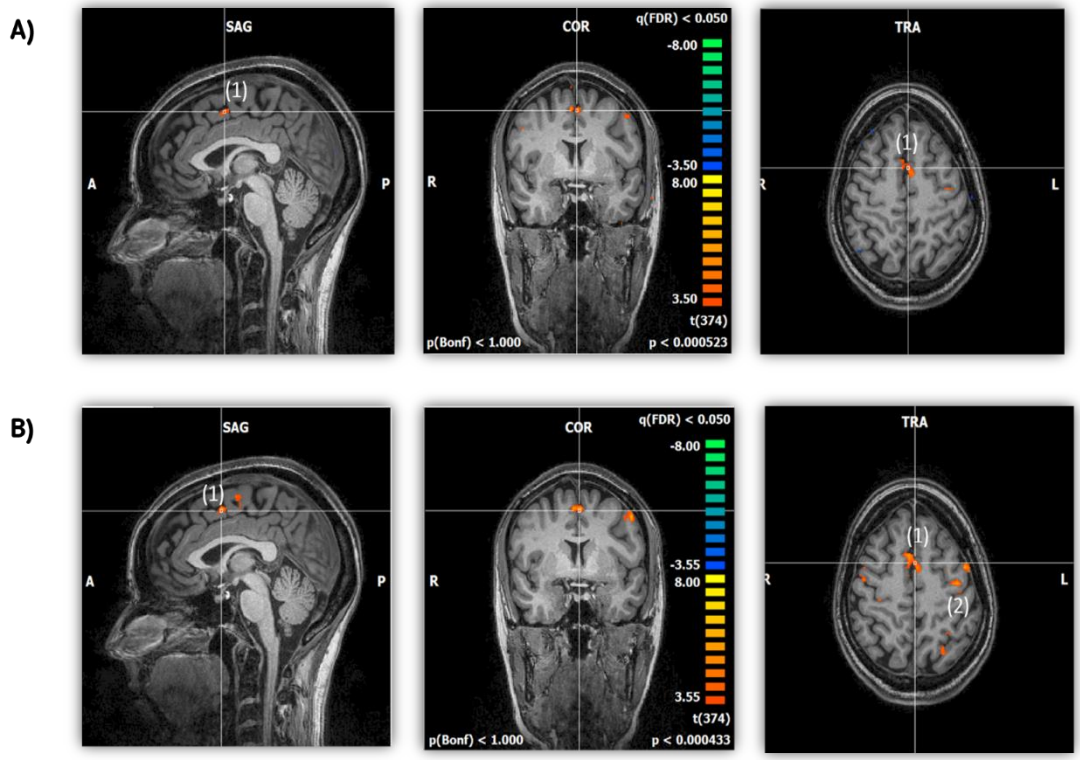

Figure 3. 27 - Cortical activations during task performance. A) Cortical regions activated during performance for Condition1 (C1). Activation of supplementary motor cortex (SMA) (1) can be seen. Bonferroni corrected (p<0.0005). Coordinates (x,y,z): 88,116,76; B) Cortical activations during performance for Condition2 (C2). Higher activations can be seen in SMA (1) and premotor cortex (2). Bonferroni corrected (p<0.0005). Coordinates (x,y,z): 88,116,76; COR: coronary; TRA: transversal; A: anterior; P: posterior; R: right; L: left.

Activation of supplementary motor area was seen for both conditions 1 and 2 (1), but a greater activation was reported for C2 (p<0.0005 Bonferroni corrected). SMA, a motor area well-reported to be involved in motor learning, movement preparation and initiation [182], projects indirectly to LC through the dorsal prefrontal cortex.

Also, a dorsal premotor cortex activation was also seen for C2 (2). According to Cisek' 'affordance competition' hypothesis, this region is activated during decision making [91] in the motor preparation interval. Therefore, it seems accurate that this activation was higher for C2 condition, the condition involving a more complex decision, in which subject had to decide which arrow to press after stimuli presentation.

Overall, a greater motocortical activation was seen for C2 blocks, which corresponded to the condition involving a more complex decision. However, these findings need further confirmation using a higher sample size.

4. CHAPTER IV. CONCLUSIONS

During motor tasks, motor cortex excitability has been well-described to be modulated by several factors as the timing of the motor response process [94], whether the response is produced by a dominant or non-dominant muscle [183] and also by the response complexity [107]. However, a role of decision complexity itself has not yet been investigated.

Our analysis of pupillometry data showed that the pupil response is differentially modulated depending on the task-related decision complexity level. Also, more importantly, our results suggest that pupil response fluctuations are related with the CS excitability probed by MEPs p-t-p amplitude. However, this result should be interpreted carefully given the small sample size and the between-subject variability in the results. This is the first study connecting pupillary response and CS excitability and appears to confirm the proposed hypothesis of pupil response as an indicator of motor cortex excitability. Thus, greater pupil size variations are related to higher excitability levels of the CS tract during motor preparation.

In contrast to the initial expectations, the results have showed that, when comparing to a passive-viewing, conditions requiring active motor responses are associated with greater CS excitability facilitation during the motor preparation period. In fact, after the cue is presented and subject becomes aware of the type of the forthcoming condition, CS excitability is differently modulated, suggesting a differently preparation according with the condition being an active or passive task.

Recent TMS research studies have reported several evidences of CS inhibition during the motor preparation interval. This motor inhibition, generally referred to as preparatory inhibition, seems to have a role in the withholding of automatic or inappropriate responses and in order to favor the wining selected-response [68, 91]. Accordingly, it would also be expected to find in our data an inhibition of motor CS excitability. The present results of CS excitability level during the delay period seem

contrary to this general finding. Nevertheless, the timing chosen for the delivery of the magnetic pulse must be considered when interpreting these results.

Studies using RT paradigms have previously reported suppression of the corticospinal tract in both task relevant and irrelevant muscles immediately before the imperative signal [68, 94, 96, 97]. This suppressive global process appears to be dependent on the delay period [184]. In the present study, the magnetic pulse was randomly applied 100-500 ms before the imperative signal (1st stimulus). This randomness did not allow to specify an early or late timing in the delay period for the pulse delivery. In fact, considering all TMS_{DELAY} blocks for the 5 subjects, the interval between pulse delivery and the imperative signal was on average 315,59±18,28 ms. Therefore, the mean CS excitability during the delay period was assessed 315,59 ms before the 1st stimulus presentation. CS suppression effects in the delay period assessed during instructed-delay CRT paradigms have, contrarily, used shorter interval times between TMS pulse and the presentation of the imperative signal (50 or 100 ms) [68, 94, 96]. Indeed, authors reported that this wide inhibition in CS pathways is stronger when probed at the end of the delay period [185]. Thus, although the present study failed to assess CS excitability in a late phase, the CS facilitation for conditions C1 and C2 seen in our results probed during the delay period, considering that CS was assessed 315 ms before the 1st stimulus, which may be considered a still early phase in the delay period, may be justified by the timing of the pulse delivery .Thus, this facilitation seen is, in fact, consistent with the excitability levels probed in early phases of the motor preparation period, showed by previous studies [68, 94, 96, 97, 185]. In future work, different timings of the pulse delivery should be tested in order to investigate the modulation of CS by decision complexity during different stages of the delay period.

Overall, the present results revealed a modulation effect of task engagement in both pupil response and the excitability levels of motor corticospinal tract. However, probably due to the small sample size, decision complexity as cognitive factor was only showed to have a modulatory effect, in the delay period, in pupil response.

Future Work

In this study, we were able to setup a complex task where behaviour, pupil diameter and motocortical excitability were measured simultaneously. Considering this to be the first study to use this setup, many features could be explored in future work, allowing a better understanding of the results here presented. As referred along the discussion, a greater sample size would overcome the statistical limitations seen for important correlations analysis regarding RT and accuracy and, more importantly, pupillary response and MEPs amplitudes. Due to the small numbers of participants included, interpretations were done with caution and a further confirmation is needed.

Furthermore, the assessment of CS excitability at different time points along the motor preparation interval, could allow a finer understanding of the corticomotor excitability processes during movement preparation. Specifically, the magnetic pulse delivery immediately before the imperative signal would perhaps elucidate cortical motor inhibition process in the delay period, widely described in earlier studies. It is possible that these preparatory processes only occur at the end of the preparation period and therefore were missed in the current study where the pulse was delivered around 315 ms before the imperative stimulus.

Since the present task paradigm involved unimanual movements of the left or right hand and only left M1 was stimulated, further investigations involving both left and right M1 stimulations and also a more homogeneous sample including also left-handers could allow perceiving hemispheric contributions to neuronal excitability modulations. This would allow us to study if modulation of motor cortical excitability occurred globally or only in task relevant muscles.

Furthermore, considering the crucial role of the LC-NE system in CS excitability modulation, studies using brain imaging techniques, as fMRI, would allow relating LC's BOLD signal with motor cortical activation.

Finally, the correlation found between MEPs amplitudes and pupillary response, if further confirmed, would give rise to a breakthrough in the indirect assessment of corticomotor excitability modulation. Considering that MEPs amplitude have been used as a marker for corticomotor impairment [69, 70, 71], this result

confirmation could allow pupillary response to become an auxiliary diagnostic tool in motor system-related disorders.

5. APPENDIX A

Luminance Measures

All visual stimuli luminance levels were measured with a PR-650 Spectra-Scan Colorimeter (Photo Research, Inc., Chatsworth, CA).

Table A. 1| Luminance measures of visual stimuli used in the task.

Light source	Luminance (candela per square metre)
Passive-viewing cue	23.7
C1 cue	24.2
C2 cue	24.3
Passive-viewing stimulus	24.0
Left arrow stimulus	24.2
Right arrow stimulus	24.1
Feedback correct	22.6
Feedback incorrect	22.5
Fixation cross	22.5
Background	23.1

6. APPENDIX B

In order to investigate whether the TMS coil moved overtime from the initial optimal position and, in the affirmative case, if this movement would affect the MEPs p-t-p amplitudes during task performance, a preliminary study was carried out in Part II.

One participant, male, aged 22 years old was tested. The visual task used in Part II was divided into 8 runs of 5 minutes duration each. At the beginning of the task, after the optimal site for stimulation was found, a spot perpendicular to the coil center was drawn with a felt-tip. At the end of each run, an inspection of the alignment of the coil center was made. No significant movement from the optimal site of stimulation neither an effect on MEPs amplitudes were seen throughout the entire task.

Behavioral, pupillometry and CS excitability results are shown below.

Reaction Time Analysis

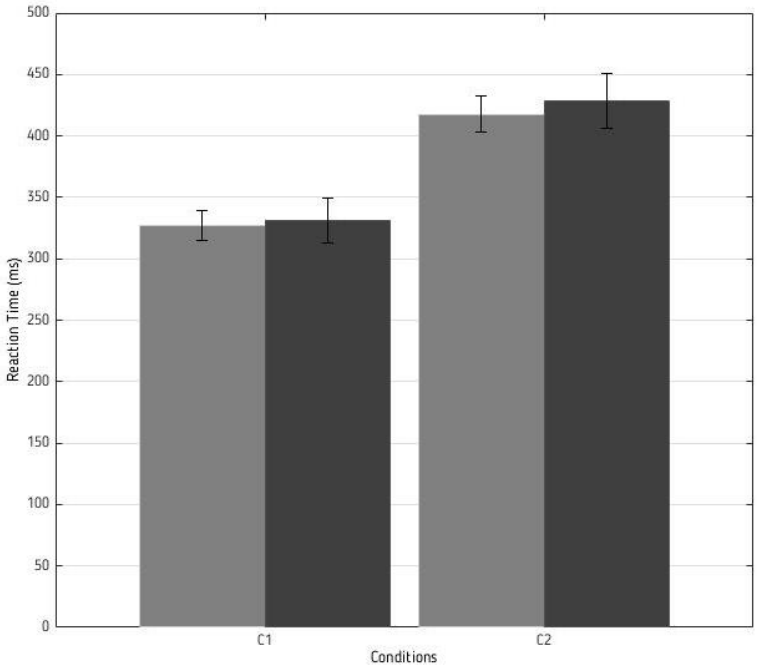

Figure B. 1 - Reaction times for the two active conditions: less difficulty condition (C1) and more difficulty condition (C2) in TMS_{DELAY} (dark grey) and NO TMS (light grey) blocks. NO TMS blocks: C1: M=321,18ms, SE=12,12ms; C2: 417,80 ms; SE=15,14 ms. TMS_{DELAY} blocks: C1: M=331,35 ms, SE=18,32 ms; C2: M=428,24 ms, SE=22,44 ms. Error bars represent standard errors.

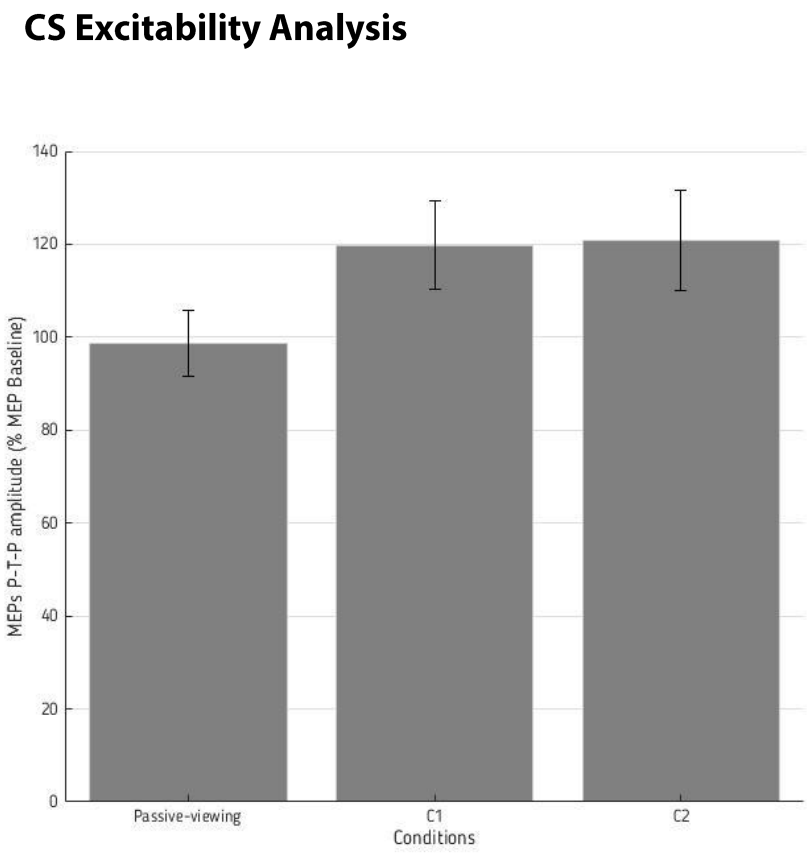

Figure B. 2 - Amplitudes percentage for the three conditions (C1, C2 and Passive-viewing) during the delay period relative to the averaged MEP Baseline value. C1: M=119,76%, SE= 9,5%; C2: M=120,82%, SE=10,94%; Passive-viewing: M=98,80%, SE=7,09%. Error bars represent standard errors.

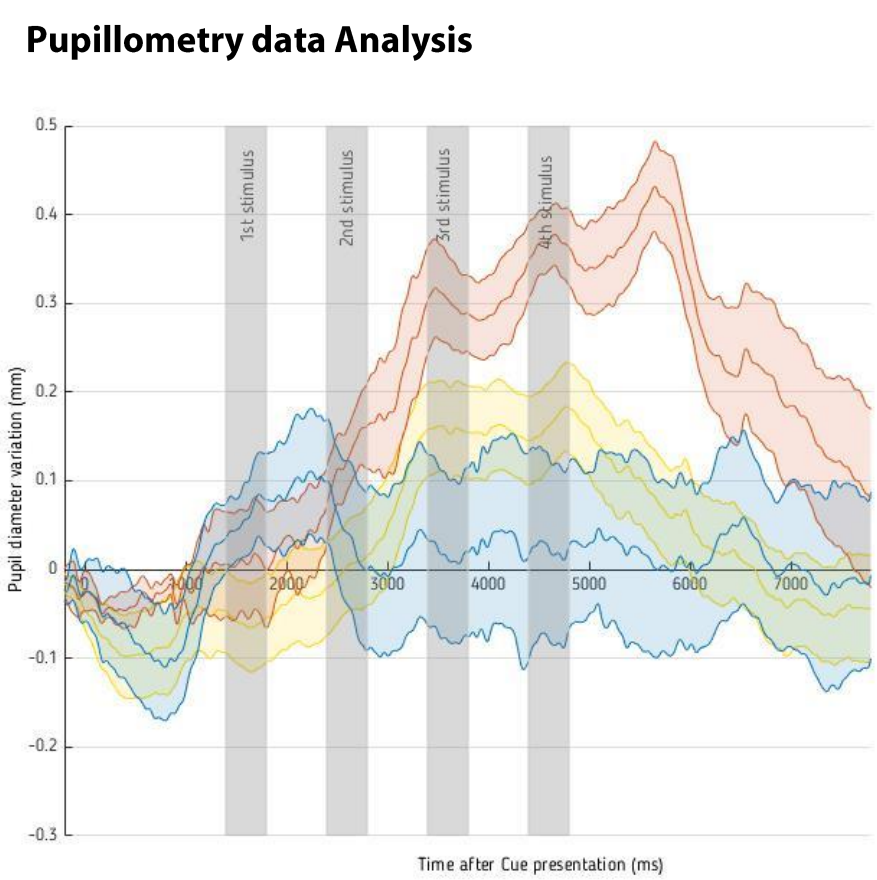

Figure B. 3 - Pupil diameter fluctuations for all three conditions during NO TMS blocks: Passive-viewing (blue trace), C1 (yellow trace) and C2 (red trace). Data is shown from [-200, 8000] ms, where the origin point corresponds to the moment of the cue offset. Time intervals in grey columns correspond to the interval time in which the according stimulus appeared. Shaded regions represent the mean ± 1 SE. C1: M=0,027mm, SE=0,058mm; C2: M=0,1745mm, SE=0,0527mm; Passive-viewing: M=0,0120mm, SE=0,0823mm.

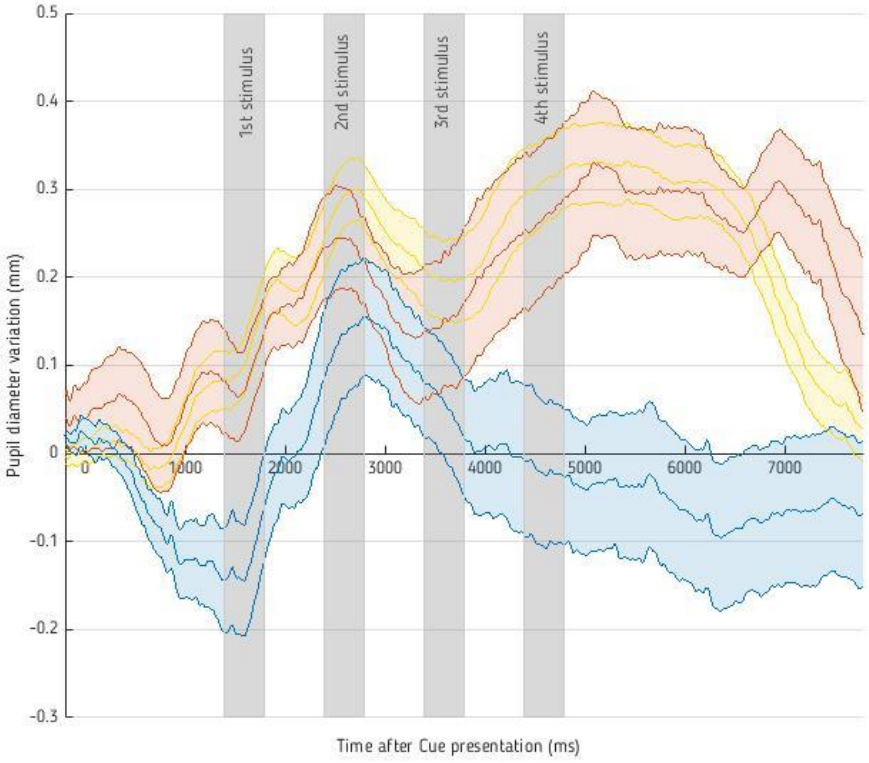

Figure B.4 - Pupil diameter fluctuations for all three conditions during TMSDELAY blocks: passive-viewing (blue trace), C1 (yellow trace) and C2 (red trace) conditions. Data is shown from [-200, 8000] ms, where the origin point corresponds to the moment of the cue offset. Time intervals in grey columns correspond to the interval time in which the according stimulus appeared. Shaded regions represent the mean ± 1 SE. C1: M=0,1899mm, SE=0,0376mm; C2: M=0,1933mm, SE=0,0644mm; Passive-viewing: M=-0,0203mm, SE=0,0659mm.

REFERENCES

1. Robinson DA. Implications of neural networks for how we think about brain function. *Behav Brain Sci*. 1992;15:644-655. doi:10.1017/CBO9780511529788.005.
2. Penfield W, Rasmussen T. The Cerebral Cortex of Man. Vol 11.; 1953. doi:10.1002/ajpa.1330110318.
3. Tallien T, Chung JY, Rousseau F, Girard N, Lefevre J, Mahadevan L. On the growth and form of cortical convolutions. Nat Phys. 2016;12:588-593.
4. Brodmann. Localisation in the Cerebral Cortex. Vol 1.; 2015. doi:10.1017/CBO9781107415324.004.
5. Hendry SH, Jones EG. Sizes and distributions of intrinsic neurons incorporating tritiated GABA in monkey sensory-motor cortex. J Neurosci. 1981;1(4):390-408.
6. Porter R, Lemon R. Corticospinal function and voluntary movement. Monogr Physiol Soc 45. 1993:1-421. doi:10.1093/acprof:oso/9780198523758.001.0001.
7. Kandel ER, Schwartz JH, Jessell TM. Principles of Neural Science. Vol 4.; 2000. doi:10.1036/0838577016.
8. Weiler N, Wood L, Yu J, Solla SA, Shepherd GMG. Top-down laminar organization of the excitatory network in motor cortex. Nat Neurosci. 2008;11(3):360-366. doi:10.1038/nn2049.
9. Jones EG. Anatomy of cerebral cortex: columnar input-output organization. In: The organization of the cerebral cortex (Schmitt FO, Worden FG, Adelmann G, Dennis SG, eds), pp 199-235. 1981. Cambridge: MIT Press.
10. Porter R, Lemon R. Corticospinal function and voluntary movement. Monogr Physiol Soc 45. 1993:1-421. doi:10.1093/acprof:oso/9780198523758.001.0001.
11. Weiler N, Wood L, Yu J, Solla SA, Shepherd GMG. Top-down laminar organization of the excitatory network in motor cortex. Nat Neurosci. 2008;11(3):360-366. doi:10.1038/nn2049.
12. Sanes JN, Donoghue JP. Plasticity and Primary Motor Cortex. Annu Rev Neurosci. 2000;23(1):393-415. doi:0147-006X.
13. Kandel ER, Schwartz JH, Jessell TM. Principles of Neural Science. Vol 4.; 2000. doi:10.1036/0838577016.

14. Dum RP, Strick PL. The origin of corticospinal projections from the premotor areas in the frontal lobe. J Neurosci. 1991;11(March):667-689. doi:S0022510X0200268X [pii].
15. Dum RP, Strick PL. Motor areas in the frontal lobe of the primate. Physiol Behav. 2002;77(4-5):677-682. doi:10.1016/S0031-9384(02)00929-0.
16. Fritsch G, Hitzig E. Electric excitability of the cerebrum. Epilepsy Behav. 2009;15(2):123-130. doi:10.1016/j.yebeh.2009.03.001.
17. Penfield W, Boldrey E. Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. Brain. 1937;60(4):389-443. doi:10.1093/brain/60.4.389.
18. Penfield W, Rasmussen T. The Cerebral Cortex of Man. Vol 221.; 1950. http://doi.apa.org/?uid=1939-00084-001.
19. Campbell NA, Reece JB, Urry LA, et al. Biology. In: Biology. ; 2008:149-177. doi:10.1007/s13398-014-0173-7.2.
20. Kocak M, Ulmer JL, Sahin Ugurel M, Gaggl W, Prost RW. Motor homunculus: passive mapping in healthy volunteers by using functional MR imaging--initial results. Radiology. 2009;251(2):485-492. doi:10.1148/radiol.2512080231.
21. Zeharia N, Hertz U, Flash T, Amedi A. Negative blood oxygenation level dependent homunculus and somatotopic information in primary motor cortex and supplementary motor area. Proc Natl Acad Sci U S A. 2012;109(45):18565-18570. doi:10.1073/pnas.1119125109.
22. Jacobs, KM; Donoghue J, Jacobs KM, Donoghue JP. Reshaping the Cortical Motor Map by Unmasking Latent Intracortical Connections. Science (80-). 1991;251(4996):944-947. doi:10.1126/science.2000496.
23. Colebatch JG, Deiber MP, Passingham RE, Friston KJ, Frackowiak RS. Regional cerebral blood flow during voluntary arm and hand movements in human subjects. J Neurophysiol. 1991;65(6):1392-1401.
24. Rao SM, Binder JR, Hammeke T a, et al. Somatotopic mapping of the human primary motor cortex with functional magnetic resonance imaging. Neurology. 1995;45(5):919-924. doi:10.1212/WNL.45.5.919.
25. Chen R. Interactions between inhibitory and excitatory circuits in the human motor cortex. Exp Brain Res. 2004;154(1):1-10. doi:10.1007/s00221-003-1684-1.
26. Emson PC. GABAB receptors: structure and function. Prog Brain Res. 2007;160:43-57. doi:10.1016/S0079-6123(06)60004-6.

27. Sah P, Hestrin S, Nicoll R a. Tonic activation of NMDA receptors by ambient glutamate enhances excitability of neurons. Science. 1989;246(4931):815-818. doi:10.1126/science.2573153.

28. MacDermott AB, Mayer ML, Westbrook GL, Smith SJ, Barker JL. NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. Nature. 1986;321(6069):519-522. doi:10.1038/321519a0.

29. Newcomer JW, Farber NB, Olney JW. NMDA receptor function, memory, and brain aging. Dialogues Clin Neurosci. 2000;2(3):219-232.

30. Stafstrom CE. Back to Basics: The Pathophysiology of Epileptic Seizures: A Primer For Pediatricians. Pediatr Rev. 1998;19(10):342 LP-351. doi:10.1542/pir.19-10-342.

31. Dutra, T. G.; Baltar, A.; Monte-Silva, K. K. (2016): Motor cortex excitability in attention-deficit hyperactivity disorder (ADHD): A systematic review and metaanalysis. Research in developmental disabilities 56, S. 1–9. doi: 10.1016/j.ridd.2016.01.022.

32. Ruohonen J (1998) Transcranial Magnetic Stimulation: Modelling and New Techniques. Dissertation, Helsinki University of Technology, Laboratory of Biomedical Engineering (BioMag).

33. Faraday M. Experimental Researches in Electricity. Philos Trans R Soc London. 1832;122(January):125-162. doi:10.1098/rstl.1832.0006.

34. Horvath JC, Perez JM, Forrow L, Fregni F, Pascual-Leone A. Transcranial magnetic stimulation: a historical evaluation and future prognosis of therapeutically relevant ethical concerns. J Med Ethics. 2011;37:137-143. doi:10.1136/jme.2010.039966.

35. Nowak R. The buzz around brain stimulation. Cosmos Magazine. 2015, May. Acessed from https://www.pressreader.com/australia/cosmos/20150528/282282433900943.

36. Kujirai T, Caramia MD, Rothwell JC, et al. Corticocortical inhibition in human motor cortex. J Physiol. 1993;471:501-519. doi:VL - 471.

37. Di Lazzaro V, Oliviero A, Pilato F, et al. The physiological basis of transcranial motor cortex stimulation in conscious humans. Clin Neurophysiol. 2004;115(2):255-266. doi:10.1016/j.clinph.2003.10.009.

38. Di Lazzaro V, Restuccia D, Oliviero A, et al. Effects of voluntary contraction on descending volleys evoked by transcranial stimulation in conscious humans. J Physiol. 1998;508(2):625-633. doi:10.1111/j.1469-7793.1998.625bq.x.

39. Di Lazzaro V, Oliviero A, Saturno E, et al. The effect on corticospinal volleys of reversing the direction of current induced in the motor cortex by transcranial magnetic stimulation. Exp Brain Res. 2001;138(2):268-273. doi:10.1007/s002210100722.

40. Di Lazzaro V, Rothwell JC. Corticospinal activity evoked and modulated by non-invasive stimulation of the intact human motor cortex. J Physiol. 2014;592:4115-4128. doi:10.1113/jphysiol.2014.274316.
41. Hannah R, Rothwell JC. Pulse Duration as Well as Current Direction Determines the Specificity of Transcranial Magnetic Stimulation of Motor Cortex during Contraction. Brain Stimul. 2017;10(1):106-115. doi:10.1016/j.brs.2016.09.008.
42. Ni Z, Charab S, Gunraj C, et al. Transcranial magnetic stimulation in different current directions activates separate cortical circuits. J Neurophysiol. 2011;105(2):749-756. doi:10.1152/jn.00640.2010.
43. Hannah R, Rothwell JC. Pulse Duration as Well as Current Direction Determines the Specificity of Transcranial Magnetic Stimulation of Motor Cortex during Contraction. Brain Stimul. 2017;10(1):106-115. doi:10.1016/j.brs.2016.09.008.
44. Barre PE, Redini F, Boumediene K, Vielpeau C, Pujol JP. Comparison of descending volleys evoked by monophasic and biphasic magnetic stimulation of the motor cortex in conscious humans. Exp Brain Res. 2001;141(1):121-127. doi:10.1007/s002210100863.
45. Di Lazzaro V, Profice P, Pilato F, Dileone M, Oliviero A, Ziemann U. The effects of motor cortex rTMS on corticospinal descending activity. Clin Neurophysiol. 2010;121(4):464-473. doi:10.1016/j.clinph.2009.11.007.
46. Chen R, Cros D, Curra A, et al. The clinical diagnostic utility of transcranial magnetic stimulation: Report of an IFCN committee. Clin Neurophysiol. 2008;119(3):504-532. doi:10.1016/j.clinph.2007.10.014.
47. Wassermann EM. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5–7, 1996. Electroencephalogr Clin Neurophysiol. 1998;108:1-16. doi:10.1016/S0168-5597(97)00096-8.
48. Rossi S, Hallett M, Rossini PM, et al. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol. 2009;120(12):2008-2039. doi:10.1016/j.clinph.2009.08.016.
49. Theodore WH. Transcranial Magnetic Stimulation in Epilepsy. EPILEPSY Behav. 2001;2(3, S):S36-S40. doi:10.1006/ebeh.2001.0216.
50. Rossini PM, Barker AT, Berardelli A, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. Electroencephalogr Clin Neurophysiol. 1994;91(2):79-92. doi:10.1016/0013-4694(94)90029-9.

51. Mills KR, Nithi KA. Corticomotor threshold to magnetic stimulation: Normal values and repeatability. Muscle and Nerve. 1997;20(5):570-576. doi:10.1002/(SICI)1097-4598(199705)20:5<570::AID-MUS5>3.0.CO;2-6.

52. Ziemann U, Lönnecker S, Steinhoff BJ, Paulus W. Effects of antiepileptic drugs on motor cortex excitability in humans: A transcranial magnetic stimulation study. Ann Neurol. 1996;40(3):367-378. doi:10.1002/ana.410400306.

53. Hallett M. Transcranial Magnetic Stimulation: A Primer. Neuron. 2007;55(2):187-199. doi:10.1016/j.neuron.2007.06.026.

54. McConnell KA, Nahas Z, Shastri A, Lorberbaum JP, Kozel FA, Bohning DE, George MS. The transcranial magnetic stimulation motor threshold depends on the distance from coil to underlying cortex: A replication in healthy adults comparing two methods of assessing the distance to cortex. Biol Psychiatry. 2001;49:454–459. doi: 10.1016/S0006-3223(00)01039-8

55. Kozel FA, Nahas ZH, DeBrux C, et al. How coil-cortex distance relates to age, motor threshold, and antidepressant response to repetitive transcranial magnetic stimulation. J Neuropsychiatry Clin Neurosci. 2000;12(3):376-384. doi:10.1176/jnp.12.3.376.

56. Mills KR, Nithi KA. Corticomotor threshold to magnetic stimulation: Normal values and repeatability. Muscle and Nerve. 1997;20(5):570-576. doi:10.1002/(SICI)1097-4598(199705)20:5<570::AID-MUS5>3.0.CO;2-6.

57. Lang N, Rothkegel H, Peckolt H, Deuschl G. Effects of lacosamide and carbamazepine on human motor cortex excitability: A double-blind, placebo-controlled transcranial magnetic stimulation study. Seizure. 2013;22(9):726-730. doi:10.1016/j.seizure.2013.05.010.

58. Ugawa Y, Terao Y, Hanajima R, Sakai K, Kanazawa I. Facilitatory effect of tonic voluntary contraction on responses to motor cortex stimulation. Electroencephalogr Clin Neurophysiol Electromyogr. 1995;97(6):451-454. doi:10.1016/0924-980X(95)00214-6.

59. Temesi J, Gruet M, Rupp T, Verges S, Millet GY. Resting and active motor thresholds versus stimulus-response curves to determine transcranial magnetic stimulation intensity in quadriceps femoris. J Neuroeng Rehabil. 2014;11(1):40. doi:10.1186/1743-0003-11-40.

60. Westin GG, Bassi BD, Lisanby SH, Luber B. Determination of motor threshold using visual observation overestimates transcranial magnetic stimulation dosage: Safety implications. Clin Neurophysiol. 2014;125(1):142-147. doi:10.1016/j.clinph.2013.06.187.

61. Herbsman T, Forster L, Molnar C, et al. Motor threshold in transcranial magnetic stimulation: The impact of white matter fiber orientation and skull-to-cortex distance. Hum Brain Mapp. 2009;30(7):2044-2055. doi:10.1002/hbm.20649.
62. Epstein CM, Wassermann EM, Ziemann U. Oxford Handbook of Transcranial Stimulation.; 2012. doi:10.1093/oxfordhb/9780198568926.001.0001.
63. Darling WG, Wolf SL, Butler AJ. Variability of motor potentials evoked by transcranial magnetic stimulation depends on muscle activation. Exp Brain Res. 2006;174(2):376-385. doi:10.1007/s00221-006-0468-9.
64. Rösler KM, Roth DM, Magistris MR. Trial-to-trial size variability of motor-evoked potentials. a study using the triple stimulation technique. Exp Brain Res. 2008;187(1):51-59. doi:10.1007/s00221-008-1278-z.
65. Kiers L, Cros D, Chiappa KH, Fang J. Variability of motor potentials evoked by transcranial magnetic stimulation. Electroencephalogr Clin Neurophysiol Evoked Potentials. 1993;89(6):415-423. doi:10.1016/0168-5597(93)90115-6.
66. Fitzgerald PB, Williams S, Daskalakis ZJ. A transcranial magnetic stimulation study of the effects of cannabis use on motor cortical inhibition and excitability. Neuropsychopharmacology. 2009;34(11):2368-2375. doi:10.1038/npp.2009.71.
67. Duque J, Greenhouse I, Labruna L, Ivry RB. Physiological Markers of Motor Inhibition during Human Behavior. Trends Neurosci. 2017;40(4):219-236. doi:10.1016/j.tins.2017.02.006.
68. Eisen A, Siejka S, Schulzer M, Calne D. Age-dependent decline in motor evoked potential (MEP) amplitude: with a comment on changes in Parkinson's disease. Electroencephalogr Clin Neurophysiol Evoked Potentials. 1991;81(3):209-215. doi:10.1016/0168-5597(91)90074-8.
69. Helfrich C, Pierau SS, Freitag CM, Roeper J, Ziemann U, Bender S. Monitoring Cortical Excitability during Repetitive Transcranial Magnetic Stimulation in Children with ADHD: A Single-Blind, Sham-Controlled TMS-EEG Study. PLoS One. 2012;7(11). doi:10.1371/journal.pone.0050073.
70. Oberman LM, Ifert-Miller F, Najib U, et al. Abnormal Mechanisms of Plasticity and Metaplasticity in Autism Spectrum Disorders and Fragile X Syndrome. J Child Adolesc Psychopharmacol. 2016;26(7):617-624. doi:10.1089/cap.2015.0166.
71. Hallett M. Transcranial Magnetic Stimulation: A Primer. Neuron. 2007;55(2):187-199. doi:10.1016/j.neuron.2007.06.026.

72. Wassermann EM. Variation in the response to transcranial magnetic brain stimulation in the general population. Clin Neurophysiol. 2002;113(7):1165-1171. doi:10.1016/S1388-2457(02)00144-X.

73. Kallioniemi E, Pitkänen M, Säisänen L, Julkunen P. Onset Latency of Motor Evoked Potentials in Motor Cortical Mapping with Neuronavigated Transcranial Magnetic Stimulation. Open Neurol J. 2015;9:62-69. doi:10.2174/1874205X01509010062.

74. Firmin L, Müller S, Rösler KM. The latency distribution of motor evoked potentials in patients with multiple sclerosis. Clin Neurophysiol. 2012;123(12):2414-2421. doi:10.1016/j.clinph.2012.05.008.

75. Möller C, Arai N, Lücke J, Ziemann U. Hysteresis effects on the input-output curve of motor evoked potentials. Clin Neurophysiol. 2009;120(5):1003-1008. doi:10.1016/j.clinph.2009.03.001.

76. Devanne H, Lavoie BA, Capaday C. Input-output properties and gain changes in the human corticospinal pathway. Exp Brain Res. 1997;114(2):329-338. doi:10.1007/PL00005641.

77. Cunningham DA, Machado A, Janini D, et al. Assessment of inter-hemispheric imbalance using imaging and noninvasive brain stimulation in patients with chronic stroke. Arch Phys Med Rehabil. 2015;96(4):S94-S103. doi:10.1016/j.apmr.2014.07.419.

78. Potter-Baker KA, Varnerin NM, Cunningham DA, et al. Influence of corticospinal tracts from higher order motor cortices on recruitment curve properties in stroke. Front Neurosci. 2016;10(MAR). doi:10.3389/fnins.2016.00079.

79. Chen R, Lozano AM, Ashby P. Mechanism of the silent period following transcranial magnetic stimulation. Exp Brain Res. 1999;128(4):539-542. doi:10.1007/s002210050878.

80. Werhahn KJ, Kunesch E, Noachtar S, Benecke R, Classen J. Differential effects on motorcortical inhibition induced by blockade of GABA uptake in humans. J Physiol. 1999;517(2):591-597. doi:10.1111/j.1469-7793.1999.0591t.x.

81. Nakashima K, Wang Y, Shimoda M, Sakuma K, Takahashi K. Shortened silent period produced by magnetic cortical stimulation in patients with Parkinson's disease. J Neurol Sci. 1995;130(2):209-214. doi:10.1016/0022-510X(95)00029-2.

82. Lefaucheur JP. Motor cortex dysfunction revealed by cortical excitability studies in Parkinson's disease: Influence of antiparkinsonian treatment and cortical stimulation. Clin Neurophysiol. 2005;116(2):244-253. doi:10.1016/j.clinph.2004.11.017.

83. Daskalakis ZJ, Christensen BK, Chen R, Fitzgerald PB, Zipursky RB, Kapur S. Evidence for impaired cortical inhibition in schizophrenia using transcranial magnetic stimulation. Arch Gen Psychiatry. 2002;59(4):347-354. doi:10.1001/archpsyc.59.4.347.

84. Levinson AJ, Fitzgerald PB, Favalli G, Blumberger DM, Daigle M, Daskalakis ZJ. Evidence of Cortical Inhibitory Deficits in Major Depressive Disorder. Biol Psychiatry. 2010;67(5):458-464. doi:10.1016/j.biopsych.2009.09.025.

85. Richter MA, de Jesus DR, Hoppenbrouwers S, et al. Evidence for Cortical Inhibitory and Excitatory Dysfunction in Obsessive Compulsive Disorder. Neuropsychopharmacology. 2012;37(5):1144-1151. doi:10.1038/npp.2011.300.

86. Alagona G, Ferri R, Pennisi G, et al. Motor cortex excitability in Alzheimer's disease and in subcortical ischemic vascular dementia. Neurosci Lett. 2004;362(2):95-98. doi:10.1016/j.neulet.2004.03.006.

87. van Kuijk A a, Pasman JW, Geurts ACH, Hendricks HT. How salient is the silent period? The role of the silent period in the prognosis of upper extremity motor recovery after severe stroke. J Clin Neurophysiol. 2005;22(1):10-24. doi:10.1097/01.WNP.0000150975.83249.71.

88. Priori A, Berardelli A, Inghilleri M, Polidori L, Manfredi M. Electromyographic silent period after transcranial brain stimulation in Huntington's disease. Mov Disord. 1994b;9:178–182. [PubMed]

89. Wu SW, Gilbert DL, Shahana N, Huddleston DA, Mostofsky SH. Transcranial Magnetic Stimulation Measures in Attention-Deficit/Hyperactivity Disorder. Pediatr Neurol. 2012;47(3):177-185. doi:10.1016/j.pediatrneurol.2012.06.003.

90. Cisek P. Cortical mechanisms of action selection: the affordance competition hypothesis. Philos Trans R Soc Lond B Biol Sci. 2007;362(1485):1585-1599. doi:10.1098/rstb.2007.2054.

91. Marr D. Vision. A Computational Investigation into the Human Representation and Processing of Visual Information. Vol 94.; 1985. doi:10.2307/2185011.

92. Cisek P. Making decisions through a distributed consensus. Curr Opin Neurobiol. 2012;22(6):927-936. doi:10.1016/j.conb.2012.05.007.

93. Bestmann S, Duque J. Transcranial Magnetic Stimulation: Decomposing the Processes Underlying Action Preparation. Neurosci. 2015:1-14. doi:10.1177/1073858415592594.

94. Cisek P, Kalaska JF. Neural correlates of reaching decisions in dorsal premotor cortex: Specification of multiple direction choices and final selection of action. Neuron. 2005;45(5):801-814. doi:10.1016/j.neuron.2005.01.027.

95. Duque J, Ivry RB. Role of corticospinal suppression during motor preparation. Cereb Cortex. 2009;19(9):2013-2024. doi:10.1093/cercor/bhn230.

96. Duque J, Lew D, Mazzocchio R, Olivier E, Richard B. Evidence for two concurrent inhibitory mechanisms during response preparation. J Neurosci. 2010;30(10):3793-3802. doi:10.1523/JNEUROSCI.5722-09.2010.Evidence.

97. Klein P-A, Olivier E, Duque J. Influence of Reward on Corticospinal Excitability during Movement Preparation. J Neurosci. 2012;32(50):18124-18136. doi:10.1523/JNEUROSCI.1701-12.2012.

98. Van Den Wildenberg WPM, Burle B, Vidal F, Van Der Molen MW, Ridderinkhof KR, Hasbroucq T. Mechanisms and dynamics of cortical motor inhibition in the stop-signal paradigm: a TMS study. J Cogn Neurosci. 2010;22(2):225-239. doi:10.1162/jocn.2009.21248.

99. Kennefick M, Maslovat D, Carlsen AN. The time course of corticospinal excitability during a simple reaction time task. PLoS One. 2014;9(11). doi:10.1371/journal.pone.0113563.

100. Pascual-Leone A. Human corticospinal excitability evaluated with transcranial magnetic stimulation during different reaction time paradigms. Brain. 2000;123(6):1161-1173. doi:10.1093/brain/123.6.1161.

101. Chen R, Hallett M. The time course of changes in motor cortex excitability associated with voluntary movement. Can J Neurol Sci. 1999;26(3):163-169. doi:10.1017/S0317167100000196.

102. Cambridge Cognition. 2017. Choice Reaction Time. [ONLINE] Available at: http://www.cambridgecognition.com/cantab/cognitive-tests/choice-reaction-time-crt/. [Accessed 3 August 2017].

103. Tanji J, Evarts E. Anticipatory activity of motor cortex neurons in relation to direction of an intended movement. J Neurophysiol. 1976;39(5):1062-1068.

104. Chen R, Yaseen Z, Cohen LG, Hallett M. Time course of corticospinal excitability in reaction time and self-paced movements. Ann Neurol. 1998;44(3):317-325. doi:10.1002/ana.410440306.

105. Quoilin C, Lambert J, Jacob B, Klein PA, Duque J. Comparison of motor inhibition in variants of the instructed-delay choice reaction time task. PLoS One. 2016;11(8). doi:10.1371/journal.pone.0161964.

106. Greenhouse I, Saks D, Hoang T, Ivry RB. Inhibition during response preparation is sensitive to response complexity. J Neurophysiol. 2015;113(7):2792-2800. doi:10.1152/jn.00999.2014.

107. Berridge CW, Schmeichel BE, Espana RA. Noradrenergic modulation of wakefulness/arousal. Sleep Medicine Reviews 2012; 16(2):187–97. doi: 10.1016/j.smrv.2011.12.003.

108. Tully K, Li Y, Tsvetkov E, Bolshakov VY. Norepinephrine enables the induction of associative long-term potentiation at thalamo-amygdala synapses. Proc Natl Acad Sci U S A. 2007;104:14146–14150.

109. Aston-Jones G, Foote SL, Bloom FE. Anatomy and physiology of locus coeruleus neurons: functional implications. In: Ziegler M, Lake CR, editors. Norepinephrine (Frontiers of Clinical Neuroscience) vol. 2. Williams and Wilkins; Baltimore: 1984. pp. 92–116.

110. Robertson SD, Plummer NW, de Marchena J, Jensen P. Developmental origins of central norepinephrine neuron diversity. Nat Neurosci. 2013;16(8):1016-1023. doi:10.1038/nn.3458.

111. Eisenhofer G, Kopin IJ, Goldstein DS. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. Pharmacol Rev. 2004;56(3):331-349. doi:10.1124/pr.56.3.1.

112. Green PR. Biological Psychology. Anim Behav. 2011;82(3):607. doi:10.1016/j.anbehav.2011.05.023.

113. Harley CW. A role for norepinephrine in arousal, emotion and learning?: Limbic modulation by norepinephrine and the kety hypothesis. Prog Neuropsychopharmacol Biol Psychiatry. 1987;11(4):419-458. doi:10.1016/0278-5846(87)90015-7.

114. Leonard BE. Stress, norepinephrine and depression. J Psychiatry Neurosci. 2001;26(SUPPL.). doi:10.1034/j.1601-5215.2002.140403.x.

115. O'Donnell J, Zeppenfeld D, McConnell E, Pena S, Nedergaard M. Norepinephrine: A neuromodulator that boosts the function of multiple cell types to optimize CNS performance. Neurochem Res. 2012;37(11):2496-2512. doi:10.1007/s11064-012-0818-x.

116. Aston-Jones G, Cohen JD. Adaptive gain and the role of the locus coeruleus-norepinephrine system in optimal performance. In: Journal of Comparative Neurology. Vol 493. ; 2005:99-110. doi:10.1002/cne.20723.

117. Berridge CW. Noradrenergic modulation of arousal. Brain Res Rev. 2008;58(1):1-17. doi:10.1016/j.brainresrev.2007.10.013.

118. Marder E. Neuromodulation of Neuronal Circuits: Back to the Future. Neuron. 2012;76(1):1-11. doi:10.1016/j.neuron.2012.09.010.

119. Nadim F, Bucher D. Neuromodulation of neurons and synapses. Curr Opin Neurobiol. 2014;29:48-56. doi:10.1016/j.conb.2014.05.003.

120. Aston-Jones G, Cohen JD. AN INTEGRATIVE THEORY OF LOCUS COERULEUS-NOREPINEPHRINE FUNCTION: Adaptive Gain and Optimal Performance. Annu Rev Neurosci. 2005;28(1):403-450. doi:10.1146/annurev.neuro.28.061604.135709.

121. Brady ST, Siegel GJ, Albers RW, Price DL. Basic Neurochemistry.; 2012. doi:10.1016/C2009-0-00066-X.

122. Wallukat G. The beta-adrenergic receptors. Herz. 2002;27(7):683-690. doi:10.1007/s00059-002-2434-z.

123. Palacios JM, Hoyer D, Cortés R. α1-adrenoceptors in the mammalian brain: similar pharmacology but different distribution in rodents and primates. Brain Res. 1987;419(1-2):65-75. doi:10.1016/0006-8993(87)90569-5.

124. Plewnia C, Hoppe J, Hiemke C, Bartels M, Cohen LG, Gerloff C. Enhancement of human cortico-motoneuronal excitability by the selective norepinephrine reuptake inhibitor reboxetine. Neurosci Lett. 2002;330(3):231-234. doi:10.1016/S0304-3940(02)00803-0.

125. Plewnia C, Bartels M, Cohen L, Gerloff C. Noradrenergic modulation of human cortex excitability by the presynaptic alpha(2)-antagonist yohimbine. Neurosci Lett. 2001;307:41-44. doi:10.1016/S0304-3940(01)01921-8.

126. Marzo A, Bai J, Otani S. Neuroplasticity regulation by noradrenaline in mammalian brain. Curr Neuropharmacol. 2009;7(4):286-295. doi:10.2174/157015909790031193.

127. Bear MF, Malenka RC. Synaptic plasticity: LTP and LTD. Curr Opin Neurobiol. 1994;4(3):389-399. doi:10.1016/0959-4388(94)90101-5.

128. Kaleem A, Hoessli DC, Haq IU, et al. CREB in long-term potentiation in hippocampus: Role of post-translational modifications-studies in silico. J Cell Biochem. 2011;112(1):138-146. doi:10.1002/jcb.22909.

129. Borodovitsyna O, Flamini M, Chandler D. Noradrenergic Modulation of Cognition in Health and Disease. Neural Plast. 2017;2017. doi:10.1155/2017/6031478.

130. Klimek V, Rajkowska G, Luker SN, et al. Brain noradrenergic receptors in major depression and schizophrenia. Neuropsychopharmacology. 1999;21(1):69-81. doi:10.1016/S0893-133X(98)00134-1.

131. Agster KL, Clark BD, Gao WJ, et al. Experimental strategies for investigating psychostimulant drug actions and prefrontal cortical function in ADHD and related attention disorders. Anat Rec. 2011;294(10):1698-1712. doi:10.1002/ar.21403.

132. McMillan PJ, White SS, Franklin A, et al. Differential response of the central noradrenergic nervous system to the loss of locus coeruleus neurons in Parkinson's disease and Alzheimer's disease. Brain Res. 2011;1373:240-252. doi:10.1016/j.brainres.2010.12.015.

133. Devilbiss DM, Berridge CW. Cognition-Enhancing Doses of Methylphenidate Preferentially Increase Prefrontal Cortex Neuronal Responsiveness. Biol Psychiatry. 2008;64(7):626-635. doi:10.1016/j.biopsych.2008.04.037.

134. Sara SJ, Roullet P, Przybyslawski J. Consolidation of Memory for Odor–Reward Association: β-Adrenergic Receptor Involvement in the Late Phase. Learn Mem. 1999;6(2):88-96. doi:10.1101/lm.6.2.88.

135. Murchison CF, Zhang XY, Zhang WP, Ouyang M, Lee A, Thomas SA. A distinct role for norepinephrine in memory retrieval. Cell. 2004;117(1):131-143. doi:10.1016/S0092-8674(04)00259-4.

136. Moore TL, Schettler SP, Killiany RJ, et al. Cognitive impairment in aged rhesus monkeys associated with monoamine receptors in the prefrontal cortex. Behav Brain Res. 2005;160(2):208-221. doi:10.1016/j.bbr.2004.12.003.

137. Chamberlain SR, Müller U, Blackwell AD, Robbins TW, Sahakian B. Noradrenergic modulation of working memory and emotional memory in humans. Psychopharmacol. 2006;188:397–407.

138. Sara SJ. The locus coeruleus and noradrenergic modulation of cognition. Nat Rev Neurosci. 2009;10(3):211-223. doi:10.1038/nrn2573.

139. Clayton EC. Phasic Activation of Monkey Locus Ceruleus Neurons by Simple Decisions in a Forced-Choice Task. J Neurosci. 2004;24(44):9914-9920. doi:10.1523/JNEUROSCI.2446-04.2004.

140. Aston-Jones G, Rajkowski J, Cohen J. Role of locus coeruleus in attention and behavioral flexibility. Biol Psychiatry. 1999;46(9):1309-1320. doi:10.1016/S0006-3223(99)00140-7.

141. Devilbiss DM, Waterhouse BD. Phasic and tonic patterns of locus coeruleus output differentially modulate sensory network function in the awake rat. J Neurophysiol. 2011;105(1):69-87. doi:10.1152/jn.00445.2010.

142. Birnbaum S, Gobeske KT, Auerbach J, Taylor JR, Arnsten AFT. A role for norepinephrine in stress-induced cognitive deficits: ??-1-Adrenoceptor mediation in the prefrontal cortex. Biol Psychiatry. 1999;46(9):1266-1274. doi:10.1016/S0006-3223(99)00138-9.

143. Arnsten AFT, Mathew R, Ubriani R, Taylor JR, Li BM. ??-1 noradrenergic receptor stimulation impairs prefrontal cortical cognitive function. Biol Psychiatry. 1999;45(1):26-31. doi:10.1016/S0006-3223(98)00296-0.

144. Bremner JD. Traumatic stress: Effects on the brain. Dialogues Clin Neurosci. 2006;8(4):445-461.

145. Sun H, Green TA, Theobald DEH, et al. Yohimbine Increases Impulsivity Through Activation of cAMP Response Element Binding in the Orbitofrontal Cortex. Biol Psychiatry. 2010;67(7):649-656. doi:10.1016/j.biopsych.2009.11.030.

146. Swann AC, Birnbaum D, Jagar AA, Dougherty DM, Moeller FG. Acute yohimbine increases laboratory-measured impulsivity in normal subjects. Biol Psychiatry. 2005;57(10):1209-1211. doi:10.1016/j.biopsych.2005.02.007.

147. Berridge CW, Waterhouse BD. The locus coeruleus-noradrenergic system: Modulation of behavioral state and state-dependent cognitive processes. Brain Res Rev. 2003;42(1):33-84. doi:10.1016/S0165-0173(03)00143-7.

148. Gilzenrat MS, Nieuwenhuis S, Jepma M, Cohen JD. Pupil diameter tracks changes in control state predicted by the adaptive gain theory of locus coeruleus function. Cogn Affect Behav Neurosci. 2010;10(2):252-269. doi:10.3758/CABN.10.2.252.

149. Kardon R. Pupillary light reflex. Curr Opin Ophthalmol. 1995;6(6):20-26. doi:10.1097/00055735-199512000-00004.

150. Piquado T, Isaacowitz D, Wingfield A. Pupillometry as a measure of cognitive effort in younger and older adults. Psychophysiology. 2010;47(3):560-569. doi:10.1111/j.1469-8986.2009.00947.x.

151. Preuschoff K, 't Hart BM, Einhäuser W. Pupil dilation signals surprise: Evidence for noradrenaline's role in decision making. Front Neurosci. 2011;(SEP):1-12. doi:10.3389/fnins.2011.00115.

152. de Gee JW, Knapen T, Donner TH. Decision-related pupil dilation reflects upcoming choice and individual bias. Proc Natl Acad Sci. 2014;111(5):E618-E625. doi:10.1073/pnas.1317557111.

153. Ferdous N, Jianu R. Using Pupil Size as an Indicator for Task Difficulty in Data Visualization. IEEE Inf Vis. 2014;(October):1-2.

154. Zénon A, Sidibé M, Olivier E. Pupil size variations correlate with physical effort perception. Front Behav Neurosci. 2014;8. doi:10.3389/fnbeh.2014.00286.

155. Costa VD, Rudebeck PH. More than Meets the Eye: The Relationship between Pupil Size and Locus Coeruleus Activity. Neuron. 2016;89(1):8-10. doi:10.1016/j.neuron.2015.12.031.

156. Varazzani C, San-Galli A, Gilardeau S, Bouret S. Noradrenaline and dopamine neurons in the reward/effort trade-off: a direct electrophysiological comparison in behaving monkeys. J. Neurosci., 35 (2015), pp. 7866-7877. doi:10.1523/JNEUROSCI.0454-15.2015

157. Joshi S, Li Y, Kalwani RM, Gold JI. Relationships between Pupil Diameter and Neuronal Activity in the Locus Coeruleus, Colliculi, and Cingulate Cortex. Neuron. 2016;89(1):221-234. doi:10.1016/j.neuron.2015.11.028.

158. Rossi S, Hallett M, Rossini PM, et al. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol. 2009;120(12):2008-2039. doi:10.1016/j.clinph.2009.08.016.

159. Bastani A, Jaberzadeh S. A Higher Number of TMS-Elicited MEP from a Combined Hotspot Improves Intra- and Inter-Session Reliability of the Upper Limb Muscles in Healthy Individuals. PLoS One. 2012;7(10). doi:10.1371/journal.pone.0047582.

160. Claffey MP, Sheldon S, Stinear CM, Verbruggen F, Aron AR. Having a goal to stop action is associated with advance control of specific motor representations. Neuropsychologia. 2010;48(2):541-548. doi:10.1016/j.neuropsychologia.2009.10.015.

161. Tobii. Tobii Eye Tracking - An introduction to eye tracking and Tobii Eye Trackers. Technology. 2010:12. http://www.tobii.com/Global/Analysis/Training/WhitePapers/Tobii_EyeTracking_Introduction_WhitePaper.pdf?epslanguage=en.

162. Kammer T, Beck S, Thielscher A, Laubis-Herrmann U, Topka H. Motor thresholds in humans: A transcranial magnetic stimulation study comparing different pulse waveforms, current directions and stimulator types. Clin Neurophysiol. 2001;112(2):250-258. doi:10.1016/S1388-2457(00)00513-7.

163. Stamoulis C, Oberman LM, Praeg E, Bashir S, Pascual-Leone A. Single pulse TMS-induced modulations of resting brain neurodynamics encoded in EEG phase. Brain Topogr. 2011;24(2):105-113. doi:10.1007/s10548-010-0169-3.

164. Delorme A, Makeig S. EEGLAB: An open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods. 2004;134(1):9-21. doi:10.1016/j.jneumeth.2003.10.009.

165. Strohl J, Luchman J, Khun J, Pierce E, Andrews K. Exploring the relationship between eye movements and pupillary response from formative user experience research. In: Lecture Notes in Computer Science (Including Subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics). Vol 9737. ; 2016:472-480. doi:10.1007/978-3-319-40250-5_45.

166. Kuchinke L, Võ MLH, Hofmann M, Jacobs AM. Pupillary responses during lexical decisions vary with word frequency but not emotional valence. Int J Psychophysiol. 2007;65(2):132-140. doi:10.1016/j.ijpsycho.2007.04.004.

167. Rajkowski J. Activation of Monkey Locus Coeruleus Neurons Varies With Difficulty and Performance in a Target Detection Task. J Neurophysiol. 2004;92(1):361-371. doi:10.1152/jn.00673.2003.

168. Rubinstein A. Response time and decision making: An experimental study. Judgm Decis Mak. 2013;8(5):540-551.

169. Gross J, Pollok B, Dirks M, Timmermann L, Butz M, Schnitzler A. Task-dependent oscillations during unimanual and bimanual movements in the human primary motor cortex and SMA studied with magnetoencephalography. Neuroimage. 2005;26(1):91-98. doi:10.1016/j.neuroimage.2005.01.025.

170. Fan, Xiaoli & Qianxiang, Zhou & Xie, Fang & Liu, Zhongqi. (2017). Effects of Time Pressure and Task Difficulty on Visual Search. 97-109. doi:10.1007/978-3-319-41959-6_9.

171. Yokota Y, Igarashi Y, Okada M, Naruse Y. Estimation of the reaction times in tasks of varying difficulty from the phase coherence of the auditory steady-state response using the least absolute shrinkage and selection operator analysis. In: Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS. Vol 2015-November. ; 2015:6662-6666. doi:10.1109/EMBC.2015.7319921.

172. Ramakrishnan A, Murthy A. Brain mechanisms controlling decision making and motor planning. Prog Brain Res. 2013;202:321-345. doi:10.1016/B978-0-444-62604-2.00017-4.

173. Wahn B, Ferris DP, Hairston WD, König P. Pupil sizes scale with attentional load and task experience in a multiple object tracking task. PLoS One. 2016;11(12). doi:10.1371/journal.pone.0168087.

174. Foltys H, Sparing R, Boroojerdi B, et al. Motor control in simple bimanual movements: A transcranial magnetic stimulation and reaction time study. Clin Neurophysiol. 2001;112(2):265-274. doi:10.1016/S1388-2457(00)00539-3.

175. Leocani, L., Cohen, L.G., Wassermann, E.M., Ikoma, K., and Hallett, M. (2000a). Human corticospinal excitability evaluated with transcranial magnetic stimulation during different reaction time paradigms. Brain 123, 1161–1173. doi: 10.1093/brain/123.6.1161

176. Farzan F, Barr MS, Hoppenbrouwers SS, et al. The EEG correlates of the TMS-induced EMG silent period in humans. Neuroimage. 2013;83:120-134. doi:10.1016/j.neuroimage.2013.06.059.

177. Seeber KG, Kerzel D. Cognitive load in simultaneous interpreting: Model meets data. Int J Biling. 2012;16(2):228-242. doi:10.1177/1367006911402982.

178. Niehaus L, Guldin B, Meyer B. Influence of transcranial magnetic stimulation on pupil size. J Neurol Sci. 2001;182(2):123-128. doi:S0022510X00004627 [pii].

179. Matthews PBC. The effect of firing on the excitability of a model motoneurone and its implications for cortical stimulation. J Physiol. 1999;518(3):867-882. doi:10.1111/j.1469-7793.1999.0867p.x.

180. Vaseghi B, Zoghi M, Jaberzadeh S. Inter-pulse interval affects the size of single-pulse TMS-induced motor evoked potentials: A reliability study. Basic Clin Neurosci. 2015;6(1):44-51.

181. Nachev P, Wydell H, O'Neill K, Husain M, Kennard C. The role of the pre-supplementary motor area in the control of action. Neuroimage. 2007;36(SUPPL. 2). doi:10.1016/j.neuroimage.2007.03.034.

182. Reid CS, Serrien DJ. Handedness and the excitability of cortical inhibitory circuits. Behav Brain Res. 2012;230(1):144-148. doi:10.1016/j.bbr.2012.02.008.

183. Lebon F, Greenhouse I, Labruna L, Vanderschelden B, Papaxanthis C, Ivry RB. Influence of Delay Period Duration on Inhibitory Processes for Response Preparation. Cereb Cortex. 2016;26(6):2461-2470. doi:10.1093/cercor/bhv069.

184. Duque J, Labruna L, Cazares C, Ivry RB. Dissociating the influence of response selection and task anticipation on corticospinal suppression during response preparation. Neuropsychologia. 2014;65:287-296. doi:10.1016/j.neuropsychologia.2014.08.006.