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# IN VIVO EVALUATION OF FAST SENSORIMOTOR INTEGRATION IN THE HUMAN MOTOR HAND AREA: FROM PHYSIOLOGY TO PATHOLOGY

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**PREFACE**

The studies described in this thesis have been designed, performed or completed during the period between 2014 and 2017 at the Department of Neurosciences, Federico II University of Napoli, Italy, (supervised by Prof. Lucio Santoro), and at the Danish Research Centre for Magnetic Resonance (DRCMR), University of Copenhagen, DK, (supervised by Prof. Hartwig Roman Siebner). All the studies here reported have Raffaele Dubbioso as first author.

## SUMMARY AND AIM OF THE THESIS

The studies included in this thesis mainly evaluated in vivo the fast sensorimotor integration in the human sensorimotor area, by using a well-known TMS (transcranial magnetic stimulation) technique, called short-latency afferent inhibition (SAI).

Section 1 will review current knowledge on the biological and physiological basis of fast sensorimotor integration and its role in mild cognitive impairment and dementia.

Section 2 will report two studies. The first one is focused on using an innovative central sulcus-based mapping technique of SAI. We showed for the first time a centre-surround organization of fast sensorimotor integration in human motor hand area (Dubbioso et al., under review). The second study is mainly focused on the role of cerebellum in the modulation of somatosensory afferent pathway (Dubbioso et al. 2015). Indeed, we demonstrated that patients with pure cerebellar atrophy had an altered capability of cerebellar filtering or processing of time specific incoming sensory volleys, influencing the sensorimotor integration and plasticity of primary motor cortex (M1).

Section 3 will report two studies where SAI has been used as a tool to investigate functional involvement of central cholinergic circuits in two different types of cognitive impairment. In the first study we showed that patients with the adult form of Niemann Pick type C (NPC) are characterized by abnormal SAI (Dubbioso et al. 2014) whereas in the second one we found that SAI is normal in Parkinson disease (PD) patients with Freezing of Gait (FOG) (Dubbioso et al. 2015). Such results indicate that cognitive decline in NPC resembles from physiologically and clinical point of view primary form of cholinergic dementia such as Alzheimer disease. On the contrary, cognitive impairment in PD patients with FOG is mainly due to the involvement of non-cholinergic circuits, resembling forms of cognitive impairment dominated mainly by executive dysfunctions such as Fronto-temporal dementia.

**PUBLICATIONS RELATED TO THE THESIS**

1. Dubbioso R, Raffin E, Karabanov A, Thielscher A, Siebner HR. Centre-surround organization of fast sensorimotor integration in human motor hand area. Under review. 2017
2. Dubbioso R, Pellegrino G, Antenora A, De Michele G, Filla A, Santoro L, Manganelli F. The Effect of Cerebellar Degeneration on Human Sensori-motor Plasticity. Brain Stimul. 2015;8:1144-1150.
3. Dubbioso R*, Manganelli F*, Iodice R, Topa A, Dardis A, Russo CV, Ruggiero L, Tozza S, Filla A, Santoro L. Central cholinergic dysfunction in the adult form of Niemann Pick disease type C: a further link with Alzheimer's disease? J Neurol. 2014;26:804-808.
4. Dubbioso R*, Picillo M*, Iodice R, Iavarone A, Pisciotta C, Spina E, Santoro L, Barone P, Amboni M, Manganelli F. Short-latency afferent inhibition in patients with Parkinson's disease and freezing of gait. J Neural Transm (Vienna). 2015;122:1533-540.

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**SECTION 1**

**SHORT LATENCY AFFERENT INHIBITION (SAI)**

## BIOLOGICAL AND PHYSIOLOGICAL BASIS OF SHORT-LATENCY AFFERENT INHIBITION (SAI)

### Introduction

Skilled finger movements are critical to carrying out many daily activities such as writing, sorting coins, preparing food or using a smartphone. These seemingly trivial actions require the coordinated activation of a set of muscles at high temporo-spatial precision as well as the integration of sensory signals from the periphery. Such integration takes place in the primary sensorimotor cortex (SM1).

Classically, the primary somatosensory cortex (S1) and primary motor cortex (M1) are considered to be functionally segregated regions. According to this view S1 is thought to process sensory input whereas M1 to encode motor output (Sanes and Donoghue 2000). In recent years, lines of research showed that both, M1 and S1 jointly contribute to sensory and motor aspects of motor control (Hatsopoulos and Suminski 2011). M1 directly receives somatosensory input enabling highly flexible, context-dependent encoding of movement kinematics (Balzamo et al. 2004; Churchland and Shenoy 2007; Ferezou et al. 2007; Hatsopoulos et al. 2007), whereas S1 actively participates in motor control, for instance driving whisker retraction in mice (Matyas et al. 2010; Petersen 2014). In accordance with these findings, it has been shown that the human M1 can be subdivided into Brodmann areas 'BA4 anterior' (4a) and 'BA4 posterior' (4p), and the posterior area 4p has been implicated in encoding tactile information (Geyer et al. 1996). Influential concepts of sensorimotor integration stress an active influence of cortical sensory input on motor output and vice versa. Somatosensory inputs inform both, reflexive and volitional actions (Friston and Kiebel 2009; Friston et al. 2009; Hommel 2009). This comprises bodily feedback generated by the movement itself and somatosensory input signalling the consequence of a movement, for instance the haptic experience when manipulating an object. Conversely, motor output impacts on perception. In addition to this "reciprocity", sensorimotor synergies have to be adjustable to the behavioural context and convey predictive "feed-forward" information to facilitate sensorimotor control: During self-generated movements, the cortex generates a motor efference copy of the descending motor command. This efference copy enables predictions about the consequences of our actions (i.e. the action-induced percept) and actively primes perception (Wolpert et al. 1995). Reciprocal sensorimotor synergies in the $SM1_{HAND}$ support cooperative interactions between associated sensory and motor events.

In human, fast sensorimotor interactions in $SM1_{HAND}$ can be probed with transcranial magnetic stimulation (TMS). At the begging of the century, in their influential study, Tokimura and colleagues (Tokimura et al. 2000) demonstrated that peripheral nerve stimulation reduced the amplitude of TMS motor evoked potentials (MEPs) when the afferent sensory input precedes few milliseconds the motor output, a process called short-latency afferent inhibition (SAI). In more details, SAI was elicited by delivering a TMS pulse over the primary motor cortex 2–8 ms after the arrival of the afferent volley in somatosensory cortex (i.e., corresponding to the N20 somatosensory evoked potential (SEP)). Such finding was supported by previous literature showing that stimulation of the median nerve at wrist suppressed EMG activity evoked in relaxed hand muscle by TMS over the motor cortex 18-21 ms later, similar effect was demonstrated after stimulation of cutaneous nerve of the index finger as well. The

suppression of EMG responses occurred at the cortical level rather than spinal level, since H-reflexes in the forearm were not affected (Delwaide and Olivier 1990). Other studies reported the same results: either TMS or electrical brain stimulation in combination with peripheral electrical stimulation applied to fingers were able to reduce motor cortex excitability at a time corresponding to the transition between the initial inhibition and subsequent facilitation observed during the cutaneo-muscular reflex (Maertens de Noordhout et al. 1992). Similar findings were reported by Palmer and Ashby (Palmer and Ashby 1992), whereas Bertolasi et al demonstrated that the activation of median nerve muscle afferents could suppress the excitability of cortical areas controlling the antagonist forearm extensor muscles acting on the hand and such inhibitory effect occurred at short latency assisting spinal pathways mediating reciprocal inhibition (Bertolasi 1998). All together these studies provided first evidence about the presence of this early and striking period of inhibition that occurs likely at cortical levels. In addition, Tokimura and colleagues speculated that such inhibition could be responsible for the initial period of inhibition evident in the cutaneo-muscular reflexes of the hand. Indeed, the minimum interval at which a digital nerve stimulus can suppress EMG response evoked by TMS is about 22 ms. Since it takes a further 20-22 ms for impulses to be conducted from cortex to the TMS targeted muscle, the effect of a digital nerve shock could be seen in muscle as early as 40-42 after is applied.

**Neuroanatomical basis of fast sensori-motor inhibition: a cortical "motor" inhibition or "sensorimotor" inhibition?**

The most direct evidence that peripheral somatosensory input modulates the TMS motor output at a cortical level in humans comes from recordings of corticospinal volleys in patients with implanted electrodes in the cervical epidural space (Tokimura et al. 2000). These showed that later I-waves (I2 and I3 waves) were reduced at an interval appropriate for SAI, whereas the early I wave (I1 wave) remained unchanged at any ISI. Since later I-waves are thought to represent local interneuronal or cortico-cortical inputs to the corticospinal output neuron in M1 (Di Lazzaro and Rothwell 2014), it seems likely that reduced corticospinal output caused by reduced cortico-cortical inputs to corticospinal cells is the cause of MEP suppression. Cortical origin of SAI has been further demonstrated by combined EEG-TMS studies: either peripheral nerve stimulation at wrist (Ferreri et al. 2012) or cutaneous digit stimulation (Bikmullina et al. 2009) are able to modulate TMS-evoked potential (TEPs): such as inhibition of cortical N100 response (Bikmullina et al. 2009; Ferreri et al. 2012), attenuation of P60 response with a motor cortex beta rhythm selective decrease of phase locking (decrease of inter-trial synchronization) (Ferreri et al. 2012). Based on these findings, SAI is thought to reflect primarily cortical processing, however the synaptic mechanisms as well as the exact anatomic circuits responsible for SAI remain obscure.

According to the classical view, sensory signal from peripheral electrical nerve stimulation travels through the dorsal lemniscus to nucleus cuneatus in the medulla oblongata and after crossing, it enters the ventral posterolateral nucleus in the rostral thalamus and project to S1 (Broadmann areas 1, 2, 3a, 3b) and S2 (Broadmann areas 40 and 43) (Strick and Preston 1982, 1983; McIntyre et al. 1984).

There are direct connections between areas 1, 2 of S1 and M1, within the same hemisphere, making it possible for sensory signal to modulate M1 excitability (Ghosh et al. 1987; Donoghue and Sanes 1994; Kaneko et al. 1994a, 1994b). However, in humans invasive recording of somatosensory evoked potentials showed that M1 directly receives somatosensory input from the hand (Slimp et al. 1986; Balzamo et al. 2004) peaking at few milliseconds (P22/P24) later respect to early negative component (N20) usually recorded in S1 (Slimp et al. 1986; Balzamo et al. 2004). Such findings support the idea that two distinct generators with different orientations are present in the sensory-motor cortex, the former (N20) places in the parietal Broadmann area 3 and the latter (P22/P24) in the motor area 4 (Desmedt et al. 1987; Spiegel et al. 1999). Since somatosensory afferents may reach precentral neurons either by cortico-cortical connections with the somatosensory cortex or by direct input from the thalamus (Jones 1983), it is still unknown whether SAI is produced by afferent inputs that reach first the sensory cortex and then, via corticortical connections, the motor cortex, or the inputs reach the motor cortex directly. However, it's interesting to observe that SAI phenomenon requires a minimum Interstimulus interval (ISI) between peripheral stimulation and TMS pulse that is about 1-2 ms longer that the N20 component of somatosensory evoked potentials produced by upper limb nerve stimulation (Tokimura et al. 2000; Di Lazzaro et al. 2005b; Di Lazzaro and Ziemann 2013) and can be obtained over a range of ISIs of N20 + 6-8 ms, that corresponds exactly to the time taken by peripheral stimulation to reach the motor cortex (Deiber et al. 1986; Balzamo et al. 2004), just before and after such ISIs the inhibition is much less consistent. Interestingly, it has been previously investigated the shifting from inhibition to facilitation in SAI curve for ISIs longer than 5 ms (Fischer and Orth 2011; Hamada et al. 2012; Dubbioso et al. 2015). The neuronal mechanisms for this gradual shift have not been well understood, but it is possible that multiple and time-dependent effects of sensory input on motor cortex may play a role. Since the cerebellum is involved in time-specific processing/filtering somatosensory signals from periphery, Hamada and colleagues (Hamada et al. 2012) tried to study the role of cerebellum on fast sensory-motor inhibition by modulating its activity trough either cathodal or anodal transcranial direct current stimulation (tDCS) in healthy subjects. Unfortunately, they failed to find any effect of cerebellum on SAI, conversely a more recent study (Dubbioso et al. 2015) showed that in patients with pure cerebellar atrophy there is a selective impairment of time specific incoming sensory volley. The authors found that in these patients, the cerebellum was not able to modulate fast sensory-motor inhibition: the SAI curve displayed a flat-shape without any shifting from inhibition to facilitation for ISI longer than 25 ms.

At the primary motor cortex level, according to the canonical microcircuit model (Di Lazzaro and Ziemann 2013), the TMS test stimulus (not preceded by peripheral stimulation) activates the axons of pyramidal neurons of layers II and III (P2 and P3) that in turn activate pyramidal neurons of layer V (P5) and the GABA cells projecting upon the layer V pyramidal cells. The activation of this complex circuit composed of excitatory and inhibitory neurons results in a repetitive discharge of corticospinal cells. In SAI protocol, the peripheral nerve stimulation might enhance the excitability of the GABAergic interneurons through the activation of thalamocortical projections, causing the suppression of the latest cortico-spinal volley (late I-waves). Indeed, GABAA activity enhancement selectively suppresses the

late I-waves (Di Lazzaro et al 2000). The role of thalamocortical projections are critical in SAI process, since either unilateral (Oliviero et al. 2005) or bilateral (Nardone et al. 2010b) paramedian infarction of thalamus can induce a marked loss of fast sensory-motor inhibition.

**Neurotransmitters involved in SAI**

Pharmacological and clinical studies demonstrated that SAI is influenced by several neurotransmitters: acetylcholine, dopamine and γ-Aminobutyric acid (GABA).

Acetylcholine influences cortical neurons in a complex manner, and how afferent input leads to cortical inhibition is not known. SAI levels are significantly reduced by scopolamine, a muscarinic cholinergic antagonist, in young healthy adults (Di Lazzaro et al. 2000) and can be improved with rivastigmine, an acetylcholinesterase inhibitors, in patients with abnormal reduction of SAI, such as Alzheimer Disease (AD) (Di Lazzaro et al. 2002). Cholinergic inhibition of pyramidal neurons have been demonstrated directly in experimental studies (Gulledge and Stuart 2005). Interestingly, this rivastigmine effect on SAI predicted the long term response to cholinesterase inhibitor (Di Lazzaro et al. 2005a). The effects of scopolamine and rivastigmine suggest that SAI may be useful to probe in vivo the functional integrity of central cholinergic circuits of the human brain.

Beyond the cholinergic transmission, dopaminergic system plays a relevant role in the modulation of SAI, since it shows strong synaptic interaction with acetylcholine in different brain areas (Di Cara et al. 2007; Millan et al. 2007).

SAI is normalized by L-dopa treatment in patients affected by restless legs syndrome (Rizzo et al. 2010) and in AD patients (Martorana et al. 2009; Nardone et al. 2014). The same effect is evident for the D2-dopamine receptors agonist, rotigotine. Indeed, rotigotine is able to restore central cholinergic transmission (Martorana et al. 2013) and normalize LTP-like cortical plasticity in AD patients (Koch et al. 2014). In addition, Nardone and colleagues demonstrated that in CADASIL ("Cerebral Autosomal-Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy"), a form of "pure" vascular dementia, the pathological reduction of SAI was not restored by the administration of L-dopa, arguing that SAI restoration induced by L-dopa can be able to differentiate AD from patients with pure form of vascular dementia (Nardone et al. 2014). Finally, it has been demonstrated that dopaminergic medication decreased SAI on the more affected side but not on the less affected side, suggesting that reduction of SAI in the PD-on groups occurs predominantly in more advanced Parkinson's disease (Sailer et al. 2003).

Regarding GABAergic system, in human cortical slices it was observed that acetylcholine activated GABA neurons and triggered GABAergic postsynaptic currents (Alkondon et al. 2000). Thus, SAI may also be mediated through the interactions between cholinergic projections and GABAergic interneurons. This also explains the findings that the administration of positive GABA-A receptor modulators has an effect on SAI. Zolpidem, a selective agonist of alpha1 subunit of GABA-A receptor, significantly reduced SAI (Di Lazzaro et al. 2005b, 2005c, 2007a), whereas diazepam, a non-selective agonist, induced a slight increase or no effect on SAI (Di Lazzaro et al. 2005c, 2007a). This observation

is presumably explained by a differential role of the different alpha subunits of GABAA receptor in the modulation of afferent inhibition with a suppression of cholinergic inhibition by alpha1 subunit activation.

**SAI as predictor of the effectiveness of plasticity-inducing protocols**

TMS can be used to investigate the neurophysiological mechanisms underlying synaptic plasticity in the human motor cortex through various repetitive TMS protocols with or without pairing of peripheral nerve stimulation. Such protocols can induce lasting changes in brain excitability that are very similar to those described in vitro studies in terms of long-term potentiation (LTP) and long-term depression (LTD; (Huang et al. 2007)).

Based on pharmacological studies, SAI is considered as a neurophysiological biomarker of cholinergic and GABAergic tone in the central nervous system (Paulus et al. 2008). Cholinergic innervation is critical in modulating cortical plasticity and LTP/like processes (Rasmusson 2000), pharmacological studies have supported an effect of acetylcholine on responses to plasticity-inducing repetitive TMS (rTMS) protocols. Indeed, cholinergic agonists, such as nicotine and the cholinesterase inhibitor rivastigmine, tend to increase and prolong facilitatory intermittent theta burst stimulation (iTBS) and paired associative stimulation (PAS) effects (Kuo et al. 2007; Swayne et al. 2009; Korchounov and Ziemann 2011a; Thirugnanasambandam et al. 2011). In contrast, the administration of a cholinergic antagonist to young adults reduces LTP-like plasticity following PAS (Korchounov and Ziemann 2011b). Similarly, experiments in animals have shown that the susceptibility to LTP/like effects in cerebral cortex is affected by the levels of GABAergic inhibition: blockade of $GABA_A$ receptors with the antagonist bicuculline, enhances LTP in horizontal connections of the motor cortex in rat (Hess et al. 1996). In addition, cell slice studies indicate that inhibition can block or reverse the polarity of plastic effects (Stanton and Senowski 1989; Elahi et al. 2012; Paille et al. 2013; Weise et al. 2013).

Recently, several studies have shown that in humans the efficacy of these plasticity inducing protocols exhibits a huge variability across subjects in terms of magnitude and direction (Hamada et al. 2013; Wiethoff et al. 2014; Murase et al. 2015). Such variability can be influenced by interactions with inhibitory intracortical circuits that are crucial with plasticity induction, functioning as plasticity gate (Thiels et al. 1994). Since SAI is able to evaluate such inhibitory circuits underpinning cortical plasticity, this raises the issue whether it restricts the efficacy of such protocols and whether interindividual variation in the strength of SAI could predict inter-individual variation in the efficacy of these TMS plasticity induction protocols. Interestingly, Cash and colleagues (Cash et al. 2016) have elegantly demonstrated that stronger inhibition at SAI was associated with weaker PAS LTP-like effects, explaining about 40% of the variability in PAS effects. It means that concurrent inhibition evoked by SAI during PAS has an inhibitory effect on plasticity induction and that individual differences in the amount of SAI contribute to the inter-individual variability of PAS effects. In another recent study (Murase et al. 2015) the authors came to the same conclusion but by using a different TMS protocol to evaluate GABAergic inhibitory circuits. They showed that the efficacy of PAS protocol

correlated positively with the level of inhibition (good inhibition) measured by SICI with the threshold tracking method. The contradictory results from the two studies mentioned above (Murase et al. 2015; Cash et al. 2016) is only apparent: SAI is able to induce disinhibition of SICI ($SICI_{SAI}$) since they are mediated through two distinct and reciprocally connected subtypes of GABAergic inhibitory interneurons (Alle et al. 2009; Cash et al. 2016). Regarding iTBS, that exhibits huge variability between individuals (Hamada et al. 2013) as well, a recent study of Young-Bernier and colleague (Young-Bernier et al. 2014) failed to find any correlation with SAI. The authors confirmed the high inter-individual variability in response to iTBS either in young or old people (only 60% of participants showed the expected facilitation of MEP responses), but in both groups SAI levels were not associated with LTP-like plasticity as assessed with iTBS.

**Somatotopy and state-dependency of SAI**

SAI is sensitive to somatotopic organization: electrical stimulation of digits close to the TMS-target muscle (i.e., homotopic stimulation) induces stronger inhibition than stimulation of digits distant to the TMS-target muscle (i.e., heterotopic stimulation) (Classen et al. 2000).

Classen and colleagues (Classen et al. 2000) further demonstrated that homotopic stimulation produced an inhibition of TMS-evoked MEP amplitudes at short ISI (25-30 ms) and a facilitation at long ISI (150-200 ms). Conversely, with heterotopic stimulation they found facilitation at short ISIs and an inhibition for long ISIs.

In addition, somatotopic property of SAI is only evident for stimulation of peripheral cutaneous nerves, but not when the stimulation is applied at mixed nerve at wrist (Fischer and Orth 2011). In this last case, the authors (Fischer and Orth 2011) found an interesting shift of the SAI curve from inhibition to facilitation at ISI longer than 5 ms, this shifting was particularly evident when the peripheral stimulation was applied at median nerve at wrist with the APB as TMS-target muscle, whereas the facilitation was less evident for the FDI muscle. Conversely, the SAI curve was completely flat, without any shifting from inhibition to facilitation, when the stimulation was applied at ulnar nerve at wrist, either for ABP or FDI muscle (Fischer and Orth 2011).

Of interest, such somatotopy is dependent on the intensity of peripheral cutaneous nerve stimulation (Tamburin et al. 2001): the maximal topographic effect was only present at 300% of perceptual threshold of peripheral stimulation applied at fingers, but not at lower and higher intensities. The lack of topographic effect for lower intensities can be due to the fact that the number of cutaneous fibers involved is too low to modulate MEP consistently, whereas for higher intensities it could be due to the spreading of peripheral stimulation to the adjacent fingers. In addition the same authors showed that SAI is influenced by the size of the receptive field (Tamburin et al. 2005), meaning that the inhibitory effect of second finger stimulation on MEP recorded from FDI or APB muscle is reduced by stimulating the first and the third fingers at the same time as the second. Based on these findings Tamburin and colleagues have also showed that in patients with focal hand dystonia and cervical dystonia the

topographic property of SAI was lost, dystonic patients exhibited a normal MEP inhibition after digit stimulation, but such inhibition did not display any somatotopic organization (Tamburin et al. 2002). Interestingly by using an innovative TMS mapping technique based on individual sulcus shape anatomy, we have demonstrated that SAI exhibited a centre-surround organization in the human hand area involving a centre inhibition and a surround facilitation (for more details see section 1).

SAI is state-specific: strong inhibition is observed at rest but not during finger movements. In previous studies, in the active muscle SAI was reduced at the onset phase of movement during both mixed and homotopic cutaneous nerve stimulation (Asmussen et al. 2013; Cho et al. 2016); on the contrary, during the maintenance phase of the movement SAI was reduced (Asmussen et al. 2013) or even normal (Cho et al. 2016).

In the surrounding muscle, SAI showed conflicting results (Voller et al. 2006; Richardson et al. 2008; Asmussen et al. 2014; Cho et al. 2016): it was reduced during the pre-movement and onset phase of the movement (Asmussen et al. 2014; Cho et al. 2016), normal during the maintence phase (Cho et al. 2016) or even increased during the onset phase of movement (Voller et al. 2006). In this last case the authors suggested a hypothetical role of SAI in the surround inhibition mechanism, even if they considered for digit SAI an ISI of 20 ms that is not sufficient to modulate consistently motor cortex.

More recently we have demonstrated that during either homotopic or heterotopic stimulation SAI is abolished in the surrounding muscles during the maintence phase of movement (see section 2).

All together these data suggest that SAI does not appear to contribute to the development of surround inhibition, since it does not provide a good contrast between the active and the surrounding muscle. Specifically, during the maintenance phase the continuing muscle activation is mediated by other feedback loop maybe longer and widespread than the SAI loop that takes only few milliseconds.

**SAI deficit as a biomarker of Mild Cognitive Impairment (MCI) and dementia**

Mild cognitive impairment (MCI) represents an intermediate state of cognitive function between the changes seen in aging an those fulfilling the criteria for dementia (Petersen 2011).

Patients with MCI can present a variety of symptoms: when memory loss is the predominant symptom it is termed "amnestic MCI" and is frequently seen as a prodromal stage of Alzheimer's disease. These patients tend to develop AD at a rate approximately 10% to 15% per year (Grundman et al. 2004). Additionally, when patients have impairments in domains other than memory it is classified as non-amnestic MCI and these individuals are believed to be more likely to convert to other dementias (e.g., fronto-temporal dementia, dementia with Lewy bodies) (Petersen 2011). However, some MCI may simply remain stable over time or even remit. Causation of the syndrome as well as its prevention and treatment remain still unknown.

Several evidence shows that alterations in cholinergic system activity occur in some type of dementia as well as in MCI patients (see Cantone et al for a review). The cortical system ascending from the nucleus basalis of Meynert (nbM) in the substantia innominata of the basal forebrain represents one of

the major cholinergic projection system in the human central nervous system. Nucleus basalis of Meynert degenerates in AD, in dementia with Lewy bodies and in PD patients (Liu et al. 2015).

Evidence shows that alterations in cholinergic system activity may also occur in MCI patients. Markers of acetylcholine esterase activity are downregulated in MCI patients, as revealed by a recent positron emission tomography (PET) study, showing that such decline correlated with cognitive functions like verbal and nonverbal memory, language comprehension and executive function (Haense et al. 2012). Similarly, a pharmacological fMRI study and a MRI diffusion study came to the same conclusion: MCI patients can present alteration either in brain activation pattern following cholinergic challenge (Goekoop et al. 2004) or atrophy of cholinergic nuclei and intracortical projecting fiber tracts (Teipel et al. 2011).

SAI is considered a surrogate measure of cholinergic activity allowing in vivo evaluation of central cholinergic circuits under the effect of ascending projection from nbM (Di Lazzaro et al. 2002). Based on this property, SAI has attracted the attention of clinicians regarding its potential use as biomarker for MCI and the subsequent development of dementia, in order to identify patients in early stage of disease who may benefit from cholinomimetic therapy, to monitor disease progression and treatment effects. SAI has been studied in Alzheimer disease: several authors found decreased SAI in AD (18 studies, 309 patients) (Di Lazzaro et al. 2002, 2004b, 2005a, 2006, 2007b, Nardone et al. 2006, 2008a, 2014; Sakuma et al. 2007; Martorana et al. 2009, 2012, 2013; Celebi et al. 2012; Marra et al. 2012a; Terranova et al. 2013; Di Lorenzo et al. 2013; Koch et al. 2014, 2015), which increases with the administration of cholinesterase inhibitors (e.g. rivastigmine) (Di Lazzaro et al. 2002, 2004b; Koch et al. 2014), L-dopa (Martorana et al. 2009; Nardone et al. 2014) or dopamine agonist (e.g. rotigotine) (Martorana et al. 2013; Koch et al. 2014). Interestingly, one study demonstrated that the increasing of SAI predicted the long-term response to cholinesterase inhibitor (Di Lazzaro et al. 2005a).

Importantly, SAI has been used as a tool to differentiate cholinergic from non-cholinergic forms of dementia. For example SAI is normal in frontotemporal dementia (Di Lazzaro et al. 2006) whereas is suppressed in patients affected from a model of “juvenile Alzheimer dementia”, such as Niemann Pick type C (Manganelli et al. 2014; Benussi A, Cotelli MS, Cosseddu M, Bertasi V, Turla M, Salsano E and Padovani A 2017), in the early phase of Huntington disease (Schippling et al. 2009), in patients with CADASIL ("Cerebral Autosomal-Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy") (Manganelli et al. 2008; Palomar et al. 2013; Nardone et al. 2014). Interestingly, in CADASIL patients, the administration of single oral dose of L-dopa does not induce normalization of SAI, that is usally observed in AD patients (Nardone et al. 2014), suggesting the potential use of L-dopa effect on SAI to differentiate these two forms of cholinergic dementia. However conflicting results have been described regarding other two forms of dementia such as Dementia with Lewy bodyes (LBD) and Vascular dementia. So far two studies have shown abnormal SAI in LBD (Di Lazzaro et al. 2007c; Marra et al. 2012b) and only one normal findings (Nardone et al. 2006). Regarding vascular dementia (VaD) vascular lesions often coexist with AD and other disorders, resulting in the overlapping syndromes. The few TMS studies targetting this disorder have focused on its subcortical form, such as subcortical vascular dementia (SVaD) that is more clinically homogeneous

(Román et al. 2002). An abnormality of SAI was found only in a small subgroup of patients with VaD (25%) which might represent a mixed form of dementia (Di Lazzaro et al. 2008) and in the study of Nardone and coworkers (Nardone et al. 2008b), targetting the subcortical VaD. Moreover, in patients with SVaD abnormal SAI values have been observed in association with microbleeds, frequently seen in this type of dementia (Nardone et al. 2011). Finally SAI performed in people at risk for developping VaD, namely vascular cognitive impairment-no dementia (VCI-ND) revealed integrity of central cholinergic system (Bella et al. 2016).

Based on these findings, SAI has been increasingly used in MCI as a tool to identify patients that have a higher risk to convert from MCI to specific forms of cholinergic dementia and can eventually benefit of a treatment with cholinesterase inhibitor.

Most of the studies performed so far have shown conflicting results regarding MCI patients. Some groups have found normal SAI either in cognitive normal patients or in MCI patients (Sakuma et al. 2007; Picillo et al. 2015; Bella et al. 2016), or predominantly abnormal SAI in MCI patients (Cucurachi et al. 2008; Manganelli et al. 2009a; Nardone et al. 2010a, 2012b, 2013; Tsutsumi et al. 2012; Young-Bernier et al. 2012).

The reason of such heterogeneity might be due to the existence of different subtypes of MCI with or without predominant involvment of memory deficit (amnestic vs non-amnestic MCI). Indeed, according to the recent evidence of the contribution of the cholinergic system to memory performance in Mild cognitive Impairment (MCI) (Peter et al. 2016), some autors have tried to figure-out if SAI was able to discern MCI subtypes with predominant memory impairment (amnestic MCI) from the one without memory deficit (non-amnestic MCI). In a systematic study a group of authors (Nardone et al. 2012a) performed SAI in 4 subtypes of MCI (amnestic and non-amnestic-MCI with and without multiple domain impairment). They fournd SAI was suppressed only in the amnestic MCI group with multiple domain impairment, meaning that cholinergic denervation occurred earlier in such group of patients making them at higher risk of conversion to AD. Interestingly, suppression of SAI was found in patients with Wernicke-Korsakoff syndrome, that usually suffer from alcohol-induced persistining amnestic disorders (Nardone et al. 2010a) and in patients with multiple sclerosis and memory disturbances (Cucurachi et al. 2008). However others groups failed to find a specific correlation of SAI with memory perfomances, but only with the overall cognitive score in patient with Multiple system atropy type C (Celebi et al. 2014), or in PD patients with frontal cognitive dysfunction and hypokinetic gait (Rochester et al. 2012).

Accordingly, SAI has been increasingly used in Parkinson's disease to identify those symptoms possibly underpinned by cholinergic dysfunction. SAI abnormalities has been found associated with dementia and Amnestic Mild Cognitive Impairment (MCI) (Celebi et al. 2012; Yarnall et al. 2013), further confirming the role of cholinergic dysfunction in the development of cognitive dysfunction in PD. Likewise SAI has been found to be reduced in PD patients with visual hallucinations (VH) or REM-sleep Behavior Disorders (RBD), suggesting that cholinergic dysfunction might be the major anatomo-functional basis for these non-motor symptoms and for VH- and RBD-associated cognitive deficits as well (Manganelli et al. 2009a; Nardone et al. 2013).

**SECTION 2**

# THE MAPPING OF SHORT LATENCY AFFERENT INHIBITION (SAI) AND THE ROLE OF CEREBELLUM IN THE MODULATION OF SENSORIMOTOR INTEGRATION AND PLASTICITY OF THE PRIMARY MOTOR CORTEX (M1).

**Evaluation of somatotopy and state dependency of SAI in human motor hand area**

*From the article: "Centre-surround organization of fast sensorimotor integration in human motor hand area" By Raffaele Dubbioso, Estelle Raffin, Anke Karabanov, Axel Thielscher, Hartwig Roman Siebner. (Under review 2017)*


**Abstract**

Dexterous movements rely on fast and efficient integration of sensory input and motor output in the sensorimotor cortex. Sensorimotor integration can be probed with transcranial magnetic stimulation (TMS) testing short-latency afferent inhibition (SAI) of the corticospinal motor output in the primary motor hand area ($M1_{HAND}$). Here we combined the SAI paradigm with a novel shape-based linear mapping approach to investigate the spatial features of fast sensorimotor integrations in human $M1_{HAND}$. We hypothesized that SAI would show a muscle-specific inhibition and facilitation depending on the site of peripheral electrical nerve stimulation and the specific muscle activated by TMS. The left index or little finger was stimulated 23 ms before TMS of the right $M1_{HAND}$. Using frameless stereotaxy, we applied biphasic TMS to one of seven stimulation spots in right M1HAND and recorded motor evoked potentials (MEPs) from left first dorsal interosseous (FDI) and abductor digiti minimi (ADM) muscles. Shape-based mapping revealed a muscle-specific somatotopic representation of SAI in $M1_{HAND}$. Homotopic stimulation applied to the finger close to the muscle targeted by TMS produced SAI. Shape-based cortical mapping showed a somatotopic expression of SAI matching the somatotopic representation of the unconditioned MEPs. Conversely, afferent heterotopic stimulation of a finger distant to the muscle targeted by TMS consistently induced a facilitation of MEPs in $M1_{HAND}$. Like homotopic SAI, heterotopic short-latency afferent facilitation (SAF) was somatotopically expressed in $M1_{HAND}$. Together, the results provide first-time evidence for a centre-surround organisation of fast sensorimotor integration in human $M1_{HAND}$.

## Introduction

The inhibition induced by the sensory peripheral stimulation on the MEP is strongest when the site of sensory stimulation is applied close to the specific muscle activated by TMS (homotopic stimulation), but it is unclear how such inhibition is expressed in TMS target muscle distant from the sensory stimulation (heterotopic stimulation) (Classen et al. 2000; Tamburin et al. 2005).

Using a novel sulcus-based M1 mapping approach (Raffin et al. 2015), we examined the possibility that SAI exhibits a specific cortical somatotopic representation within $M1_{HAND}$. We hypothesized that $M1_{HAND}$ integrates sensory and motor signals through topographically specific interactions and displays a centre-surround organization. Therefore, we expected to find the well-known inhibition (SAI) of the motor output during homotopic stimulation and a "surrounding" facilitatory effect on the MEP amplitude for heterotopic (short-latency afferent facilitation, SAF). We further anticipated that such centre-surround organization of the sensorimotor integration would depend on the functional sensorimotor state. We therefore predicted that pre-activation through voluntary muscle contraction would selectively abolish the homotopic centre inhibition and heterotopic surround facilitation observed at rest.

## Materials and methods

### Participants

Fourteen healthy volunteers (mean age: 27.8 ± 1.7 SE, 5 women) participated in the first experiment. Ten subjects also participated in the second experiment (mean age: 28.7 ± 2.0 SE, 4 women). All participants were right handed as assessed by the Edinburgh handedness inventory (Oldfield 1971) and had no history of neurological or psychiatric disorders. All subjects were screened for contraindications to TMS (Rossi et al. 2009). They all gave written informed consent to the experimental procedures. The study complied with the Helsinki declaration on human experimentation. The study was approved by the Ethics Committee of the Capital Region of Denmark (H-15000551).

### Shape-based neuronavigated TMS of the primary motor cortex

On the same day of the TMS experiment, participants underwent structural high-resolution magnetic resonance imaging (MRI) of the whole brain at 3-Tesla (TIM Verio scanner, Siemens, Erlangen, Germany). Structural MRI employed a three-dimensional, T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence consisting of 192 sagittal slices with 1 $mm^3$ isotropic voxel resolution (TR/TE = 2300/2.98 ms, TI = 1100 ms; 256 × 256 matrix, flip angle 9°).

For shape-based TMS mapping, participants were seated comfortably in a chair and the TMS coil position was continuously controlled by a frameless neuronavigation system (Localite, Sankt Augustin, Germany). The brain surface was automatically reconstructed from the T1-weighted images using neuronavigation software (Localite, Sankt Augustin, Germany). The root mean square of difference between the co-registered anatomical landmarks estimated by the neuronavigation software was set below 2 mm for each subject to maintain positioning accuracy all along the experiment.

TMS target locations in the precentral gyrus were marked prior to the experiment on the segmented brain of each subject. The right $M1_{HAND}$ was identified by a trained investigator (RD) using the characteristic knob-like shape of the sulcus ("hand knob") as anatomic landmark (Yousry et al. 1997). The investigator placed seven targets in the posterior part of the crown of the precentral gyrus within the $M1_{HAND}$. The seven M1-targets matched the curvature of the hand knob, forming a line of equidistant targets every 10 mm. Target 4 corresponded to the centre of the "hand knob" (Fig. 1). For each target, coil orientation was adjusted to produce a current direction perpendicular to the central sulcus. The individual coil positioning parameters were stored in the neuronavigation software. Table 1 reports the MNI normalized mean coordinates.

**Surface electromyography (EMG)**

We recorded the electrical muscle activity of the left first dorsal interosseus (FDI) and abductor digiti minimi (ADM) muscle with surface electrodes (Ambu Neuroline 700, Ballerup, Denmark) arranged in a bipolar belly-tendon montage. The signals from the EMG electrodes were amplified, bandpass filtered (5 – 3000 Hz), digitized at a frequency of 5 kHz, and stored in a laboratory computer for later offline analysis, using Signal software and CED 1401 hardware (Cambridge Electronic Design, Cambridge, UK).

**Peripheral electrical digit stimulation**

Peripheral electrical stimuli were given to the fingers through bipolar ring electrodes strapped around the left $2^{nd}$ and $5^{th}$ fingers. We applied square pulses of 200 µs duration with the cathode positioned at the proximal and the anode positioned at the distal interphalangeal joint (Digitimer stimulator, Model DS7A, Hertfordshire, England). The peripheral stimulation was applied 23 ms prior to the TMS pulse to elicit SAI (Tokimura et al., 2000). In each participant, perceptual threshold (PT) was determined for the $2^{nd}$ and $5^{th}$ fingers by delivering a series of stimuli at increasing intensity from 2 mA in steps of 1 mA. The PT was defined as the minimal intensity of stimulation perceived by the participant in 10 of 10 consecutive stimuli (Manganelli et al. 2013). Stimulation intensity was set to 100%, 200% or 300% of individual PT (PT100%, PT200%, PT300%) of each finger for electrical digit stimulation in the first experiment (rest condition) and to PT300% in the second experiment (contraction condition). We didn't used higher stimulus intensities to preserve a topographically confined conditioning effect in $M1_{HAND}$ (Tamburin et al. 2001). None of the subjects perceived the peripheral nerve stimulation as painful.

**Transcranial magnetic stimulation**

Single-pulse TMS was performed using a MagPro X100 stimulator (Magventure, Skovlunde, Denmark) connected to a cooled-MC-B35 figure-of-eight coil with windings of 35 mm diameter. In order to keep TMS as spatially confined as possible, we used a biphasic pulse configuration generating an antero-posterior followed by postero-anterior (AP–PA) current in the brain, because this pulse configuration allowed effective suprathreshold stimulation of $M1_{HAND}$ at the lowest possible stimulation intensity (Lang et al. 2006). We first located the target position among our seven predefined locations (Fig. 1A)

where TMS elicited the maximal MEP in the left ADM (i.e. the ADM hotspot). We then placed the coil on the ADM hotspot with a 90-degree angle relative to the individual central sulcus shape (Fig. 1A) and determined the intensity of TMS stimulator in order to get an average MEP amplitude in the ADM muscle around 0.2-0.5 mV by using the Maximum-Likelihood Strategy Parameter Estimation by Sequential Testing (MLS-PEST) approach (Awiszus 2003). This intensity was then used for single-pulse TMS during all measurements in Experiments 1 and 2. It had been previously shown that a test MEP amplitude varying between 0.2 and 1 mV did not influence the relative magnitude of SAI (Udupa et al. 2009, 2014).

**Mapping procedures**

Experiments 1 (rest condition) and 2 (contraction condition) were carried out on separate days at least one week apart.

For each of the seven targets, we applied 20 pulses delivered at inter-stimulus intervals jittered between 4 and 5 s. In each stimulation block, the order of conditioned (10 pulses preceded by electrical stimulation) and unconditioned MEPs (Test stimulus alone, 10 pulses) was pseudo-randomized. Within each session, the order of stimulated targets was pseudo-randomized and counterbalanced across subjects.

***Experiment 1 (Rest condition)***

The experiment was designed to evaluate the effect of different conditioning peripheral stimulation intensities (100%, 200% and 300% of the individual PT) on the somatotopic representation of SAI during heterotopic and homotopic stimulation. For homotopic stimulation, we applied the conditioning peripheral stimulus to the digit close to the muscle targeted by TMS. Conversely, for heterotopic stimulation we delivered the conditioning stimulus to the digit distant from the muscle targeted by TMS. For example, electrical digit stimulation of the index finger was homotopic with respect to the FDI muscle and heterotopic with respect to the ADM muscle (Fig. 1B). Likewise, electrical digit stimulation of the little finger was homotopic with respect to the ADM muscle and heterotopic with respect to the FDI muscle (Fig. 1B).

***Experiment 2 (Contraction condition)***

Experiment 2 employed shape-informed linear SAI mapping during tonic muscle contraction to test how motor activity of one intrinsic hand muscle impacts on the SAI profile of the "surrounding" hand muscle staying relaxed. We performed SAI mapping during two active conditions: (1) isometric contraction of the FDI muscle with the "surrounding" ADM muscle being relaxed, (2) isometric contraction of the ADM muscle with the "surrounding" FDI muscle being relaxed (Fig. 1B).

Participants maximally abducted their fingers against a force-sensor device and then we computed the 10% of their maximum voluntary contraction (MVC). This measure was completed for the $2^{nd}$ and $5^{th}$ finger separately. We trained participants to keep an isolated isometric contraction at around 10% of MVC, using a visual feedback displayed on an oscilloscope, while keeping the surrounding muscle

completely relaxed. Participants continuously received feedback of the EMG activity of the two muscles. Trials in which background EMG activity in the "surrounding" muscle exceeded 0.1mV were excluded from analyses.

To avoid fatigue, each sequence of isometric contractions lasted 45 s followed by a 30-second break. We allowed additional resting periods, if needed by the subject. The two pre-activation conditions were counter-balanced across subjects.

**Data analyses**

Single MEP trials were visually inspected and trials with visible voluntary motor activity were removed in recordings performed at rest. For each trial, peak-to-peak MEP amplitude of each MEP was determined in the time window between 10 and 30 ms after the TMS stimulus (Signal software, version 6.04 for Windows, Cambridge Electronic Design, Cambridge, UK). We generated muscle excitability profiles for the test stimulus alone (unconditioned MEP) and the MEP preceded by peripheral stimulation along the seven targets (conditioned MEP) under the various conditions.

Using the conditioned or unconditioned mean MEP amplitude for each subject as the dependent variable, we computed a repeated measure ANOVA to model the distribution of the conditioned and unconditioned MEPs amplitudes recorded from the ADM and FDI muscles across targets and to test for differences between conditions at the group level using a repeated measure ANOVA. We computed separate ANOVAs for each experiment. In experiment 1, the ANOVA included the within-subject factors cortical *target* (target site 1 - 7), *muscle* (ADM vs FDI muscle), *intensity* of peripheral electrical stimulation (100%, 200%, or 300% of individual PT), and *site* of peripheral stimulation (index versus little finger).

In experiment 2, the ANOVA included the within-subject factors cortical *target* (target site 1 - 7), *muscle* (ADM vs FDI muscle), *site* of peripheral stimulation (index versus little finger), and *state* (tonic contraction versus relaxation).

We computed two additional indicators of cortical excitability. First, we calculated the *area under the curve* (AUC) to assess the effect of different peripheral stimulation intensities during heterotopic and homotopic stimulation. The AUC was calculated according to the following formula:

$$\boldsymbol{AUC = \int_{1}^{7} f(x)dx}$$

The term f(x) is the function that the curve represents. The limits of the curve are given by target 1 and target 7. This indicator reflects the up or down regulation of the global corticospinal excitability recorded from a single muscle (Raffin et al. 2015). A ratio between the AUC of the conditioned muscle profiles ($AUC_c$) and the AUC of the unconditioned muscle profiles ($AUC_u$) higher than 1 would indicate a facilitatory effect of the peripheral stimulation on the MEP amplitudes whereas a value lower than 1 an inhibitory effect.

Second, we computed the amplitude-weighted mean position (denoted here as "weighted mean position", WMP) of each muscle profile to return the one-dimensional muscle location along $M1_{HAND}$ (Raffin et al. 2015). The weighted mean (WMP) was calculated according to the following formula:

$$WMP = \frac{\sum_{k=1}^{7} \textbf{Target(k)} * \textbf{Mean MEP Amplitude Target(k)}}{\sum_{k=1}^{7} \boldsymbol{Mean\ MEP\ Amplitude\ Target}\textbf{(k)}}$$

Target(k) refers to each target's number (from 1 to 7) and Mean MEP Amplitude Target (k) refers to the mean peak-to-peak Motor-Evoked-Potential amplitudes at each target (from target 1 to target 7). Distinct weighted mean positions associated with the two muscle profiles suggest distinct corticomotor representations for the two muscles. Along the same line of reasoning, differences in WMP across conditions for a single muscle indicate context-dependent shifts of the muscle excitability profile. The ratio $AUC_c/AUC_u$ and the Weighted Mean Positions of each muscle were entered into two separate ANOVAs with different within-subject factors according to the aim of the analysis (see Results part).
Finally, we performed a correlation analysis between the peak value of the $AUC_C$/ $AUC_U$ ratio of the homotopic (inhibitory) and heterotopic (facilitatory) stimulation centred on targets 3, 4 and 5 of the two muscles using Pearson correlation coefficient. We chose these three targets, since these targets were located exactly in the medial, central, and lateral part of the "hand knob", respectively.
All statistical analyses used IBM SPSS Statistics software (Version 22 for Windows, New York City, USA). Normal distribution of all variables was verified by means of Kolmogorov and Smirnov test. Alpha inflation due to multiple comparisons was controlled using Bonferroni correction when appropriate. We used the Mauchly's Test to test for sphericity and the Greenhouse-Geisser correction method to correct for non-sphericity. Group data are presented as mean ± standard error of the mean (SEM).

## Results

### Experiment 1

Experiment 1 addressed the somatotopy of short-latency sensorimotor integration in $M1_{HAND}$ during heterotopic and homotopic stimulation.

#### *Somatotopic representation of the unconditioned MEPs*

We first performed a repeated measure ANOVA which used the unconditioned MEP amplitude as independent variable to confirm our previous results regarding the somatotopic representation of the FDI and ADM muscle (Raffin et al. 2015) and to assess the impact of the intensity and site of peripheral stimulation on these representations. We treated cortical *target*, *muscle*, *intensity*, and *site* of peripheral stimulation as within-subject factors. In agreement with our previous work (Raffin et al. 2015), we found a somatotopic gradient of muscle arrangement along the hand motor area, with the FDI muscle being represented more laterally than the ADM muscle (Fig. 2A'). Accordingly, the ANOVA yielded a main effect of *muscle* ($F_{(1,13)} = 23.256$, $p < 0.001$), *target* ($F_{(2.62,34.1)} = 15.643$, $p < 0.001$; Mauchly's Test of Sphericity: $\chi^2_{(20)} = 87.1$, $p = <0.001$; Greenhouse-Geisser correction: $\varepsilon = 0.437$) and a significant interaction between *muscle* and *target* ($F_{(2.1,27.3)} = 9.094$, $p = 0.001$; Mauchly's Test of Sphericity: $\chi^2_{(20)} = 123.05$, $p = <0.001$; Greenhouse-Geisser correction: $\varepsilon = 0.350$). The spatial dissociation of the two

muscle profiles was further corroborated by a significant main effect of *muscle* in the ANOVA comparing the weighted mean positions of each of the two curves *muscle* ($F_{(1,13)} = 42.873$, $p < 0.001$). Importantly, we found no significant impact of the *intensity* and *site* of peripheral stimulation on the somatotopy of the unconditioned muscle representations (i.e., in the absence of peripheral stimulation). Using the mean MEP amplitude as dependent variable, ANOVA showed neither an interaction among *target, intensity* of peripheral stimulation, and *muscle* nor *target, site* of peripheral stimulation, and *muscle* ($p > 0.6$). There was also no interaction between *intensity* of peripheral stimulation and *muscle* or *site* of peripheral stimulation and *muscle* in the ANOVA testing condition-specific effects on weighted mean positions ($p > 0.5$).

***Somatotopic representations of short-latency afferent inhibition***

The main ANOVA focused on the conditioned MEPs to test for a somatotopic representation of SAI treating *target, muscle, site* and *intensity* of peripheral stimulation as within-subject factors. We found a distinct medio-lateral distribution of the conditioned MEPs amplitudes for the FDI and ADM muscles as demonstrated by a significant interaction between *target* and *muscle* ($F_{(6,78)} = 8.843$, $p < 0.001$). This muscle-specific somatotopic representation was influenced by the site and intensity of peripheral electrical stimulation as reflected by a significant interaction among *target, site* and *intensity* of peripheral stimulation, and *muscle* ($F_{(4.48,58.26)} = 3.589$, $p = 0.009$; Mauchly's Test of Sphericity: $\chi^2_{(20)} = 87.1$, $p = <0.001$; Greenhouse-Geisser correction: $\varepsilon = 0.437$).

To quantify the effect of different PT intensities during heterotopic and homotopic stimulation on the SAI profiles, we computed the AUC for each of the conditioned MEP profiles (see data analysis). We then computed the ratio between the AUC of the conditioned MEP amplitudes ($AUC_c$) and the AUC of the unconditioned MEP amplitudes ($AUC_u$). We then computed a repeated measure ANOVA with $AUC_c/AUC_u$ as dependent variable and *muscle*, *site* and *intensity* of peripheral stimulation as within-subject factors. The analysis revealed an intensity-dependent effect of the type of stimulation (i.e. homotopic or heterotopic stimulation) on the SAI profiles recorded from the two muscles as reflected by an interaction between *site* and *intensity* of peripheral stimulation and *muscle* ($F_{(2,26)} = 17.812$, $p < 0.001$).

For homotopic stimulation, a higher intensity of peripheral stimulation caused greater inhibition of the conditioned MEP amplitude. Bonferroni-corrected paired comparisons showed that homotopic stimulation induced a reduction of the SAI ratio at 300% of PT for both muscles compared to the two other intensities, indicating a dose-dependent inhibitory effect (Fig. 2B). There was a consistent SAI at an intensity of 300%, but not at 100% PT. The inhibitory conditioning effect at 300% PT was significantly greater than SAI at 100% PT for the FDI muscle (PT300%: mean= 0.72 ± 0.05; PT100%: mean=1.04 ± 0.06; p= 0.001), and ADM muscle (PT300%: 0.86 ± 0.04; PT200%: 1.07 ± 0.05; p= 0.041).

We also found an intensity dependent effect for heterotopic stimulation. In contrast to homotopic stimulation, heterotopic stimulation had a facilitatory effect on $AUC_c/AUC_u$ ratio (Fig. 2C). In analogy to SAI, we refer to this conditioning effect as short-latency afferent facilitation (SAF). Like SAI, SAF

depended on the intensity of peripheral stimulation (Fig. 2C). For both muscles, post-hoc testing revealed a significant increment of the $AUC_c/AUC_u$ ratio at an intensity of 300% PT compared to 200% PT (FDI: PT300%: 1.15 ± 0.06; PT200%: 0.89 ±0.06; p= 0.026; ADM: PT300%: 1.09 ± 0.05; PT200%: 0.94 ± 0.04; p= 0.019).

***Somatotopic arrangement of SAI and SAF at rest***

We measured the spatial dissociation of the two SAI profiles using the weighted mean positions to examine the muscle-specificity of sensorimotor integration. Since the largest modulatory effect on the conditioned MEP amplitude was present at a stimulus intensity of 300% PT, we only considered the homotopic SAI and heterotopic SAF profiles evoked with an electrical digital stimulus that matched 300% of individual PT.

We computed a repeated measure ANOVA on the weighted mean position with *site* of peripheral stimulation and *muscle* as within-subject factors. We found a mediolateral dissociation of the sensorimotor integration associated with the FDI and ADM muscle as evidenced by a significant main effect of "Muscle" ($F_{(1,13)}$ = 25.209, p < 0.001) with the FDI muscle being located more laterally and the ADM muscle more medially along the central sulcus (Fig. 3A-C). Importantly we did not find any main effect of *site* of peripheral stimulation ($F_{(1,13)}$ = 0.521, p = 0.483) or a *site* of peripheral stimulation by *muscle* interaction ($F_{(1,13)}$ = 0.366, p = 0.555), suggesting that the somatotopic arrangement were similar for the homotopic or heterotopic stimulation (Fig. 3C-D).

**Experiment 2**

Experiment 2 assessed how tonic muscle contraction of one of the two target muscles impacts on the sensorimotor interactions in the "surrounding" relaxed muscle revealed by heterotopic and homotopic stimulation.

***Cortical motor representations without preceding afferent stimulation***

We first tested whether the site of peripheral finger stimulation (stimulation of index versus little finger) or the motor context (rest versus tonic contraction of the non-target muscle) influenced the somatotopy of corticomotor representations, as revealed by the cortical test pulse alone without afferent conditioning stimulation. To this end, we computed a repeated measure ANOVA with the unconditioned MEP amplitude as dependent variable and *target, muscle, site* of peripheral stimulation and *state* as within-subject factors. Like in experiment 1, there was a main effect of *muscle* ($F_{(1,8)}$ = 21.343, p = 0.002), *target* ($F_{(6,48)}$ = 14.747, p = 0.001) as well as an interaction between *muscle* and *target* ($F_{(1.8,14.41)}$ = 7.779, p = 0.006; Mauchly's Test of Sphericity: $\chi^2_{(20)}$ = 74.301, p = <0.001; Greenhouse-Geisser correction: ε = 0.300). The ANOVA revealed no higher-order interactions between *muscle* and *target*, involving additional factors (i.e., *site* of peripheral stimulation or *state*).

***Effect of tonic muscle contraction on heterotopic SAF and homotopic SAI***

We computed a repeated measure ANOVA to examine whether facilitation (SAF) or inhibition (SAI) that is present at rest is also present during tonic contraction of a different hand muscle. For the three cortical sites corresponding to the hand knob (i.e., target positions 3, 4, and 5), we pooled the $AUC_c/AUC_u$ ratios of the relaxed surrounding muscle together and used this value as dependent variable. The factors *muscle, site* of peripheral stimulation and *state* were within-subject factors. We found that selective tonic contraction of an intrinsic hand muscle abolished both, the facilitatory effect of heterotopic stimulation and the inhibitory effect of homotopic stimulation in the surrounding relaxed muscles (Fig. 4A). This effect resulted in a significant interaction between *muscle* and *site* of stimulation ($F_{(1,9)} = 62.038$, $p < 0.001$) and among *muscle, site* of peripheral stimulation and *state* ($F_{(1,9)} = 72.471$, $p < 0.001$). The Bonferroni-corrected paired comparisons for each muscle confirmed that tonic contraction induced a significant decrease in SAF evoked by heterotopic stimulation (FDI muscle: paired t-test: $t_{(9)} = -4.963$, $p < 0.001$; ADM muscle: paired t-test: $t_{(9)} = -2.780$, $p = 0.021$). The same was the case for SAI evoked by homotopic stimulation (FDI muscle: paired t-test: $t_{(9)} = 6.291$, $p < 0.001$; ADM muscle : paired t-test: $t_{(9)} = 3.612$, $p = 0.006$).

We tested whether contraction-induced reduction of SAF in the relaxed heterotopic muscle and SAI in the relaxed homotopic muscle had a specific spatial distribution. The conditioned MEP amplitudes recorded from the relaxed muscle were entered in a repeated measure ANOVA with *target, muscle,* and *site* of peripheral stimulation as within-subject factors. We found a main effect of *target* ($F_{(6,48)} = 20.161$, $p < 0.001$), *muscle* ($F_{(1,8)} = 6.908$, $p = 0.03$) and a interaction between *target* and *muscle* ($F_{(6,48)} = 3.614$, $p = 0.046$). These results show that the contraction induced decrease in SAF and SAI showed a spatial pattern that is specific to the cortical muscle representation (Fig.4C). The spatial specificity of reduced SAF and SAI was also evident for the weighted mean positions of the linear cortical representations of the FDI and ADM muscles (Fig. 4D). There was a spatial dissociation between the two muscles for both, heterotopic stimulation (paired t-test: $t_{(9)} = -3.372$,$p = 0.008$) and homotopic stimulation (paired t-test: $t_{(9)} = 2.298$, $p = 0.047$ ).

**Relationship between short-latency facilitation and inhibition**

We explored whether the magnitude of SAF, induced by heterotopic stimulation, scaled with the magnitude of SAI, induced by homotopic stimulation, within and between the two muscles. We identified the peak change in $AUC_c/AUC_u$ ratio evoked at an intensity of 300% PT in the handknob targets (i.e., target positions 3, 4 and 5) for heterotopic (facilitatory) and homotopic (inhibitory) stimulation.

The highest $AUC_c/AUC_u$ ratio evoked by heterotopic stimulation reflected maximal SAF, whereas the lowest $AUC_c/AUC_u$ ratio evoked by homotopic stimulation indicated maximal SAI. When the little finger was stimulated, the magnitude of homotopic SAI scaled positively with heterotopic SAF (Fig. 5). The stronger homotopic SAI in the ADM muscle, the stronger was heterotopic SAF in the FDI muscle, resulting in a significant negative correlation between maximal $AUC_c/AUC_u$ ratios elicited by heterotopic stimulation in the FDI muscle and by homotopic stimulation in the ADM muscle ($r = -0.791$, $p_{corrected} = 0.005$). This was not the case for stimulation of the index finger, where the magnitude of

homotopic SAI in the FDI muscle showed no linear relationship with the magnitude of heterotopic SAF in the ADM muscle ($r = -0.104$, $p_{uncorrected} = 0.724$). The absence of a similar relationship might be explained by the fact that SAF measured in the ADM muscle was less consistent across subjects, being present in 9 of 14 subjects, whereas SAF was consistently expressed in the FDI muscle in all 14 participants.

We also found that the maximal amount of SAI evoked in the intrinsic hand muscle correlated with each other (Fig.5). There was a strong positive correlation between maximal SAI evoked by homotopic stimulation in the FDI muscle and homotopic stimulation in the ADM muscle ($r = 0.803$, $p_{corrected} = 0.003$). No other significant correlations were found between the individual magnitudes of maximal SAI and SAF. Especially maximal SAF in one hand muscle did not predict maximal SAF in the other hand muscle. Further, the individual magnitude of SAF and SAI in the same hand muscle did not show a significant linear relation.

**Discussion**

We demonstrated that our sulcus-based sensorimotor-mapping approach can capture the somatotopy of sensorimotor integration in vivo. This specific spatial organization was evident for both homotopic and heterotopic stimulation. In addition, we provide first evidence about the existence of a centre-surround organization of the human sensorimotor system. Such centre-surround organization is dominated by centre-specific inhibitory and surround facilitatory mechanisms and such effects are state dependent (i.e abolished during tonic muscle contraction).

**Somatotopic organization of the sensorimotor integration in the hand motor area**

Our results are in accordance with previous data documenting the "somatotopy-like" organization of the MEP inhibition to cutaneous afferences (Classen et al. 2000; Tamburin et al. 2001; Tamburin et al. 2005). Classen and colleagues demonstrated that such somatotopy-like organization was modulated differently in homotopic versus heterotopic stimulation and it was maximal when the conditioning stimulation was applied around 25-30 ms and 150-200 ms prior to TMS (Classen et al. 2000). Moreover, such somatotopy was dependent on the intensity of peripheral stimulation (Tamburin et al. 2001) and was influenced by the size of the receptive field (Tamburin et al. 2005)

However, such previous results only provided indirect and rough estimates of the topological organization of the sensorimotor integration. By using a sulcus-based neuronavigated-SAI mapping technique, we demonstrate in vivo the cortical topographic distribution of the sensorimotor integration within $M1_{HAND}$ for either homotopic or heterotopic cutaneous afferents in humans. Homotopic stimulation is characterized by inhibitory effect on the conditioned MEP, whereas heterotopic stimulation is characterized by facilitatory effects.

A diffusion tractography study in humans (Catani et al. 2012) has recently demonstrated that the motor and somatosensory homunculi are directly connected through short U-shaped fibres running beneath the central sulcus. The pattern of distribution of these fibres follows the topographical organization of M1 and S1. As a consequence, a larger amount of connections exists between homotopic body parts.

This typical organization in humans is consistent with previous reports in animal models (Fabri and Burton 1991; Izraeli and Porter 1995) where the projections from S1 are topographically organized and terminate mainly in homologous M1 body part representations. These same studies also indicate that focal sites in M1 cortex receive projections from S1 areas that represent neighboring body parts. Such findings have functional significance, because they suggest that projections from S1 to M1 might be organized in order to allow the coordination of multiple motor representations. Likewise, in our study, we found that both homotopic stimulation, which can be considered as the direct connection of homotopic sensorimotor regions (e.g. II finger stimulated-II finger muscle), and heterotopic stimulation, S1 stimulation from neighboring finger is connected to the same motor focal site of the homotopic stimulation (e.g. V finger stimulated-II finger muscle), exhibit topographic distribution. This theory is true if the inhibition induced by peripheral electrical stimulation passes through the somatosensory cortex and then reaches the motor cortex. Unfortunately, the precise mechanisms underlying SAI are not fully understood.

Of interest, topographic distribution for homotopic and heterotopic stimulation was evident only for the highest intensity of PT (300% PT), while the topographic distribution was much less evident with lower intensities. Our findings are consistent with Tamburin et al. (Tamburin et al. 2001) who demonstrated that the maximal topographic effect was only present at 300% of PT, but not at lower and higher intensities. The lack of topographic effect for lower intensities can be due to the fact that the number of cutaneous fibers involved is too low to modulate MEP consistently.

Although the projetions from SI are topographically organized and terminate mainly in homologous MI body part representations (Fabri and Burton 1991; Burton and Fabri 1995; Izraeli and Porter 1995), these same studies also indicate that focal sites in MI cortex receive projections from SI areas that represent neighboring body parts. Such findings have functional significance, because they suggest that projections from SI to MI might be organized in ways that coordinate multiple motor representation

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**Centre-surround organization of the sensorimotor cortex and the effect of tonic muscle contraction**

Our results support the idea that a centre-surround organization can also be demonstrated between the somatosensory and motor cortices. The communication between these two cortical areas seems to rely on centre specific homotopic inhibition and surrounding heterotopic facilitation.

An influential study on sensorimotor integration in the human hand area (Classen et al. 2000) came to the same conclusion: inhibition measured in the homotopically stimulated muscle at short ISIs (around 25 ms) was less pronounced or replaced by facilitation in the heterotopically stimulated muscle.

Conversely, at longer ISIs (around 200 ms) the authors found the opposite results. They reported inhibition for heterotopically stimulated muscle and facilitation for the homotopic muscle. Together those findings and ours point out to the fact that intracortical interneurons might be involved in the input-specific inhibition and surround facilitation at shorter ISIs.

According to the canonical microcircuit model, SAI might be produced by excitatory thalamic inputs to GABAergic cells projecting upon corticospinal cells in M1 (Di Lazzaro et al. 2012). Thus, as counterpart of homotopic SAI, the surround facilitation we observed for heterotopic SAF profiles can be explained by a GABAergic mediated disinhibition in the hand motor area.

Of interest, surround facilitation has been already demonstrated in the visual cortex when the centre of the receptive filed is shown at low contrast respect to the periphery or for crossed oriented centre and surround stimuli. The possible mechanisms underlying visual surround facilitation are the selective activation of excitatory neurons so surround inputs are amplified, or disinhibition of local interneurons, via the activation of another pool of inhibitory neurons selective to the same orientation as the surround stimulus (Seriès et al. 2003).

From a biological point of view, this centre-specific inhibition and surround facilitation may be an important mechanism to amplify the contrast between the centre and the periphery of the sensorimotor receptive field. Importantly, this equilibrium can be disrupted in neurological diseases, such as focal hand dystonia where the precise spatial processing of sensory and motor stimuli is completely distorted (Tamburin et al. 2002). Interestingly, the application of our mapping technique could be considered as a neurophysiological biomarker to monitor the effect of treatment with botulinum toxin injection on the stability of cortical representation maps and eventually predict the clinical worsening. So it might allow individually tuned therapies by identifying the exact individual time interval between two re-injections, avoiding the reappearance of the clinical symptoms.

We have also demonstrated that the facilitatory (heterotopic stimulation) and inhibitory (homotopic stimulation) mechanisms observed at rest are abolished in the surrounding muscles during tonic muscle contraction. Importantly, previous studies have shown conflicting findings. While some have demonstrated reduced homotopic SAI in the active muscle during tonic muscle contraction (Asmussen et al. 2013), others have shown that homotopic SAI is normal in both active and surrounding muscles (Cho et al. 2016). Our data supports the idea that SAI is abolished in the surrounding muscles during tonic contraction either for heterotopic or homotopic stimulation. In addition, since it has been already demonstrated an abolishment of SAI in the active muscle as well (Asmussen et al. 2013, 2014), we can argue that SAI during tonic muscle contraction does not provide a good contrast between the active and the surrounding muscle, the reason can be due to the fact that the feedback loop involved during the movement maintenance phase is maybe longer and widespread than the SAI loop that takes only few milliseconds.

**Conclusion and Outlook**

The human sensorimotor system exhibits a specific spatial organization for the integration of sensory input and motor stimuli. This precise organization is characterized by the equilibrium of centre-specific

inhibitory input and surround facilitation. The alteration of the spatial organization and the equilibrium between these two opposite mechanisms (inhibition and facilitation) can be considered to play a pivotal role in neurological diseases characterized by aberrant sensorimotor integration, such as Parkinson's disease or focal hand dystonia. In more general terms, this protocol has the potential to provide a model to study the dynamic of neural representations and the adaptive synergies between the motor and sensory cortices underlying fine motor control

**Figure legend**

**Figure 1. Experimental design.**

**Panel A.** Schematic illustration of linear sulcus-based mapping of short-latency afferent sensorimotor interactions. Using frameless stereotaxy, we applied single-pulse TMS to one of seven cortical target sites (yellow numbers) in right $M1_{HAND}$ and recorded motor evoked potentials (MEPs) from left first dorsal interosseous (FDI) and abductor digiti minimi (ADM) muscles. The coil orientation was always perpendicular to the (yellow numbers) individual shape of the right central sulcus. Cortical targets 3, 4 and 5 are located in the centre of the hand knob, the macro-anatomical landmark of the $M1_{HAND}$. The blue medial area illustrates the core cortical representation of the ADM muscle, while the purple lateral area corresponds to the core cortical presentation of the FDI muscle. Note that these muscle representations are overlapping.

**Panel B.** Peripheral nerve stimulation was given 23 ms before a suprathreshold TMS pulse applied to $M1_{HAND}$. The conditioning effects of peripheral stimulation on the motor evoked potentials (MEPs) elicited by the suprathreshold TMS pulse were assessed at each cortical stimulation site to probe short-latency sensorimotor integration in $M1_{HAND}$. Two different types of peripheral stimulation were applied. Homotopic stimulation (labelled as red flash symbol) and heterotopic stimulation (labelled as green flash symbol). Experiment 1 was performed during rest condition. Experiment 2 was performed during tonic muscle contraction (black arrows) of the second or the fifth finger to evaluate the sensorimotor modulatory mechanisms in the surrounding muscles drawn as filled ovals (FDI: pink and ADM: blue).

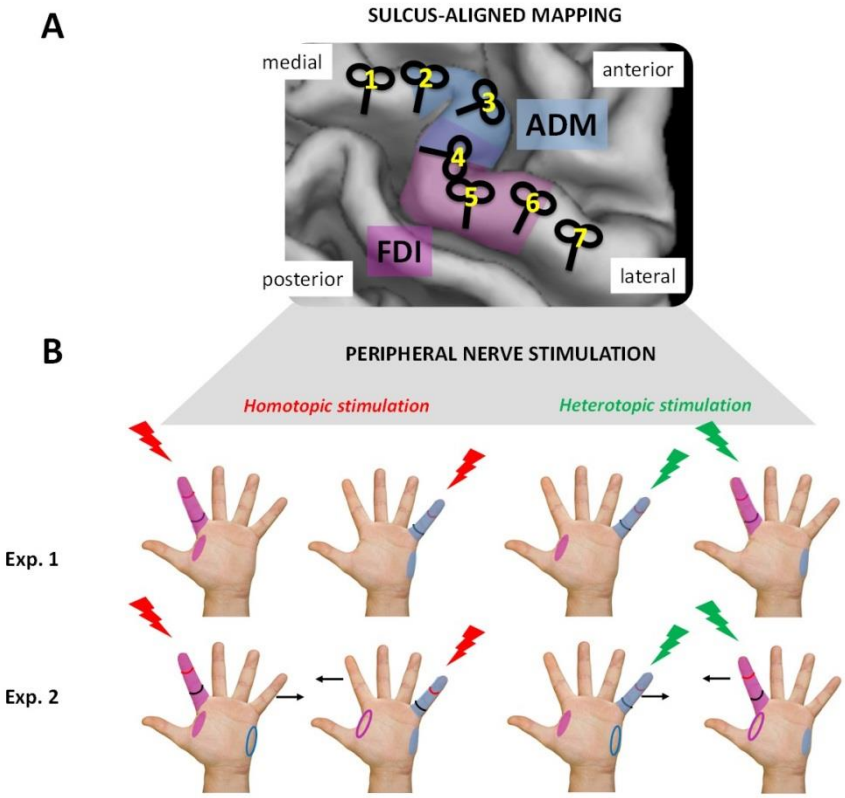


**Figure 2**

**Panel A.**

A. Homotopic stimulation (in red): the peripheral nerve stimulation is applied close to the TMS muscle target (filled ovals). Heterotopic stimulation (in green). the peripheral nerve stimulation is applied far from the TMS muscle target (filled ovals). ADM= blue. FDI= pink. Homotopic stimulation= full line; heterotopic stimulation = dotted line.

A'. Stability of the spatial dissociation (measured as Weigheted Mean Positions) of the two muscle profiles (ADM and FDI) for the unconditioned MEP across the six experimental blocks (Index and little finger stimulated at 100%-200%-300% of perceptual threshold, PT).

**Panel B and C.**

Ratio between the Area under the curve (AUC) of the conditioned MEP amplitudes ($AUC_C$) and of the unconditioned MEP amplitudes ($AUC_U$) at three different intensities of peripheral stimulation (100%, 200%, 300% of perceptual threshold, PT) for homotopic (B) and heterotopic stimulation (C)

For the homotopic stimulation a different modulation of the inhibitory curves (ratio of the $MEP_C$ and $MEP_U$) along the seven targets at different PT intensities was evident either for FDI muscle (B') or ADM muscle (B''). Regarding the heterotopic stimulation, the inhibition is replaced by facilitation (SAF), with the highest facilitatory effect for PT300 in FDI(C') and ADM msucles (C'').

A value higher than 1 indicates a facilitatory effect of the peripheral stimulation on the MEP whereas a value lower than 1 an inhibitory effect. (*) Indicates the significant modulatory effect of the intensity of the peripheral stimulation and of the finger stimulated (homotopic vs heterotopic stimulation) at 300% of PT. $MEP_C$= conditioned MEP; $MEP_U$= unconditioned MEP; Homotopic stimulation= full line; heterotopic stimulation = dotted line. SAI= short afferent inhibition; SAF= short afferent facilitation.

**Figure 2**

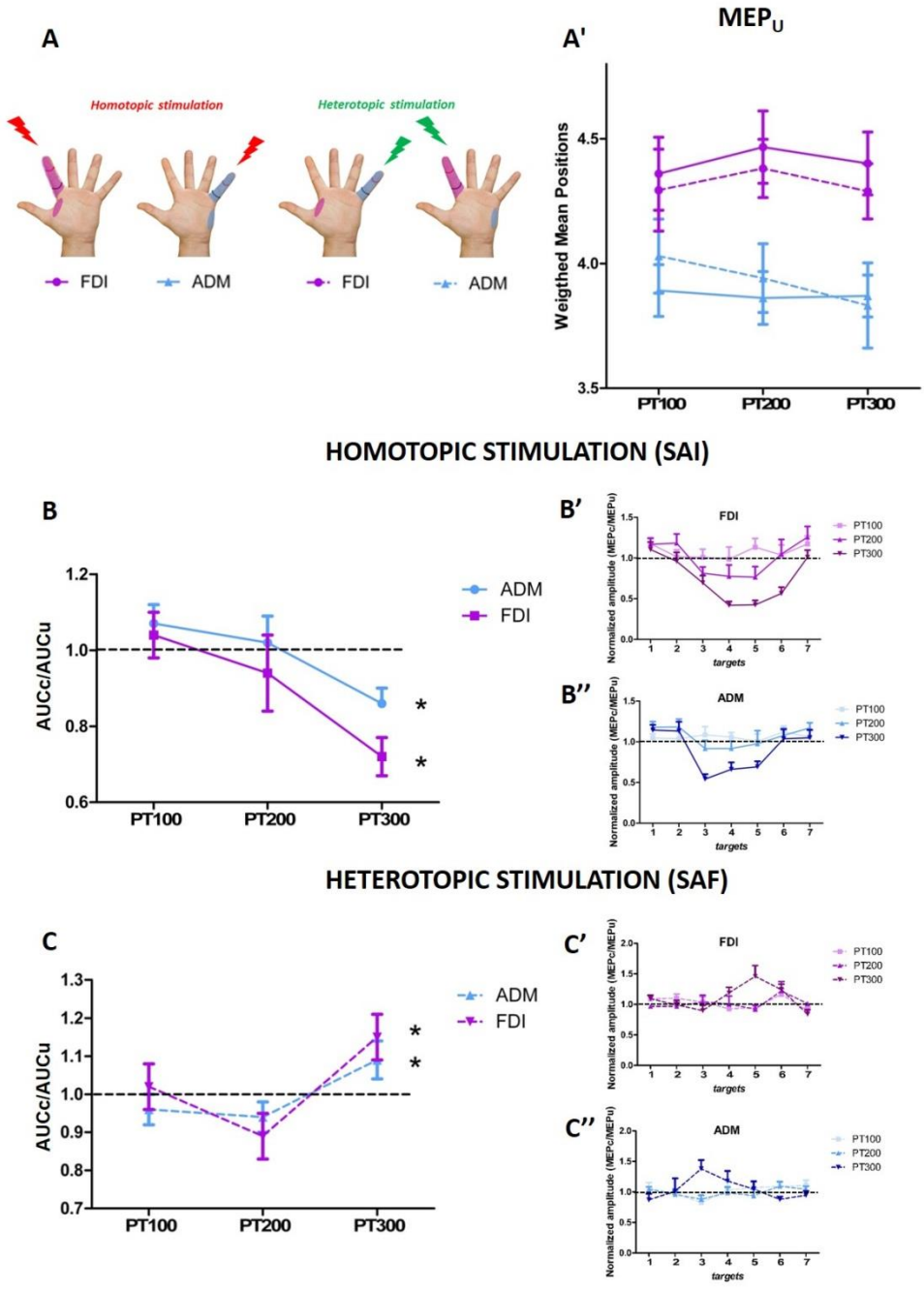

A
Homotopic stimulation
Heterotopic stimulation
FDI
ADM
FDI
ADM
A'
MEP$_U$
Weigthed Mean Positions
4.5
4.0
3.5
PT100
PT200
PT300
HOMOTOPIC STIMULATION (SAI)
B
AUCc/AUCu
1.2
1.0
0.8
0.6
PT100
PT200
PT300
ADM
FDI
*
*
B'
FDI
Normalized amplitude (MEPc/MEPu)
targets
PT100
PT200
PT300
B''
ADM
HETEROTOPIC STIMULATION (SAF)
C
AUCc/AUCu
1.3
1.2
1.1
1.0
0.9
0.8
0.7
PT100
PT200
PT300
ADM
FDI
*
*
C'
FDI
C''
ADM

**Figure 3**

**Panel A and B.** Mediolateral dissociation of muscle excitability profiles for the conditioned MEP (bold line) and unconditioned MEP (light line), with the FDI muscle located more laterally respect to the ADM muscle either during the homotopic (A) or heterotopic stimulation (B) at PT of 300%. Homotopic stimulation= full line; heterotopic stimulation = dotted line. SAI= short afferent inhibition. SAF= short afferent facilitation.

**Panel C and D.** Weighted Mean Positions of the two muscles profiles referred to the conditioned MEP. (*) Indicates the significant spatial dissociation during homotopic (C) vs heterotopic stimulation (D). Note the somatotopic arrangement is similar either for the homotopic or heterotopic stimulation.

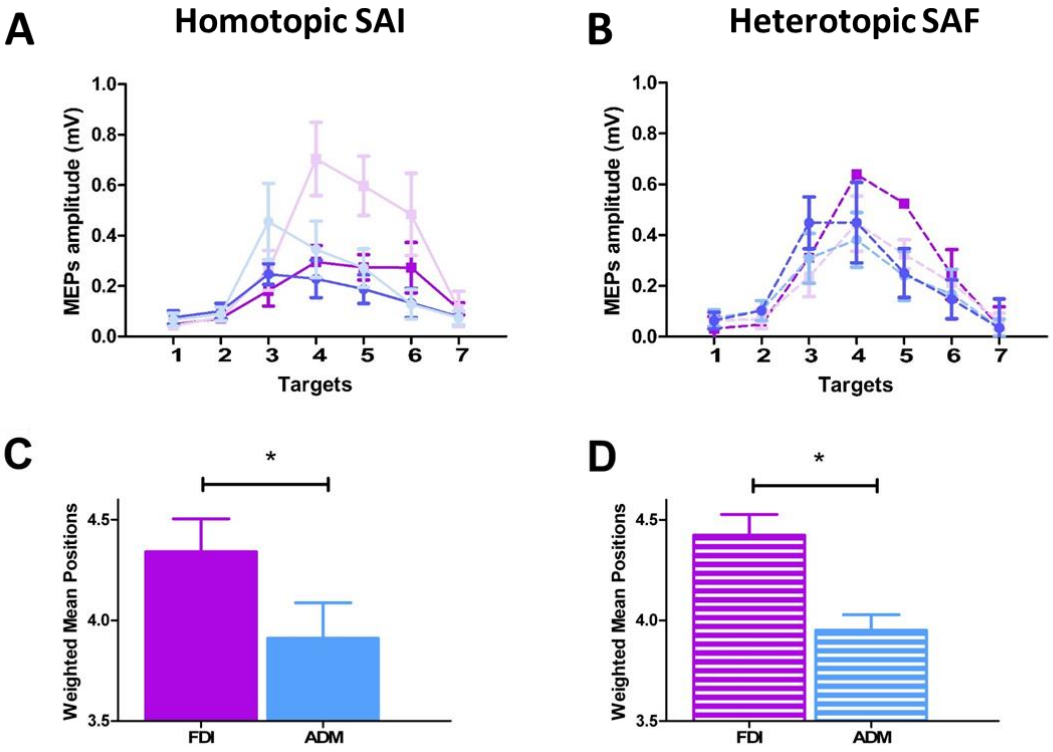


**Figure 4**

**Panel A.** Homotopic and heterotopic stimulation during tonic muscle contraction (black arrows) of the second or the fifth finger to evaluate the sensorimotor modulatory mechanisms in the surrounding muscles drawn as filled ovals (FDI: pink and ADM: blue).

**Panel B**. Ratio between the area under the curve (AUC) of the conditioned MEP amplitudes (AUCc) and of the unconditioned MEP amplitudes (AUCu) at rest and during selective tonic muscle contraction of a intrinsic hand muscle (Movement) in the relaxed surrounding muscles (ADM muscle= blue line; FDI muscle= pink line). (*) indicates the significant abolishment of the homotopic inhibitory and heterotopic facilitatory effects observed at rest condition. HET= heterotopic; HOM= homotopic.

**Panel C.** Linear cortical representation profiles of the surrounding muscles as revealed by the MEP evoked by homotopic or heterotopic stimulation along the seven cortical target positions in $M1_{HAND}$. The bold lines indicate unconditioned MEP, while the light lines represent the conditioned MEP.

**Panel D.** Weighted mean positions of the conditioned MEP for surrounding muscles profiles during tonic muscle contraction. The suppression of the facilitation and inhibition was somatotopically expressed with a significant (*) spatial dissociation between the two muscles.

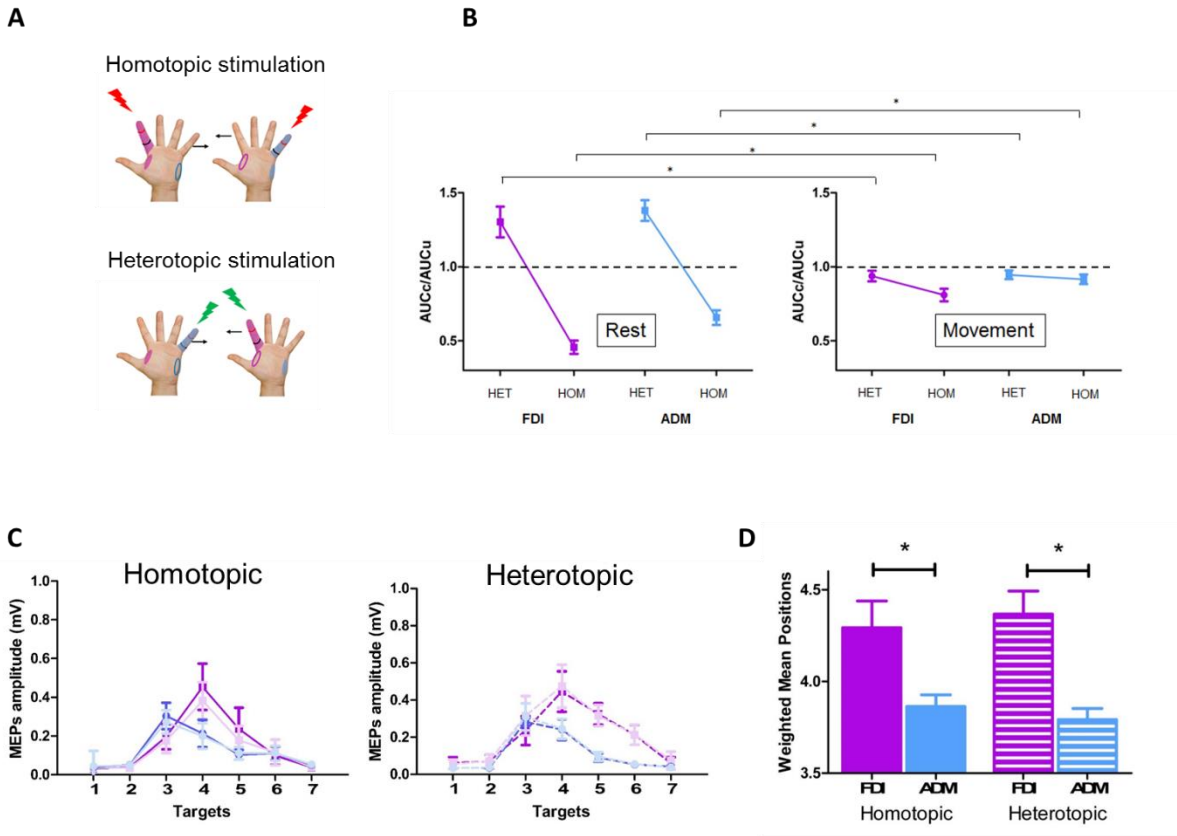


**Figure 5**

**Panel A.** Correlation between heterotopic FDI facilitation and homotopic ADM inhibition (Pearson correlation, r= -0.791, corrected p= 0.005).

**Panel B.** Correlation between homotopic FDI inhibition and homotopic ADM inhibition (Pearson correlation, r = 0.803, corrected p = 0.003).

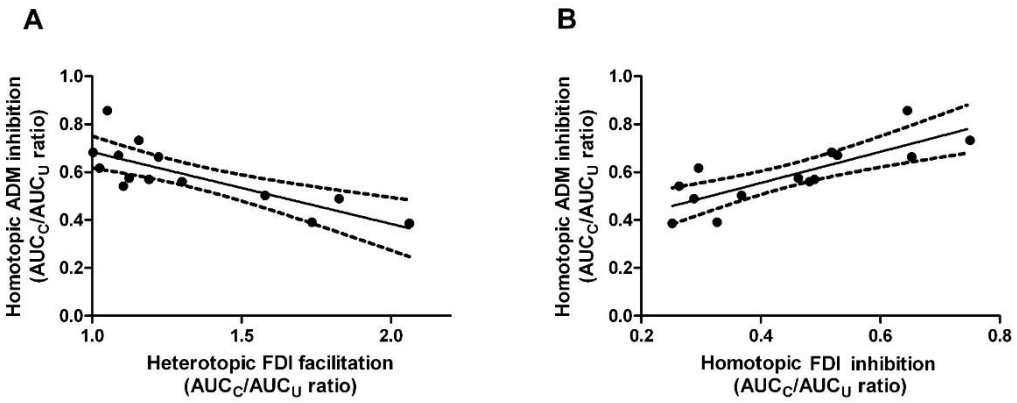


**Table 1**

Normalized mean coordinates (SD) of stimulation targets. Coordinates were first normalized to the Talairach Atlas using the Localite software and then normalized to the MNI template with a rigid transformation.

| | X ± SD | Y ± SD | Z ± SD |
|---|---|---|---|
| **Target 1** | 16.3 ± 7.0 | -17.0 ± 7.2 | 73.1 ± 2.5 |
| **Target 2** | 23.4 ± 8.7 | -17.0 ± 6.7 | 71.6 ± 3.1 |
| **Target 3** | 30.5 ± 7.9 | -16.9 ± 9.3 | 68.9 ± 3.2 |
| **Target 4** | 36.1 ± 7.2 | -15.3 ± 9.6 | 67.8 ± 3.3 |
| **Target 5** | 40.4 ± 6.8 | -10.4 ± 9.5 | 61.1 ± 6.6 |
| **Target 6** | 43.9 ± 8.3 | -6.0 ± 9.8 | 55.7 ± 6.9 |
| **Target 7** | 50.1 ± 6.7 | -4.6 ± 8.7 | 51.0 ± 8.1 |

**The Role of Cerebellum in the fast somatosensori-motor integration**

*From the article: "The Effect Of Cerebellar Degeneration On Human Sensori-Motor Plasticity" By Raffaele Dubbioso, Giovanni Pellegrino, Antonella Antenora, Giuseppe De Michele, Alessandro Filla, Lucio Santoro, Fiore Manganelli. (Brain Stimulation 2015)*

**Abstract**

Plasticity of the primary motor cortex (M1) has a critical role in motor control and learning. The cerebellum facilitates these functions using sensory feedback. We investigated how cerebellar degeneration influences the plasticity of the M1 by using PAS (paired associative stimulation) technique. PAS involves repeated pairs of electrical stimuli to the median nerve and transcranial magnetic stimulation (TMS) of the motor cortex. If the interval between peripheral and TMS stimulation is around 21–25 ms, corticospinal excitability is increased via a long term potentiation (LTP)-like effect within M1. Our aims were: (i) to explore the presence of a time-specific influence of cerebellar degeneration on human associative plasticity; (ii) to evaluate the role played by somatosensory pathway on cerebellar modulation of sensory-motor plasticity. We studied 10 patients with pure cerebellar atrophy and 10 age-matched healthy subjects. Motor-evoked-potentials amplitudes, short-afferent inhibition (SAI), motor thresholds, I/O curves, somatosensory-evoked-potential (SEP) were measured before, just after and 30 min after PAS at ISIs (interstimulus intervals) of 21.5 and 25 ms. Cerebellar patients show a selective lack of LTP-like effect induced by PAS25 ms, but not at 21.5 ms. SAI was overall not truly modulated by PAS but clearly differed between cerebellar patients and healthy subjects for ISIs around 25 ms (+6 ms and +8 ms) ($p< 0.01$). SEPs showed the amplitude of P25 wave was markedly reduced in patients with a more severe clinical and radiological impairment of cerebellum. Cerebellar patients have an altered capability of cerebellar filtering or processing of time-specific incoming sensory volleys, influencing the plasticity of M1.

**Introduction**

The cerebellum is traditionally considered as a motor structure; however it has increasingly understood to play a wider role due to its connections with association cortex, such as the parietal and prefrontal lobes. This shift has been driven by studies in animals that have demonstrated that sensory feedback arising from movements and interactions with the environment are processed by the cerebellum to facilitate motor control and promote motor learning (Nixon 2003; Chen and Wolpaw 2005; Oulad Ben Taib and Manto 2006). Recently non-invasive brain stimulation studies (Hamada et al. 2012; Popa et al. 2013) have demonstrated in humans that cerebellar processing of sensory afferent information influences the plasticity of the primary motor cortex (M1). This phenomenon has been evaluated interfering with cerebellar function by means of repetitive Transcranial Magnetic Stimulation (rTMS) or Transcranial Direct Current Stimulation (tDCS) and testing the performance of LTP-like plasticity induction techniques, such as Paired Associative Stimulation (PAS) (Stefan et al. 2000) and intermittent Theta Burst Stimulation (iTBS) (Huang et al. 2005), on primary motor cortex (M1). The role of the afferent system can be disentangled because while M1 PAS LTP-like induction depends upon peripheral sensory input (Stefan et al. 2000), iTBS LTP-like effects do not (Di Lazzaro and Ziemann 2013). Indeed, Popa and colleagues reported that only M1 PAS (and not M1 TBS) is sensitive to changes of cerebellar excitability induced by theta burst stimulation (TBS) (Popa et al. 2013) and, with a similar approach, Hamada and colleagues had shown how the modulation of cerebellar activity using both anodal and cathodal (tDCS) can abolish motor cortex PAS-induced plasticity. Only the so called M1 PAS25 (Inter-stimulus interval between peripheral stimulus and TMS 25ms) is abolished, regardless of the cerebellum tDCS employed (cathodal/inhibitory vs anodal/excitatory), while M1 PAS21.5ms performance does not change significantly. Thus, despite both PAS21.5ms and PAS25ms induce Long Term Potentiation (LTP)-like effects (Hamada et al. 2014a; Strigaro et al. 2014), they involve different circuits and mechanisms. This is supported by the direct evaluation of the effects of PAS 25 and 21.5 on the corticospinal activity evoked by TMS, while both PAS 25 and PAS 21.5 enhances MEP amplitude, only PAS25 enhances the late I-wave amplitude (Di Lazzaro et al. 2009) while PAS 21.5 has no effect on these waves (Hamada et al 2014a). This strongly supports the hypothesis that the modulation of different cortical circuits is responsible for the effects of PAS 25 and PAS 21.5. The possibility that the cerebellar stimulation influences sensory processing in the primary somatosensory cortex was ruled out in these studies (Hamada et al. 2013; Popa et al. 2013) by the lack of cortical somatosensory-evoked potentials changes after cerebellar stimulations.

The low spatial specificity and great variability among subjects which characterize neuromodulation techniques (Di Lazzaro et al. 2011; Hamada et al. 2013) makes very difficult the interpretation of the influence of cerebellum and somatosensory pathways on sensory-motor cortex plasticity. However, on the basis of these findings, we hypothesize that cerebellar patients suffer from a selective lack of M1 plasticity. To test this hypothesis, we recruited a group of "pure" cerebellar patients suffering from primitive cerebellar degeneration and evaluated the effects of PAS21.5 and PAS25 compared to healthy controls. We considered this subset of "pure" cerebellar patients as a good model to explore in vivo the

influence of cerebellar degeneration on motor cortex plasticity. In summary, our aims were: (i) to explore the effect of cerebellar degeneration on human associative plasticity; (ii) to evaluate the role played by somatosensory pathway on cerebellar modulation of sensory-motor plasticity.

## Methods

### Patients

A total of 10 right-handed patients with cerebellar degeneration (five males, mean age 38.1 ± 13.9 years, mean disease duration 10.8 ± 11.5 years) and an equal number of right-handed age- and sex- matched healthy participants (four males, mean age 36.9 ± 16.4 years) took part in the study. The study was approved by the local ethics committee of the Department of Neuroscience, University Federico II of Naples and the research was conducted in accordance with the 1964 Declaration of Helsinki. All the subjects gave their written informed consent prior to the participation in the study. Inclusion criteria were: (i) the presence of cerebellar syndrome as result of a slowly progressive disease; (ii) Magnetic Resonance Imaging (MRI) evidence of cerebellar atrophy. Exclusion criteria were: (i) clinical involvement of the motor (weakness, parkinsonism, dystonia, amyotrophy) or sensory systems (such as somatosensory and visual systems), (ii) dementia or mild cognitive impairment, (iii) Electromyography (EMG) and Nerve Conduction Studies abnormalities. All MRI studies were carried out at 3-tesla on the same MRI scanner and included T1, T2-weighted and FLAIR (Fluid Attenuated Inversion Recovery) sequences. All patients were characterized on the basis of clinical, biohumoral, genetic, neurophysiological, neuropsychological and MRI features. Severity of ataxia was evaluated by using the Scale for the Assessment and Rating of Ataxia (SARA) (Schmitz-Hübsch et al. 2006). MRI scans were reviewed by a neuroradiologist, who was blind to the clinical status of the patients. For the qualitative rating of the MRI scans, emphasis was placed on the appearance of the cerebellar hemispheres, as the cerebellar vermis is variable within normal control population (Koller et al. 1981). The degree of atrophy was judged by the size of the sulci and folia. Based on the degree of cerebellar atrophy seen on MRI, the patients were divided in three groups: group I, mild atrophy; group II, moderate atrophy; group III, severe atrophy (table 1).

### Experimental design

Both healthy and cerebellar patients underwent PAS21.5 and PAS25 in two different TMS sessions, separated by a week. The order of the tested PAS (21.5 vs 25) was randomized between subjects. Before, immediately after (T0) and 30 minutes after (T30) PAS we acquired a panel of measures to fully characterize M1 brain excitability and plasticity: Resting Motor Threshold (RMT), Active Motor Threshold (AMT), 1 mV Motor Evoked Potentials (MEP), input/output recruitment curves (I/O curves) to investigate changes of excitability and LTP-like induction, Short-Latency Afferent Inhibition (SAI) and Somatosensory Evoked Potentials (SEP) to unveil potential effects of the cerebellum on the somatosensory pathway and sensory-motor cortex function (Fig. 1).

**Electromyographic recordings**

EMG recordings were acquired from the right abductor pollicis brevis (APB) and right abductor digiti minimi (ADM) muscles on the side contralateral to stimulated cortex with Ag-AgCl surface electrodes using a belly-tendon montage. The signals from the EMG electrodes were amplified, bandpass filtered (20 Hz–3 kHz), digitized at a frequency of 5 kHz, and stored in a laboratory computer for later offline analysis by Signal software and CED 1401 hardware (Cambridge Electronic Design, Cambridge, UK).

**Transcranial magnetic stimulation (TMS)**

TMS of the left primary motor cortex was applied using a high-power magnetic stimulator with a biphasic current waveform (MagPro X100, Medtronic, Denmark) connected to a standard figure-of-eight coil. The "hot spot" was defined as the optimal scalp position for eliciting MEPs of maximal amplitude in the contralateral abductor pollicis brevis (APB) muscle (target muscle). The same hot spot was used for assessing the MEPs in abductor digiti minimi (ADM) muscle (Rossini et al. 1994).

**Corticospinal excitability**

AMT and RMT for the target muscle were determined according to the standard definitions (Rossini et al. 2015). Single MEPs were recorded using a stimulus intensity adjusted to produce MEP amplitude of approximately 1 mV in the relaxed APB muscle (1 mV MEPs) and this intensity was kept constant for assessment of MEPs after PAS. For recruitment curves (I/O curves), the intensities of the single TMS stimuli were individually expressed relative to RMT at baseline. Ten MEPs were recorded at 100, 120 and 140% RMT stimulation intensity. For each subject, the peak-to-peak amplitudes were measured on each single trial to calculate the mean amplitude at each stimulus intensity.

**Somatosensory evoked potentials (SEPs)**

SEPs were elicited by electrical stimulation (square wave pulse; stimulus duration, 0.2 ms) of the right median nerve at the wrist (cathode proximal) at an intensity of 1.2 times motor threshold (defined as the minimum stimulation intensity able to produce a small twitch of the APB in about 5 out of 10 stimuli) and at a frequency of 3 Hz using a constant current generator (Digitimer, WelwynGarden City, UK). Two recording electrodes were placed at the C3' (2 cm posterior to C3 of International 10–20 system) with the ear-lobe as reference. The impedance between the electrodes was kept below 5 kΩ. SEPs were recorded in epochs from −10 to 100 ms triggered by the electrical stimuli. The sampling rate was set at 8 kHz, and the potentials were amplified and filtered between 10 and 3000 Hz. We collected and averaged 2 blocks of 1000 trials to ascertain the reproducibility. The amplitudes of N20 and P25 components were measured from the preceding peaks in each trial.

**Short latency afferent inhibition (SAI)**

SAI was examined at interstimulus intervals (ISIs) ranging from 2 to 8 ms after N20 latency, in steps of 2 ms (Tokimura et al. 2000). The median nerve was stimulated at wrist through bipolar surface electrodes (cathode proximal, rectangular pulse of 0.2 ms duration). Stimulus intensity was adjusted to produce a slight thumb twitch. The intensity of the test stimulus (TS) was set at an intensity required to elicit a 1 mV MEP ($SI_{1mV}$). Ten trials were recorded for each condition and randomly intermixed with 32 trials of TS alone. Stimuli were given every 4.5–5.5 s. TS intensity was adjusted after intervention, if required, in order that the MEP had the same size as at baseline. The ratio of the mean amplitude of the conditioned response to that of the TS response was calculated for each condition and ISI in each subject. Data of patients and controls, obtained at the ISIs 2, 4, 6 and 8, were analyzed and averaged to obtain a grand mean ratio of SAI.

**Paired associative stimulation (PAS)**

PAS consisted of 180 electrical stimuli of the right median nerve at the wrist paired with a single TMS over the hotspot of the right APB muscle at a rate of 0.2 Hz. Electrical stimulation (square wave pulse; stimulus duration, 0.2 ms) was applied at an intensity of three times the perceptual threshold using a constant current generator (Digitimer). TMS was applied at $SI_{1mV}$. The effects of PAS given with an interstimulus interval of 25 ms (PAS25) and of 21.5 ms (PAS21.5) between peripheral and TMS stimuli were tested. Both protocols have been shown previously to induce a long lasting increase in MEP amplitude (Stefan et al. 2000; Seidel et al. 2012). Subjects were instructed to look at their stimulated hand and count the peripheral electrical stimuli they perceived. The MEPs evoked in the APB were displayed online during the intervention to control for the correct coil position and stored for off-line analysis.

**Data analysis and statistics**

Main aim of the statistical analysis was to assess differences of M1 plasticity between Groups (Cerebellar vs Healthy) and PAS protocols (PAS21.5 vs PAS25). The investigation of the effect of PAS protocols on ADM muscle is ancillary, motivated by Popa's study (Popa et al. 2013), and has been performed separately to further evaluate the spatial specificity of PAS-induced effects. Data were analyzed using SPSS v. 19.0 for Windows (SPSS Inc.). Normal distribution was verified by means of Kolmogorov and Smirnov test. Group matching regarding age and gender was tested by means of independent sample t-test and $\chi^2$ respectively. After checking the underlying assumptions, we performed an analysis of variance to assess the role of multiple sources of variation on excitability and plasticity measures. As preliminary step, we ensured that baseline neurophysiological measures did not differ across Groups and PAS protocols. Then, for RMT, AMT, MEP amplitude, SAI, slope of recruitment curve and SEP components, we applied a mixed model ANOVA with *PAS_protocol* (two levels: PAS21.5 and PAS25) and *Time* (three levels: Pre, T0 and T30) as within subject factors and *Group* (two levels: Cerebellar and Healthy) as between subject factor. When dealing with SAI, being multiple ISI available, we also inlcuded the factor *ISI* (4 levels: 2ms, 4ms, 6ms, 8ms) as within subject factor. The

Greenhouse–Geisser method was used to correct for non-sphericity whenever necessary. The slope of the recruitment curve was quantified by a linear regression analysis for all data points between 100 and 140% RMT as described by others (Cirillo et al. 2009). Correlation between SARA scale and degree of atrophy (ranged from 1: mild atrophy to 3: severe atrophy) and neurophysiological measures were evaluated with Spearman's correlation coefficients. p values < 0.05 were considered significant. Alpha inflation due to multiple comparisons was controlled according Bonferroni's approach when appropriate. Descriptive statistic is reported as mean±standard error of the mean (SEM).

**Results**

**Clinical, radiological and demographic data**

Neurological examination showed a pure cerebellar syndrome in all patients. Four patients with an autosomal inheritance pattern underwent molecular analysis in order to exclude the most common spinocerebellar ataxia subtypes (SCA1, 2, 3, 6, 7, and 17) in which basal ganglia affection has been demonstrated (Seidel et al. 2012). Three patients presented with an autosomal recessive inheritance pattern and were negative for GAA expansion in FRDA gene and had normal levels of ceruloplasmin, CK, α-fetoprotein, cholesterol and albumin. The remaining three patients, after excluding the acquired causes (metabolic alterations, vitamin E deficiency, neoplasms, and malabsorption) were defined as apparently sporadic idiopathic cerebellar ataxia. In none of the subjects atrophy of cerebral cortex, midbrain, pons and medulla, lesions of basal ganglia or white matter were identified. All our patients presented a diffuse and homogenous cerebellar hemispheric atrophy without any asymmetry, further clinical and neuroimaging details are reported in table 1.

**Effect of Cerebellar Degeneration on primary motor cortex PAS**

Patients and healthy subjects completed the two sessions (PAS21.5 and PAS25) without complications. No differences for physiological data at baseline (RMT, AMT, MEP size, test MEP for SAI, N20 and P25 latencies and amplitudes) were found across sessions and groups ($p>0.100$), see supplementary Table 1. Individual RMTs and the intensities used to evoke 1mV MEPs are reported in supplementary Table 2.

**MEPs amplitude**

The two PAS paradigms were overall different [*PAS_paradigm* main effect $F(1,19)=5.163$, $p=0.036$] and differed between *Groups* [*PAS_paradigm by Group interaction* $F(1,18)=8.748$, $p=0.008$]. PAS changed brain excitability [*Time* main factor: $F(2,36)=67.770$, $p=0.000$] in a different fashion in the two *Groups* [*Time by Group interaction* $F(2,36)=6.160$, $p=0.005$] and for the two paradigms [*Time by PAS_paradigm interaction:* $F(2,36)=5.864$, $p=0.013$]. This was further supported by the *PAS_paradigm by Time by Group interaction* [$F(2,36)=6.352$, $p=0.004$]. The main factor *Group* was not significant [$F(1,18)=2.140$, $p=0.161$]. We therefore computed two mixed model ANOVA for the two PAS paradigms with factor *Time* as within subject factor and *Group* as between subject factor.

While PAS21.5 produced a significant change of brain excitability [*Factor Time F(2,36)=44,673, p=0.000*], similar for both Cerebellar patients and healthy controls [*Factor Group F(1,18)=0.15, p=0.904* and *Time by Group interaction F(2,36)=0.047, p=0.954*], PAS25 still produced an overall increase of excitability [*Factor Time F(2,36)=19.024, p=0.000*], but the effect was different in the two *Groups* [*Factor Group F(1,18)=6.712, p=0.018* and *Time by Group interaction F(2,36)=14.552, p=0.000*]. In details, the effect of PAS25 on Cerebellar patients vs Healthy subjects is significantly lower at T0 [*Cerebellar=1.06±0.20; Healthy=1.67±0.15; p=0.048*] and at T30 [*Cerebellar=1.00±0.20; Healthy=1.95±0.15; p=0.001*]. As additional test we also verified that in the Cerebellar group PAS25 was not effective at all [*T0 vs Pre and T30 vs Pre p>0.200 consistently*]. See Fig. 2A.

**RMT and AMT**

Neither RMT nor AMT showed any significant modulation in dependence of *PAS_protocol*, *Time*, *Group* or their interaction (*p>0.100*).

**Recruitment curve**

The significant effect of the factor *Time* [*F(2,36)=7.811, p=0.002*] suggested an overall effect of PAS on the recruitment curve which, notably, depended upon *PAS_paradigm* and *Group* [*PAS-paradigm by Time by Group interaction: F(2,36)=4.287, p=0.021*]. The ANOVA performed on each PAS_protocol with *Time* as within subject factor and *Group* as between subject factor unveiled a significant factor *Time* [*F(2,36)=9.651, p=0.002*] for PAS21.5, suggesting that this stimulation produced an effect on the recruitment curve similar for the two groups at different time points (lack of significant factor *Group* and *Time by Group interaction*, p>0.100 consistently). Conversely, for PAS25 only the *Time by Group interaction* [*F(2,36)=6.136, p=0.005*] was significant, putting forward a different effect of the stimulation in the two groups. Indeed, the post hoc comparison showed that, compared to healthy subjects, Cerebellar patients have a smaller slope of the recruitment curve at T30 (p=0.038). Additionally, we also found that, in agreement with the behavior of MEPs, Cerebellar patients' recruitment curve was not modulated by PAS25 at all [*T0 vs Pre and T30 vs Pre p>0.200 consistently*]. See Fig. 2B.

**SAI**

The ANOVA mixed model only revealed an expected factor *ISI* [*F(3,54)=28.880, p=0.000*], a global difference between groups [*Factor Group: F(1,18)=9.662, p=0.006*] and an *ISI by Group interaction* [*F(3,54)=17.412, p=0.000*] suggesting a different modulation of the inhibition at different ISI for the two groups. There is an overall lack of PAS effect on this parameter, as suggested by the lack of significance for the factor *Time* and for all the interactions containing it. Notably, the two groups differed for ISI 6 ms and ISI 8 ms (p=0.000 consistently). Regarding grand mean ratio of SAI, we computed a mixed model ANOVA with PAS protocol (two levels:PAS21 and PAS25) and Time (three levels: Pre, T0 and T30) as within subject factor and Group (two levels: Cerebellar and Healthy) as

between subject factor. We found a significant main factor Group [F(1,18)=8.203, p=0.010] and a significant Time factor [F(2,36)=4.348, p=0.020] showing that there was a PAS effect, similar for both protocols. In details, the only significant difference is for PAS21 comparing in the healthy group the SAI pre PAS vs SAI at T30 (p=0.012). See Fig. 3A, Fig. 3B.

**Effect on SEP components (N20 and P25)**

While for N20 the ANOVA mixed model did not show any modulation of N20 in dependence of *PAS_paradigm*, *Time* and *Group*, the same model revealed a P25 modulation in dependence of PAS [*Factor Time: F(1,17)=5.449, p=0.032*] regardless of the kind of protocol and Group (p>0.200).

**Effect of Cerebellar Degeneration on topographic specificity (ADM muscle)**

In order to explore the topographic specificity we analyzed the MEPs and recruitment curve of the non-target muscle ADM. Neither MEP amplitude nor recruitment curves showed any significant modulation in dependence of *PAS_protocol*, *Time*, *Group* or their interaction (*p>0.100*) indicating a preserved topographic specificity also in cerebellar patients.

**Correlation of cerebellar clinical score (SARA) and degree of atrophy with motor cortex excitability parameters and SEPs**

Correlation analysis between clinical score (SARA) and degree of atrophy disclosed that patients with a more severe cerebellar disease are associated with a higher degree of cerebellar atrophy (SARA, ρ=0.768, p=0.005). Moreover we detected for PAS25 an inverse correlation between SARA and the slope of recruitment curves at T30 (SARA, ρ=-0.689, p= 0.028) and a negative association between SARA/degree of atrophy and the amplitude of MEP at T30 (SARA, ρ=-0.634, p=0.048; degree of atrophy, ρ=-0.711, p=0.022). Regarding SEP components, we found a negative correlation between SARA, degree of atrophy and P25 amplitudes for all considered sessions (see supplementary Table 3). No other correlation between SEP measures and clinical features were found.

**Discussion**

In this study we showed the effect of cerebellar degeneration on the plasticity of the sensorimotor cortex. In particular, we demonstrated that: (i) in patients affected by primitive cerebellar degeneration sensorimotor plasticity is selectively abolished for PAS25; the loss of plasticity is correlated with clinical impairment and degree of atrophy; (ii) the worse are clinical condition and cerebellar atrophy, the worse is the sensory-motor processing of afferent information, as probed by the correlation of clinical and morphological measures with P25 component. This datum suggests the altered capability of cerebellum to filter or process of time-specific incoming sensory volleys.

Both PAS21.5 and PAS25 are accepted techniques for induction of LTP-like effect on the motor cortex (Stefan et al. 2000; Weise et al. 2006). Recently, several studies (Hamada et al. 2013, 2014b; Strigaro

et al. 2014) have demonstrated on healthy subjects that even though the techniques induce the same amount of plastic effect on motor cortex, the mechanisms underlying PAS at these two interstimulus intervals are different, being PAS25 effect dependent upon the cerebellum. For the first time in a group of well selected pure cerebellar patients we show that sensorimotor plasticity is abolished at PAS25, while it is preserved at PAS21.5. Moreover, for patients with a higher clinical impairment demonstrated by SARA scale and with a more severe cerebellar atrophy at MRI, the loss of plasticity was more evident. A recent study of Kishore (Kishore et al. 2014b) and colleagues showed that in elderly people, where the presence of cerebellar atrophy/impairment is well-known, aging causes alterations in the cerebellar modulation of the response of motor cortex to plasticity-induction and this is likely to reflect the altered capability of cerebellar processing of incoming sensory inputs.

In our study, SEPs showed that the amplitude of P25 wave is markedly reduced in patients with a more severe clinical and radiological impairment of cerebellum. Such results are consistent with a previous work where unilateral cerebellar lesions reduce the amplitude of the P24 component (sometimes labelled P25) of the SEP without changing earlier responses (Restuccia et al. 2001). In contrast, recent studies (Hamada et al. 2012; Popa et al. 2013) failed to demonstrate that cerebellar stimulation may influence sensory processing in the primary somatosensory cortex, because of the lack of change in cortical somatosensory-evoked potentials. It is possible that in healthy subjects, non-invasive brain stimulation techniques (i.e. tDCS and TBS) are unable to induce detectable changes in SEPs, whereas in our cohort , cerebellar atrophy might lead to an alteration of cortical subcortical circuit underlying the P25 component. This, in turn, would support the role of cerebellum on the time specific processing of somatosensory pathway.

In addition, observations on SAI are consistent with the idea that sensory input can have both early and late effects on motor cortex. However the inhibitory effects of SAI decline at longer intervals (Tokimura et al. 2000) and are replaced by facilitation at around 25 ms, which about corresponds to the so called SAI 6 ms or SAI 8 ms (Fischer and Orth 2011). The neuronal mechanisms for this gradual shift from inhibition to facilitation have not been well understood, but it is possible that multiple and time-dependent effects of sensory input on motor cortex may play a role. Interestingly, in our SAI study we found that in cerebellar patients there was no shifting from inhibition to facilitation around 25 ms (SAI 6 ms and SAI 8 ms), enhancing the possible role of the cerebellum in the late effect of sensory input on motor cortex.

It is therefore possible that sensory input to cortex, arriving via this transcerebellar route, contributes to PAS at 25 ms. Sensory information, such as the one from the median nerve stimulation in PAS, is conveyed through the dorsal column–medial leminiscal system to the thalamic nuclei and from there to M1 (direct pathway) via either a relay in sensory cortex or direct thalamic input to M1 (Stefan et al. 2000). However, sensory information from median nerve can concomitantly activate afferent pathways projecting to the cerebellum through the spino-inferior olivary (IO) fasciculus and the spino-cuneo-cerebellar tract (indirect pathways). Both these indirect pathways send excitatory projections to the cerebellar cortex and to the cerebellar nuclei in a somatotopic manner (De Zeeuw et al. 1998). The peripheral electrical stimulation during PAS could act as a stream of non-selfgenerated afferent impulses

that activate the olivo-dentatothalamo-cortical (Fig. 4A) system and keep it in a hyperresponsive state. The TMS stimuli applied to the motor cortex during PAS could exploit this hyperresponsive state to facilitate a LTP in M1. In other words, the direct pathway might involve PAS at short intervals (21.5 or N20 latency), whereas the indirect pathway might involve PAS at longer interval (25 or P25 latency). In our patients this indirect pathway might be impaired, since cerebellar cortex and the dentate nucleus are not able to activate the thalamo-cortical relay, which could then not mediate the PAS response efficiently (Fig. 4B). Interestingly, some studies (Deiber et al. 1986; Balzamo et al. 2004) have demonstrated that later component after N20 of somatosensory inputs from the hand projected directly to M1, suggesting a "motor" generator for P25 wave and enhancing the link of cerebellum with P25 wave and its modulation on M1 plasticity.

Although our study supports altered sensory processing in patients with cerebellar degeneration, the impairment of sensory pathway cannot exclusively explain the results obtained here. Indeed, Hamada and colleagues (Hamada et al. 2014b) have recently demonstrated the cerebellar tDCS stimulation modified cortical excitability assessed by anterior-to-posterior current during target muscle contraction. Their result suggests that cerebellar tDCS seems to change the subpopulation of neurons (i.e. later I-waves) in the primary motor cortex. Therefore an alternative explanation to the lack of LTP like changes observed in our patients might be due to substantial changes in later I-waves in patients with cerebellar degeneration.

**Conclusion**

We demonstrated that cerebellar degeneration can influence associative plasticity in a timing-specific manner. This cerebellar time specific effect on sensory-motor plasticity could be mediated by gating of sensory information arriving via this transcerebellar route, the pathway seems to be the same of that generating P25 wave. Our study sheds light on the cerebellum as an active component of sensorimotor circuits and shows the importance of cerebellar loop with motor cortex that is relevant to cognitive processing as well as generation and control of movement. The present results have important implication for understanding the pathophysiology of neurological disorders in which cerebellar impairment is assumed, i.e. dystonia (Sadnicka et al. 2012), essential tremor (Deuschl 2000), Parkinson's disease (Kishore et al. 2014a), cortical myoclonus (Tijssen et al. 2000) and Huntington's disease (Crupi et al. 2008).

**Legend**

**Figure 1**

**Experimental design**

Ten patients with cerebellar atrophy underwent paired associative stimulation (PAS) protocol at interstimulus intervals (ISI) of 21.5 and 25 ms in two transcranial magnetic stimulation (TMS) sessions separated by 1 week. In each session we first measured baseline corticospinal excitability (resting motor threshold [RMT], active motor threshold [AMT], input/output [IO] curve, motor evoked potential [MEP]) and short latency afferent inhibition (SAI) at ISIs ranged from 2 to 8 ms after N20 latency, in steps of 2 ms. We then applied conditioning PAS protocol and assessed the effect of PAS on MEP, RMT, AMT, IO curve, SAI at 2 time points: 0 minutes, and 30 minutes after PAS. Somatosensory evoked potentials (SEPs) were tested before and 30 minutes after PAS. For assessment of 1 mV MEP the TMS intensity was kept constant throughout the experiment.

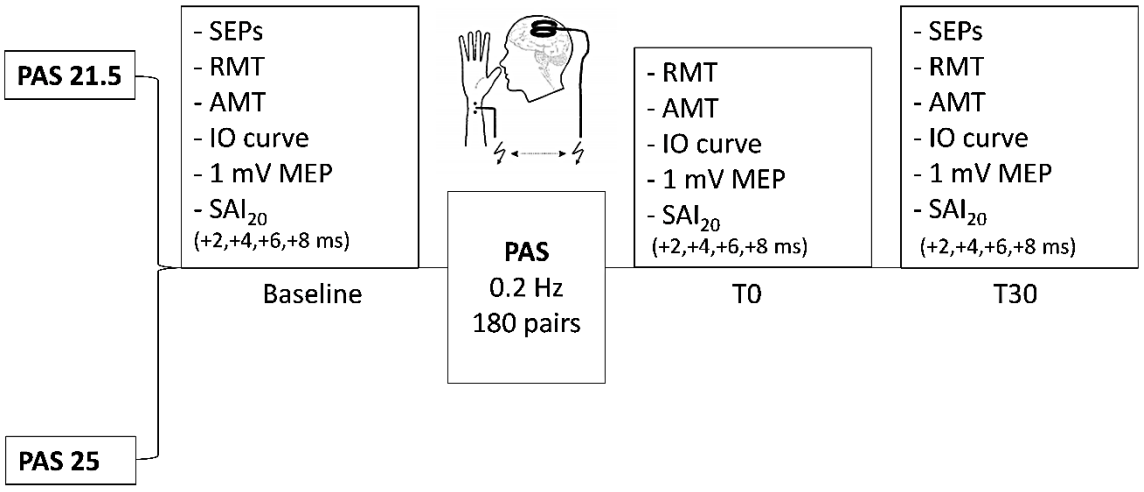


**Figure 2**

**PAS21.5 and PAS25 LTP-like effects on brain excitability and recruitment curve in healthy subjects (HS) and cerebellar patients (C)**

Panel A. Effects on MEP amplitude. PAS21.5 produced similar LTP-like plasticity effects in Cerebellar patients (C) and healthy controls (H). Conversely, PAS25 produced different effects in the two Groups being not effective for cerebellar patients. Panel B. Effects on recruitment curve. Results on the recruitment curve confirmed the findings achieved on the study of MEP amplitude. While PAS21.5 increases the slope of the recruitment curve in a similar fashion in healthy subjects and cerebellar patients, PAS25 is not effective in cerebellar patients compared to healthy subjects and does not produce significant changes of the recruitment curve in the cerebellar group.

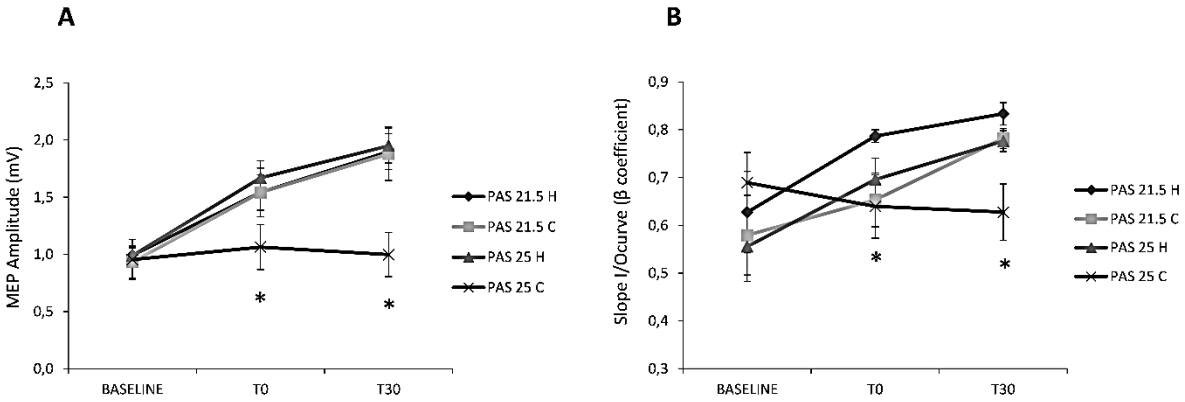


**Figure 3**

**Short latency afferent inhibition (SAI) in healthy subjects (H) and cerebellar patients (C)**

Panel A. SAI (% of test MEP) was overall not truly modulated by PAS but clearly differed between C vs HS for ISIs +6 ms and +8 ms ($p < 0.01$). Panel B. Grand mean of SAI showed a significant difference between the two groups ($p=0.010$).

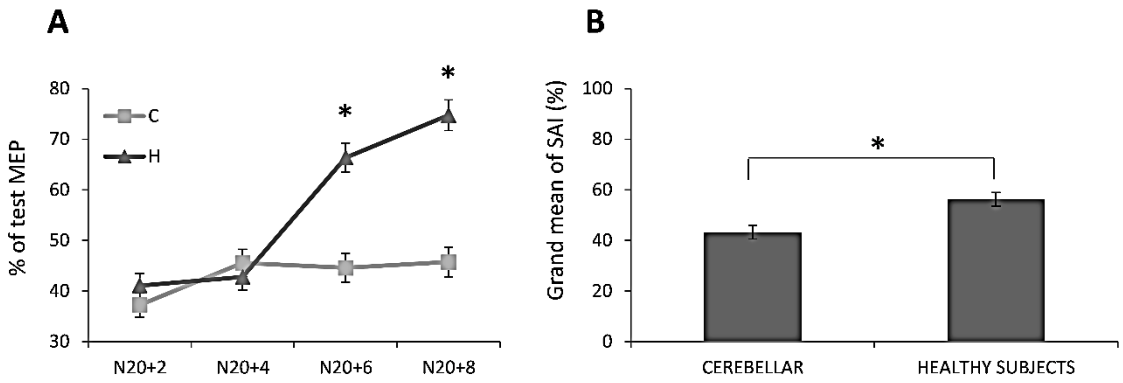
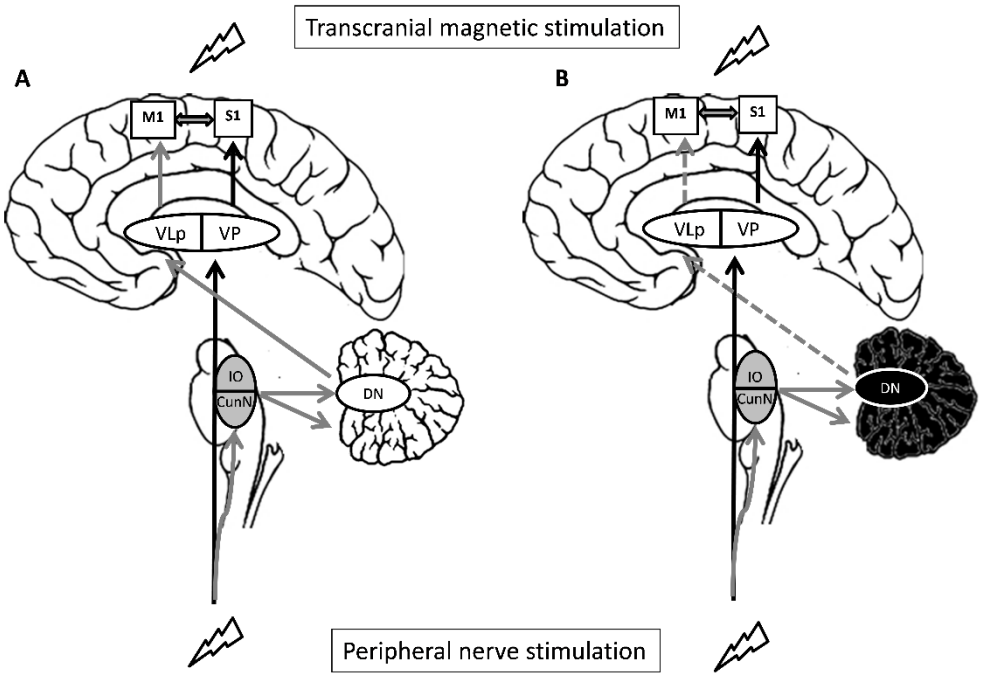


**Figure 4**

**Schematic representation of the spino-cerebello-thalamo-cortical circuit models controlling the peripheral afferent information flow to motor cortex (M1).**

(A) Healthy subjects, (B) cerebellar patients. Afferent inputs are conveyed through inferior olive to the dentate nuclei to interact with the cerebello-thalamo-cortical system. (S1, somatosensory cortex; DN, dentate nucleus; IO, inferior olive; VLp, posterior part of the ventrolateral thalamic nucleus; VP, ventral posterior thalamic nucleus, pars caudalis). Black line: sensory information arriving to cerebral cortex directly through the thalamic nuclei (direct pathway); gray line: sensory information arriving to cerebral cortex via transcerebellar route (indirect pathway); dashed lines indicates the possible pathway impaired in cerebellar patients.



**SECTION 3**

# SHORT LATENCY AFFERENT INHIBITION (SAI) AS A TOOL TO EVALUATE IN VIVO CENTRAL CHOLINERGIC NETWORK IN PATIENTS WITH DEMENTIA AND MILD COGNITIVE IMPAIRMENT

**The role of SAI as a tool to evaluate in vivo central cholinergic dysfunction in a model of juvenile Alzheimer's Dementia**

*From the article: "Central Cholinergic Dysfunction In Adult Form Of Niemann Pick Disease Type C: A Further Link With Alzheimer's Disease?" By Fiore Manganelli,[*] Raffaele Dubbioso,[*] Rosa Iodice, Antonietta Topa, Andrea Dardis, Cinzia Valeria Russo, Lucia Ruggiero, Stefano Tozza, Alessandro Filla and Lucio Santoro. (Journal of Neurology 2014)*

**Abstract**

Adult patients with Niemann-Pick disease type C (NPC) usually develop cognitive impairment progressing to dementia, whose pathophysiology remains still unclear. Noteworthy parallels exist in cognitive impairment and cellular pathology of NPC and Alzheimer's disease (AD). In particular, alterations of cholinergic system, that represent one of the pathological hallmarks and contribute to cognitive deterioration in AD, have been recently demonstrated in a human brain autopsy and in an experimental model of NPC. This finding raised the issue that central cholinergic circuits dysfunction may contribute to pathophysiology of cognitive impairment also in NPC and prompted us to evaluate the cholinergic functional involvement in NPC patients by applying a neurophysiologic technique, named short-latency afferent inhibition (SAI).

We describe clinical, biochemical, molecular and neuropsychological features, and SAI findings in three patients affected by NPC. Diagnosis of NPC was assessed by demonstration of cholesterol accumulation in cultured skin fibroblasts and/or by the molecular analysis of the *NPC1* gene. The main clinical features were cerebellar ataxia, vertical supranuclear gaze palsy and a variable degree of cognitive impairment ranging from only memory impairment to severe dementia. Electrophysiological evaluation revealed a reduced SAI in all three patients.

Our SAI findings provide evidence of cholinergic dysfunction in patients with adult form of NPC, supporting that cholinergic alterations may play a role in cognitive impairment in NPC and strengthening the similarities between NPC and AD.

## INTRODUCTION

Niemann-Pick disease type C (NPC) is a rare neurodegenerative disorder that develops from a failure of cholesterol trafficking within the endosomal-lysosomal pathway. It is inherited in an autosomal-recessive fashion and caused by mutation of either NPC1 (95%) or NPC2 gene. The clinical spectrum of the disease ranges from a neonatal rapidly fatal disorder to an adult onset chronic neurodegenerative disease.

In adult forms of NPC the main clinical features are cerebellar ataxia, vertical supranuclear gaze palsy, dysarthria, cognitive impairment, movement disorders, visceromegaly, psychiatric disorders and dysphagia (Sévin et al. 2007).

Cognitive dysfunction is highly variable from patients with mild cognitive impairment to severely demented patients in later stage of disease (Klarner et al. 2007; Stampfer et al. 2013a).

Interestingly NPC has been proposed as a model of "juvenile Alzheimer's disease" (Borbon et al. 2012). In fact, intriguing similarities exist in the cellular pathology of NPC and Alzheimer's disease (AD), including neurofibrillary tangle formation, prominent lysosome system dysfunction, accumulation and aggregation of amyloid beta protein and influences of apolipoprotein E ε4 genotype (Horoupian and Yang 1978; Love et al. 1995a; Yamazaki et al. 2001a; Saito et al. 2002; Nixon 2004a). In addition, basal forebrain cholinergic system alterations have been recently demonstrated in human and in experimental model of NPC1-deficient mice (Cabeza et al. 2012a; Chiba et al. 2014) raising the issue that cholinergic dysfunction may contribute to pathophysiology of cognitive impairment in NPC and expanding the similarities with AD.

In vivo evaluation of cholinergic central circuits can be assessed by means of a neurophysiologic technique, named short-latency afferent inhibition (SAI) (Tokimura et al. 2000), that can be used as a non-invasive additional tool for discriminating between cholinergic and non-cholinergic forms of dementia (Di Lazzaro et al. 2006).

Such assumptions prompted us to evaluate the cholinergic functional involvement in NPC disease, by applying SAI technique, on three patients with adult form of NPC1 and cognitive decline.

## PATIENTS AND METHODS

We describe three patients (P1, P2 and P3), belonging to two families, affected by NPC1. All patients underwent clinical, biochemical, molecular, neuropsychological and neurophysiological evaluation. Written consent to participate in the study was obtained from all subjects. The protocol was approved by the local ethics committee, and the research was conducted in accordance with the 1964 Declaration of Helsinki.

### *Biochemical and molecular studies*

The diagnosis of NPC disease was confirmed by demonstration of cholesterol accumulation in cultured skin fibroblasts through filipin staining (Blanchette-Mackie et al. 1988) and/or by the molecular analysis

of the *NPC1* gene (Fancello et al. 2009). In all cases the genotype was confirmed by the analysis of the identified mutations in the patient's parents.

*Neuropsychological assessment*

The neuropsychological evaluation tapped selected cognitive abilities by means of Italian standardized tests. Mini-Mental State Examination (MMSE) was used to assess general cognitive abilities, and Frontal Assessment Battery (FAB) to screen frontal functions. In addition, we used the following specific neuropsychological tests to evaluate selected cognitive domains: (1) Corsi's block-tapping test and verbal span for words to assess short-term memory, (2) Rey's immediate and delayed recall of 15 words and of a short passage to evaluate long-term memory and learning, (3) Raven's 47 Coloured Progressive Matrices (RCPM), to evaluate nonverbal intelligence, (4) phonological fluency tasks to assess cognitive flexibility and (5) a copying test for geometrical figures to assess spatial organization and visuoconstructional skills (Dubbioso et al. 2012).

*Neurophysiological assessment*

Short-latency afferent inhibition was studied using the technique described by Tokimura (Tokimura et al. 2000). Conditioning stimuli were single pulses of electrical stimulation applied to the median nerve at the wrist. The intensity of the conditioning stimulus was set at just over motor threshold for evoking a visible twitch of the thenar muscles. The intensity of the test cortical magnetic shock was adjusted to evoke a muscle response in the relaxed first dorsal interosseus muscle with an amplitude of ~1 mV peak-to-peak. The conditioning stimulus to the peripheral nerve preceded the magnetic test stimulus. Interstimulus intervals (ISIs) were determined relative to the latency of the N20 component of the somatosensory evoked potential evoked by stimulation of the median nerve. ISIs from the latency of the N20 plus 2 ms to the latency of the N20 plus 8 ms were investigated in steps of 2 ms. Five stimuli were delivered at each ISI. The amplitude of the conditioned motor evoked potential (MEP) was expressed as the percentage of the amplitude of the test MEP. The percentage inhibition of the conditioned responses at the five different ISIs was averaged to obtain a grand mean. None of the patients were treated with anti-cholinergic and/or anti-depressant medications. SAI has been performed in patients P1 and P2 before starting miglustat treatment and on treatment with miglustat in patient P3. SAI was also performed in 11 healthy, control subjects (5 females; mean age $\pm$ SD: 27.8 $\pm$ 5.5 years). All control subjects showed the inhibition of MEPs at ISI from 2 to 8 ms after N20 latency (mean value and SD: 46.4 $\pm$ 11.8% of basal MEP amplitude; range: 21%-62%). Upper normal limit of SAI was considered to be the mean plus 2 SD of control values (70%).

**RESULTS**

*Clinical, neuropsychological, radiological and neurophysiological findings*

The first patient (P1) was a 23-year-old woman. Clinical history was unremarkable until adolescence when during high school she needed help because of mild difficulties in learning and attention. Since

she was 20 years old she started complaining of unsteady gait with frequent falls and occasional episodes of urinary incontinence.

When she was admitted to our hospital, neurological examination showed gait ataxia, left upper limb dystonia, dysarthria, mild dysphagia, dysmetria and supranuclear gaze palsy.

Neuropsychological examination showed decreased global efficiency, with a Mini-Mental State Examination (MMSE) score consistent with a mild dementia. There was a severe deficit in short and long term memory and executive functions (table 1). Brain magnetic resonance imaging (MRI) revealed moderate atrophy affecting the cerebellum without signal abnormalities. SAI was reduced (72% of basal MEP; normal value $\leq$70%).

The second patient (P2) a 26-year-old woman was the older sister of P1. Her psychomotor development was normal and her schooling was unremarkable apart from slight memory difficulties and poor concentration capacities. Neurological examination showed a wide based gait, slight dysarthria and impaired vertical eye movement.

Neuropsychological examination showed a normal MMSE score with a selective impairment of short and long term memory (table 1). Brain MRI revealed atrophy of the cerebellum with periventricular white matter alterations.

SAI was reduced (79% of basal MEP; normal value $\leq$70%).

The third patient (P3) was a 33-year-old woman. Her medical history was unremarkable until she was 18 years old when she started to experience clumsy gait, poor upper limb coordination, learning difficulties, memory disturbances and psychiatric troubles (visual hallucinations). She has progressively deteriorated over the years and subsequently she has become totally depending. Treatment with miglustat was started at the age of 28 years.

Neurological examination showed severe gait ataxia, dysarthria, dysphagia, dysmetria and supranuclear gaze palsy.

Neuropsychological evaluation showed a severe and diffuse cognitive impairment with disorientation in time and space, memory and executive dysfunctions, and visuoconstructive and ideomotor apraxia (table 1). Brain MRI revealed atrophy of the cerebral cortex and cerebellum with periventricular white matter alterations.

SAI was reduced (86% of basal MEP; normal value $\leq$70%).

*Laboratory findings*

Molecular analysis of *NPC1* gene enabled the identification of the following genotype in patients P1 and P2: [c.2974G>C (p.G992R); c.2130dupG (p.R711EfsX3)]. To our knowledge, the c.2130dupG (p.R711EfsX3) mutation has not been previously reported. Biochemical analysis was performed in cultured skin fibroblasts of index case (P1) and filipin staining showed a massive accumulation of unesterified cholesterol in perinuclear vesicles consistent with a “classical phenotype”. The patient P3 was a compound heterozygous for the already reported mutations: c.3493G>A (p.V1165M) and c.3019C>G (p.P1007A); the latter being usually associated with the “variant biochemical phenotype”.

Filipin staining evidenced a mild accumulation of unesterified cholesterol in perinuclear vesicles, consistent with a "variant phenotype".

Abdominal ultrasonography disclosed splenomegaly in all three patients associated with hepatomegaly in two of them (P1 and P3). This rate is higher than that observed by Sevin and colleagues (Sévin et al. 2007) that found hepatomegaly and splenomegaly in 53.8 and 92.3% of their patients, respectively. Moreover, we also found visceromegaly and psychiatric disturbance in patient P3 who had a variant phenotype that is generally less associated with such features (Sévin et al. 2007).

**DISCUSSION**

Data from this paper show, for the first time, a reduced SAI in three NPC1 patients, suggesting a dysfunction of central cholinergic system and providing interesting insights in understanding cognitive impairment in NPC.

Most patients with NPC develops cognitive impairment progressing to dementia (Stampfer et al. 2013b) and the commonest features in NPC encompass dysexecutive syndrome, attentive dysfunction and memory impairment (Klarner et al. 2007; Sévin et al. 2007). Nonetheless, the pathophysiology of the cognitive decline in NPC remains still poorly understood.

Such cognitive impairment associated with behavioral disturbances, as frequently reported in NPC patients, may remind the clinical picture of frontotemporal dementia (FTD), as well as the presence of dysexecutive syndrome associated with a vertical gaze palsy may address toward another type of dementia, such as progressive supranuclear palsy (PSP).

However, closer parallels exist in cognitive impairment and cellular pathology of NPC and Alzheimer's disease. Notably, in addition to the Alzheimer-like lesions (neurofibrillary tangle formation, accumulation and aggregation of amyloid beta protein) (Horoupian and Yang 1978; Love et al. 1995b; Yamazaki et al. 2001b; Saito et al. 2002; Nixon 2004b), cholinergic alterations in the basal forebrain have been recently reported in an autopsy case of NPC (Chiba et al. 2014) and in an experimental model of NPC1-deficient mice (Cabeza et al. 2012b). These evidences induce interesting speculations about the cholinergic system imbalance and cognitive impairment in patients with NPC.

In fact, the cerebral cortex receives dense cholinergic innervation originating from the basal forebrain, and the disconnection of cortical areas from their source of cholinergic innervation in the basal forebrain could be responsible for mental-state impairment (Everitt and Robbins 1997; Selden 1998). The degeneration of cholinergic neurons in the basal forebrain is a major neuropathological feature in AD (Muir et al. 1993) and it contributes significantly to the deterioration of cognitive function (Mufson et al. 2003). Furthermore, cholinergic dysfunction has attracted attention in relation to cognitive impairment in dementia with Lewy bodies (LBD) (Londos et al. 2002) and more recently in Parkinson's disease (Manganelli et al. 2009a; Celebi et al. 2012).

It is noteworthy that SAI technique (Di Lazzaro et al. 2006) gives the opportunity to test non-invasively cholinergic circuits in the human cerebral motor cortex (Chen et al. 2008) and SAI has been proposed as a suitable tool for differentiating cholinergic from non-cholinergic dementias (Di Lazzaro et al. 2006). Accordingly, SAI has been found abnormal in cholinergic dementias such as AD (Di Lazzaro et

al. 2006) and LBD (Marra et al. 2012a), while it has been found normal in non-cholinergic types of dementia such as FTD (Di Lazzaro et al. 2006) and PSP (Nardone et al. 2005).

Thus, our SAI findings support that cholinergic alterations may play a role in cognitive impairment also in NPC and strengthen the similarities between NPC and AD.

SAI was abnormal in all our NPC patients, even though the extent of SAI reduction appeared, at least partially, independent from the degree of cognitive impairment. In fact, whilst the patient P3 was severely demented and showed the lowest SAI reduction, the patient P2, despite having only memory impairment, showed a greater SAI impairment than patient P1 with mild dementia. Anyway, it can be drawn from these findings, consistently with data from literature in patients with mild cognitive impairment, that SAI technique may be useful in early identification of individuals in whom cholinergic degeneration is occurred (Nardone et al. 2008a).

Moreover, the SAI value of 72% in patient P1, given a reference value of less than 70%, is only apparently scarcely significant. Indeed, the significance of SAI reduction is typically based on comparison of means between two groups (for example AD versus healthy subjects) and if we consider the mean value of SAI in all our three patients we have a value of 79% that is clearly different from controls ($46.4 \pm 11.8$) but in keeping with that observed in Alzheimer disease. Accordingly, the finding of a value exceeding the upper normal limit on a single patient indicates *per se* a significant SAI reduction.

Finally, the patients P2 an P3 with the most significant SAI reduction had, unlike P1, periventricular white matter abnormalities at MRI. In this respect, since data from volumetric MRI study have suggested that disrupted myelination and axonal structure predate changes to the neuronal cell body, (Walterfang et al. 2010) it could be argued that the disruption of cholinergic subcortical pathways secondary to white matter pathology may have influenced SAI findings in both these patients.

**CONCLUSIONS**

Our findings provide evidence of cholinergic dysfunction in patients with adult form of NPC and cognitive decline. However, the role of the central cholinergic system in NPC needs to be further investigated in a larger cohort of patients in view of possible implications for treatment with cholinesterase inhibitors.

**Table 1** Neuropsychological data of NPC patients.

| | P1 | P2 | P3 | Normal cut-off score |
|---|---|---|---|---|
| **Age at examination, years** | 23 | 26 | 33 | |
| **Educational level, years** | 13 | 18 | 13 | |
| **Neuropsychological measures** | | | | |
| **Screening tests** | | | | |
| Mini-Mental State Examination | **22.59** | 27.07 | **10.75** | 23.8 |
| FAB | **9.74** | 14.75 | **2.3** | 13.68 |
| **Spatial and verbal working memories** | | | | |
| Corsi's test | **3.75** | **3.75** | **1** | 3.97 |
| Verbal span | **2.25** | **2** | **2** | 3.6 |
| **Long-term memory** | | | | |
| 15-word Immediate recall | **23.9** | **22.4** | **0** | 28.53 |
| 15-word Delayed recall | **3.5** | **5.4** | **0** | 6.77 |
| Story recall test | **0** | **6.75** | **0** | 7.01 |
| **Non-verbal intelligence** | | | | |
| RCPM | **15.2** | 28.6 | **3** | 21.03 |
| **Cognitive flexibility** | | | | |
| Phonological fluency | **17.2** | 17.6 | **0** | 17.35 |
| **Visuospatial skill** | | | | |
| Copying task | 11.5 | 10.5 | **5.5** | 7.75 |

P1= patient 1; P2= patient 2; P3= patient 3; NPC= Niemann-Pick type C; FAB= Frontal Assessment Battery; RCPM= Raven's 47 Coloured Progressive Matrices; values reported in bold denote a score below normal cut-off score.

**The role of SAI as a tool to evaluate in vivo central cholinergic system in PD patients with freezing of gait and executive dysfunctions.**

*From the article: "Short Latency afferent inhibition in patients with Parkinson's disease and Freezing of Gait" By Marina Picillo[*] Raffaele Dubbioso,[*] Rosa Iodice, Alessandro Iavarone, Chiara Pisciotta, Emanuele Spina, Lucio Santoro, Paolo Barone, Marianna Amboni, Fiore Manganelli. (J Neural Transm 2015).*

**Abstract**

Freezing of gait (FOG) is one of the most common gait disturbances in patients with Parkinson's disease (PD). Recently, a PET study has documented that PD patients with FOG display cholinergic deficits selectively driven by nucleus basalis of Meynert (nbM)-neocortical denervation and not by peduncolopontine nucleus (PPN)-thalamic degeneration. Short-latency afferent inhibition (SAI) is a neurophysiological technique that allows evaluating major cholinergic sources in the central nervous system in vivo.

We sought to determine whether central cholinergic circuits, evaluated by means of SAI testing, are impaired in patients with PD with FOG (FOG+) as compared to those without (FOG-).

SAI and neuropsychological data were collected in 14 FOG+ and 10 FOG-. SAI was also performed in 11 healthy control subjects. Demographic, clinical and cognitive data were compared by using non parametric tests. Parametric tests were used to compare electrophysiological results among groups.

FOG+ and FOG- had similar SAI without significant differences with controls (p=0.207). None of the PD patients had SAI values outside the normal range (>72%). FOG+ presented poorer executive and visuospatial performances as compared to FOG-.

Despite the presence of cognitive deficits, SAI failed to detect any significant decrease of cholinergic activity in FOG+. However, nbM-related cholinergic dysfunction cannot be ruled out. In fact, integrity or even increased activation of PPN-related cholinergic circuits may mask an eventual nbM dysfunction thus resulting in normal SAI findings. Indeed, selective PPN cholinergic neurons sparing maybe a distinctive features of FOG. Alternatively, or complementary, FOG pathophysiology is underpinned by non-cholinergic neurotransmitters dysfunction.

**Introduction**

Freezing of Gait (FOG) is a common and disabling symptom of Parkinson's disease (PD). It is characterized by the episodic feeling of feet "glued" to the floor preventing the generation of effective stepping despite the intention to walk (Giladi and Nieuwboer 2008). FOG is one of the most common gait disturbances in PD and is frequently associated to balance and speech dysfunction as to frontal cognitive impairment (Amboni et al. 2008; Naismith et al. 2010). Based on the dopaminergic responsiveness, it is possible to identify: a) FOG which appears in the off state, and disappears in the on state (treatment responsive FOG); b) FOG indistinctly present in off and on states (treatment non-responsive FOG); c) FOG present during on state and absent in the off state (drug-induced FOG), the rarest form (Nieuwboer and Giladi 2013a). Despite different models have been proposed, the mechanisms underlying FOG and gait disturbances in PD are not fully understood (Nutt et al. 2011). It has been hypothesized that various degrees of alteration in the interplay between the peduncolopontine nucleus (PPN) and both fronto-striatal and basal ganglia circuits may generate a wide range of gait disturbances in PD, including FOG (Nieuwboer and Giladi 2013b). Recently, it has become increasingly evident that a close interplay between gait and cognition exists (Tessitore et al. 2012) and that multiple neurotransmitter deficits may contribute to determine both gait and cognitive dysfunction in PD, with a major role exerted by cholinergic networks (Yarnall et al. 2011). Indeed, beyond its direct role in cognition, recent studies provide compelling evidence that cholinergic system may crucially contribute to gait dysfunction (Rochester et al. 2012). There are two major cholinergic projection systems in the brain: the subcortical system originating in the PPN in the brainstem and the cortical system ascending from the nucleus basalis of Meynert (nbM) in the substantia innominata of the basal forebrain. Overall, cholinergic activity in the brain can be estimated with short latency afferent inhibition (SAI), a technique that non-invasively assesses an inhibitory circuit in the sensory-motor cortex. In fact, SAI is considered a surrogate measure of cholinergic activity allowing in vivo evaluation of central cholinergic circuits under the effect of ascending projection from nbM (Di Lazzaro et al. 2002) and PPN (Oliviero et al. 2005). Accordingly, SAI has been increasingly used to identify those symptoms possibly underpinned by cholinergic dysfunction in a variety of neurodegenerative diseases. Indeed, in PD, SAI abnormalities have been linked with slower gait disturbances (Rochester et al. 2012), with dementia (Celebi et al. 2012) and Mild Cognitive Impairment (MCI) (Yarnall et al. 2013), further confirming the role of cholinergic dysfunction in the development of both gait and cognitive dysfunction in PD. Likewise SAI has been found to be reduced in PD patients with visual hallucinations (VH) or REM-sleep Behavior Disorders (RBD) (Manganelli et al. 2009b; Nardone et al. 2012b), suggesting that cholinergic dysfunction may be the major anatomo-functional basis for VH- and RBD-associated cognitive deficits as well.

To our knowledge, studies evaluating SAI in PD patients with FOG as compared to those without are lacking. Thus, based on these premises, we sought to determine whether central cholinergic circuits, evaluated by means of SAI testing, are impaired in PD patients with FOG, namely with treatment non-responsive FOG. We also evaluated the cognitive profile of all the patients enrolled.

## Patients and Methods

### Subjects

Patients with a diagnosis of PD according to the clinical diagnostic criteria of the United Kingdom Parkinson's Disease Society Brain Bank (Hughes et al. 1992) were consecutively screened at the Movement Disorders Centre, University Federico II, Naples, Italy. Consecutive series screening methodology was used only for screening purposes. Inclusion criteria were: (1) age of 40 years or older in order to exclude early onset parkinsonism; (2) a Hoehn & Yahr (H&Y) stage equal or less than 2.5 while in on state; (3) disease duration less than 10 years; (4) antiparkinsonian treatment at a stable and optimized daily dosage during the 4 weeks prior to study entry. Exclusion criteria were: (1) treatment with cholinesterase inhibitors, benzodiazepines, neuroleptics, anti-cholinergic or anti-depressant drugs; (2) major depression according to the Diagnostic and statistical manual of mental disorders fourth edition criteria; (3) dementia according to clinical diagnostic criteria proposed by the Movement Disorders Society (MDS) commissioned Task Force (Emre et al. 2007); (3) presence of VH or RBD; (4) clinically significant or unstable medical conditions including serious cardiovascular or cerebrovascular disease or other conditions possibly affecting gait. The recruitment lasted for 2 years and involved all the clinics performed in our centre (4 clinics per week).

Among eligible subjects, we identified patients with treatment non-responsive FOG according to the following 3 steps assessment: 1) identification of off and on states based on brief semi-structured interview aimed at detecting the periodic reappearance of motor and non-motor symptoms, and their response to medication intake; 2) identification of FOG based on the following two conditions that had all to be fulfilled: (a) score >0 to item 3 of the FOG questionnaire (FOG-Q) (Giladi et al. 2000), (b) patients' recognition of the condition when it was demonstrated to them by an experienced clinician mimicking the phenomenon; 3) detection of FOG subtypes based on the following question *"When do you experience FOG?"* (a) *only during the best moments of the day (on state);* (b) *only during the worst moments of the day (off state);* (c) *in both (on and off state)"*. Treatment non-responsive FOG was defined as FOG present during both on and off state (point 3, response c). Absence of FOG was defined when neither condition of the point 2 was fulfilled. Patients presenting FOG either only during on state or off state were not included in this study.

### Clinical evaluation

Clinical and motor functions were evaluated with the H&Y, parts I–IV of the Unified Parkinson's disease Rating Scale (UPDRS) and the FOG-Q. In order to compare the amount of the administered dopaminergic drugs, levodopa equivalent daily dose (LEDD) was computed for each patient.

### Electrophysiological evaluation

### Magnetic stimulation

Transcranial magnetic stimulation (TMS) of the motor cortex was performed with a high-power magnetic stimulator (MagPro X100, Medtronic, Denmark). A figure-of-eight coil (with external loop

diameters of 9 cm) was positioned at the scalp over the right or left (according to the more affected side) hand motor area to evoke motor responses [motor-evoked potentials (MEPs)] in the contralateral first dorsal interosseous (FDI) muscle. MEPs were recorded through surface electrodes with the active electrode on the motor point of the muscle and the reference electrode on the metacarpophalangeal joint of the index finger. MEPs were amplified and filtered (bandwidth 3 Hz to 3 kHz) using a Keypoint electromyograph (Medtronic).

**Short latency afferent inhibition (SAI)**

SAI was investigated by applying the technique described by Tokimura and colleagues (Tokimura et al. 2000). Conditioning stimuli were single electrical pulses (200 ms) applied through bipolar electrodes to the median nerve at the wrist (cathode proximal). The intensity of the conditioning peripheral stimulus was set at just over the motor threshold to evoke a visible twitch of the thenar muscles. The N20 wave of cortical somatosensory response was recorded with active electrode attached 2 cm behind C4/C3 (10–20 International System) and reference electrode 2 cm behind C3/C4. A total of 500 responses were averaged twice and superimposed to identify the latency of the N20 peak. The intensity of the test cortical magnetic shock was adjusted to evoke an MEP in relaxed FDI muscle with peak-to-peak amplitude of ~1 mV. SAI was tested at different interstimulus intervals (ISIs) determined on the basis of the N20 wave latency. ISIs ranged from 2 to 8 ms after N20 latency and were investigated in steps of 2 ms. For each ISI, we calculated the amplitude of basal MEP (average of five consecutive responses obtained after cortical stimulation alone) and the amplitude of conditioned MEP (average of five consecutive responses obtained after the conditioning peripheral electrical stimulus). The amplitude of conditioned MEP, expressed as a percentage of the basal MEP amplitude at each ISI, was used to evaluate the amount of SAI. All subjects utilized audiovisual feedback of EMG signal at high gain to maintain complete relaxation during experiments. However, patients with tremor score >1 were not included in this experiment. Electrophysiological tests were performed on the more affected side and on patients taking dopaminergic medication. This protocol was decided because it reduces the discomfort level and SAI modifications in patients with Parkinson's disease have been reported on the more affected side, both on and off medication (Di Lazzaro et al. 2004a). SAI was also performed in 11 healthy control subjects (five females; mean age ± standard deviation: 62.4 ± 6.2 years). Data of patients and controls, obtained at the ISIs 2, 4, 6 and 8, were analyzed and averaged to obtain a grand mean of SAI in order to reduce the data variation. Upper normal limit of SAI was considered to be the mean + 2 SD of control values (72%).

**Neuropsychological evaluation**

All subjects were screened with the Mini-Mental State Examination (MMSE) and underwent a comprehensive neuropsychological battery of tests to assess four cognitive domains: (i) executive functions, evaluated by means of phonological verbal fluency and Frontal Assessment Battery (FAB); (ii) memory, evaluated by means of Rey's auditory 15-word learning test (15-RAWLT), both immediate and delayed recall; (iii) visuospatial functions, evaluated by means of Ten-point Clock test (TPCT) and

constructional apraxia; and (iv) attention/working memory, evaluated by means of attentional matrices and the interference task of Stroop test. Furthermore, patients were classified as affected or not affected by Mild Cognitive Impairment (MCI+ or MCI-) according to the Level I of the MDS commissioned Task Force (i.e. cognitive deficit not causing a significant functional decline and impairment in at least 2 neuropsychological tests demonstrated by at least 1.5 Standard Deviation below the expected age and education corrected score) (Litvan et al. 2012). Motor and cognitive functions were each evaluated by two different raters. The neuropsychological battery was administered by a trained neuropsychologist blinded to the presence/absence of FOG. All enrolled subjects signed the informed consent form according to the Declaration of Helsinki and the study was approved by the local Ethics Committee.

**Statistical analysis**

Differences in the distribution of categorical variables among groups were assessed by the chi-square test. Demographic, clinical and cognitive variables of FOG+ and FOG- were compared using the Mann-Whitney test. Normal distribution of neurophysiological parameters was verified by means of Kolmogorov and Smirnov test. Grand mean ratio of SAI entered into one- way analysis of variance (ANOVA) with a main factor of Group (three levels: Controls, FOG+, FOG-) to examine differences among the three groups of this value. Moreover, we used a repeated-measures ANOVA with the factor ISI (4 levels: 2ms, 4ms, 6ms, 8ms) as within subject factors and Group (three levels: Controls, FOG+, FOG-) as between subject factor to evaluate the effect of groups on the change in SAI at different ISIs. Greenhouse-Geisser's correction was used when sphericity assumption was violated. Bonferroni's post hoc test was used for further analysis.

Computation was supported by the Statistical Package for the Social Sciences (SPSS 19.0) software. Significance threshold was set to $p < 0.05$.

**Results**

**Clinical evaluation**

Twenty-four PD patients were enrolled including 14 exhibiting treatment non-responsive FOG (FOG+) and 10 without FOG (FOG-), matched for age, disease duration and gender. The two groups did not differ in UPDRS-I, UPDRS-III, UPDRS-IV scores and antiparkinsonian treatment. The UPDRS-II score was significantly higher in FOG+ as compared to FOG- ($p=0.004$), due to the fact that the scale includes two gait-related items (item 14: freezing and item 15: walking) whose score is significantly higher in presence of FOG. Median H&Y differed significantly between the two groups ($p= 0.019$). As expected, the FOG-Q score was significantly higher in FOG+ ($p<0.001$) (Table 1).

**Electrophysiological evaluation**

**Short latency afferent inhibition**

Regarding grand mean ratio of SAI, all healthy subjects showed the inhibition of MEPs (mean ± SD: 47.09 ± 12.51 of basal MEP amplitude). FOG+ and FOG- had normal grand mean ratio of SAI (48.63 ± 12.78 and 56.42 ± 12.45) without significant differences with controls (p= one- way ANOVA,

F(2,34)=1.658, p=0.207) (figure 1A)None of the PD patients had grand mean ratio of SAI outside the normal range (>72%). In addition, the repeated-measures ANOVA only revealed an expected factor ISI [F(1,32)=72.879, p<0.001], without any statistical difference among groups [F(2,32)=2.403, p=0.107] and an ISI by Group interaction [F(2,32)=1.532, p=0.232] (figure 1B). Post-hoc comparison revealed that SAI at ISIs +6 ms and +8 ms was significantly different respect to ISIs +2ms and +4 ms (p<0.001 consistently) for all three groups (figure 1B). These data suggest the presence of the same modulation of the inhibition at different ISIs for the three groups (figure 1B).

**Neuropsychological evaluation**

There were significant differences between the two groups in executive and visuospatial functions. FAB and phonological verbal fluency z-scores were significantly lower in FOG+ (p= 0.006 and p= 0.026, respectively) as well as constructional apraxia and TPCT z-scores (p= 0. 001). Other tests z-scores were similar between FOG+ and FOG- (Table 2). Based on the results of the neuropsychological test battery, 11 out of 24 enrolled PD patients met the criteria for MCI (MCI+). The remaining 13 patients did not meet the criteria, and were therefore considered cognitively intact (MCI-). MCI+ were significantly more prevalent among FOG+ as compared to FOG- (71.4% vs 10%, p=0.005).

**Discussion**

To the best of our knowledge, this is the first study evaluating in vivo cholinergic circuits' functional integrity in PD patients with FOG by means of SAI technique. Our findings do not show significant SAI values differences between PD patients with and without FOG and control subjects.

SAI evaluates an inhibitory phenomenon in the motor cortex that is believed to depend mainly on both central cholinergic sources (i.e. nbM and PPN) (Di Lazzaro et al. 2002; Oliviero et al. 2005). The PPN, that is part of the mesencephalic locomotor region, establishes connections with cortex, basal ganglia, cerebellum and spinal cord, and plays a crucial role in the initiation and control of locomotion (Pahapill and Lozano 2000). PPN is a heterogeneous structure in terms of both topography and neurochemical composition of its neurons consisting of both non-cholinergic and cholinergic neurons (Benarroch 2013). Cholinergic neurons loss within the PPN has been associated with slowing of gait, postural instability and falls in PD patients (Karachi et al. 2010; Rochester et al. 2012). Conversely, in a recent PET study Bohnen et al. documented that PD patients with FOG display cholinergic deficits selectively driven by nbM-neocortical denervation and not by PPN-thalamic degeneration (Bohnen et al. 2014). Our neurophysiological findings show overall preserved cholinergic activity in PD patients with FOG. However, nbM-related cholinergic dysfunction cannot be ruled out (Bohnen et al. 2014). We hypothesize the presence of a possible compensatory balance in cholinergic circuits as a peculiar pathophysiological mechanism underlying FOG. In fact, we speculate that the PD-associated overinhibition of PPN non-cholinergic neurons (Tattersall et al. 2014) coupled with the sparing of PPN cholinergic neurons (Bohnen et al. 2014) may result in a normal or even increased PPN cholinergic output possibly masking nbM dysfunction and, thus, resulting in normal SAI findings (Snijders et al. 2011). This hypothesis would account also for the discrepancy between neurophysiological and

neuropsychological findings detected in FOG+, who, contradicting previous evidence (Yarnall et al. 2013), display normal SAI values despite the presence of cognitive impairment (10 out of 14 FOG+ meet MCI criteria with predominant executive and visuospatial dysfunction). An alternative or complementary explanation is the possibility that both FOG and the pattern of cognitive impairment detected in FOG+ are mostly underpinned by non-cholinergic neurotransmitters dysfunction. Indeed, SAI has been also proposed as a suitable tool for differentiating cholinergic from non-cholinergic cognitive impairment (Di Lazzaro et al. 2006). From a neurochemical based perspective, cholinergic abnormalities typically correlate with degree of memory impairment (Terry Jr and Buccafusco 2003) whereas executive functions are tightly coupled to the dopaminergic system (Floresco and Magyar 2006). Consistently, our findings showing predominant executive and visuospatial dysfunction may support a major role for non-cholinergic neurotransmitters in determining the cognitive performances of FOG patients. Differently from previous evidence (Rochester et al. 2012), we failed to detect significant differences is SAI between the whole PD population and controls. SAI results may be significantly affected by many variables associated to PD (i.e. VH, cognitive impairment, RBD) (Manganelli et al. 2009, Nardone et al. 2013, Yarnall et al. 2013). Thus when analyzing SAI in an heterogeneous group of PD patients, a wide range of responses can be detected (Rochester et al. 2012), reflecting the heterogeneity of the group and resulting in an overall significant difference as compared to healthy controls. We tried to exclude such variability in our study by excluding patients with VH or RBD. There are some limitations to this study that should be acknowledged. First, SAI technique measures sensorimotor integration which is a neurophysiological property modulated, beyond cholinergic transmission, by different neurotransmitters including dopamine (Sailer et al. 2003, Sailer et al. 2007) and GABA (Di Lazzaro et al. 2005) which could have influenced our results. Indeed, SAI is normalized by dopaminergic treatment, in patients affected by RLS (Rizzo et al. 2010) and in AD patients (Nardone et al. 2014) while it is decreased by dopaminergic drugs on the more affected side in patients with Parkinson's disease (Sailer et al. 2003). Accordingly, since we have found normal SAI in PD patients ON medication, it is likely that our results are not related to dopaminergic treatment. Second, SAI is also modulated by movement itself. Unfortunately, we performed our experiment only in a rest condition, but it would have been interesting to evaluate if during finger movements we were able to capture some differences in the amount of sensorimotor inhibition between FOG+ and FOG-. Third, the number of trials for each condition was low (5) and they were not delivered randomly as commonly performed in TMS studies. We are aware that it could have influenced our results, however, in a previous report, using the same technical procedure; we were able to detect a reduced SAI in PD patients with visual hallucinations, believed to reflect degeneration of cholinergic neurons in the pedunculopontine nucleus (Manganelli et al. 2009). Fourth, biased recall may have influenced self-report of FOG in our patients. As a matter of fact, clinical assessment of FOG is challenging because the behavior is episodic and largely unpredictable and patients who frequently experience FOG during their daily life may suppress it during testing. It is therefore possible that we have tested patients with mild FOG (only referred and objectified) and failed to see a difference in SAI for this reason. To confirm our findings, SAI should be repeated in patients with observed FOG, ideally evaluated with objective

assessments (i.e. gait analysis). In conclusion, our study shows that PD patients with treatment non-responsive FOG present normal SAI values, suggesting overall preserved cholinergic activity. Ultimately, nbM-related cholinergic dysfunction cannot be ruled out since integrity or even increased activation of PPN-related cholinergic circuits may mask an eventual nbM dysfunction thus resulting in normal SAI findings. Finally, our data further raise questions about the role of cholinergic and non-cholinergic circuits in the pathophysiology of FOG and gait disturbances in PD (Devos et al. 2010). Additional investigations are warranted to better characterize the neurochemical profile of PD patients with FOG.

**Table 1. Demographic and clinical features of PD patients with (FOG+) and without Freezing of Gait (FOG-).**

| | **FOG+ (=14)** | **FOG-(=10)** | **p** |
|---|---|---|---|
| **Age, years** | 63 (12) | 65 (10) | 0.8 |
| **Gender, M/F (%)** | 9/5 (64,3/45,7) | 7/3 (70/30) | 0.9 |
| **Disease duration, years** | 6.5 (4) | 5 (2) | 0.6 |
| **UPDRS-I** | 2 (2) | 1 (2) | 0.1 |
| **UPDRS-II** | 10 (5) | 6 (5) | **0.004** |
| **UPDRS-III** | 17 (12) | 13.5 (8) | 0.074 |
| **UPDRS-IV** | 3 (2) | 2 (2) | 0.074 |
| **H&Y** | 2 (1) | 1.5 (1) | **0.019** |
| **FOG-Q** | 10 (4) | 2 (2) | **<0.001** |
| **LEDD** | 1022.5 (771.2) | 560 (255) | 0.096 |

Data are listed in median (Interquartile Range, IQR), unless otherwise specified. Significant differences between groups are in bold. Abbreviations: F:female; FOG-Q: Freezing of Gait Questionnaire; H&Y: Hoehn and Yahr scale; LEDD: levodopa equivalent daily dose; M:male; UPDRS: Unified Parkinson's disease rating scale.

**Table 2. Neuropsychological features of PD patients with (FOG+) and without Freezing of Gait (FOG-).**

| | **FOG+ (=14)** | **FOG- (=10)** | **p** |
|---|---|---|---|
| *Global cognition* | | | |
| MMSE (raw scores) | 27.1 (5) | 27.6 (2) | 0.2 |
| *Executive domain* | | | |
| Phonological verbal fluency | -0.1 (1.4) | 0.8 (4.5) | **0.026** |
| FAB | -2 (1.2) | -0.3 (5) | **0.006** |
| *Memory domain* | | | |
| 15-RAWLT, immediate recall | -0.1 (1.8) | 0.1 (1.7) | 0.5 |
| 15-RAWLT, delayed recall | 0.3 (1.4) | -0.2 (1.4) | 0.9 |
| *Visuospatial domain* | | | |
| TPCT | -1.8 (2.3) | 0.3 (1.4) | **0.001** |
| Constructional apraxia | -1.1 (2.6) | -0.1 (0.9) | **0.001** |
| *Attention/working memory domain* | | | |
| Attentional matrices | 0.08 (1.1) | -0.04 (0.7) | 0.8 |
| Stroop test-interference task | -0.7 (0.5) | -0.2 (0.9) | 0.1 |

Cognitive performances are displayed as z-scores, unless otherwise specified. Values are listed as median (Interquartile range, IQR), unless otherwise specified. Significant differences between groups are in bold. Abbreviations: FAB: Frontal Assessment Battery; MMSE: Mini-Mental State Examination; TPCT: Ten-point Clock test; 15-RAWLT: Rey's auditory 15-word learning test.

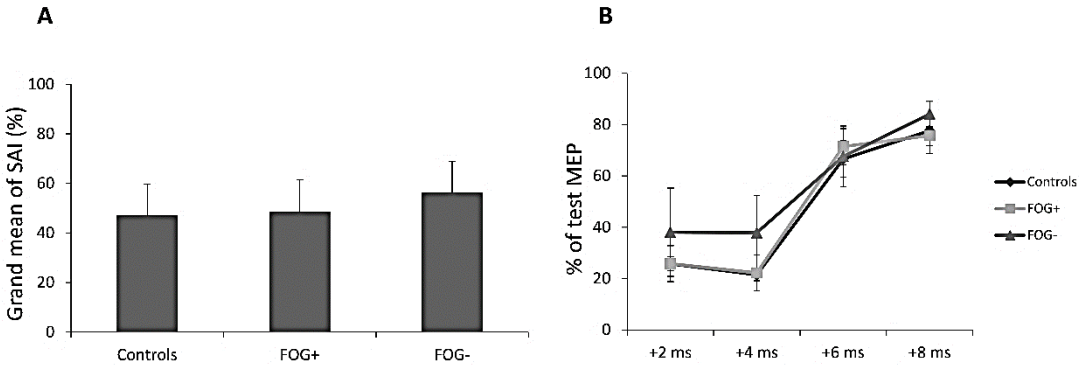
**Figure legend**

Figure 1

**Short latency afferent inhibition (SAI) in control subjects, patients with (FOG+) and without freezing of gait (FOG-)**

A. Grand mean of SAI (% of test MEP) in Parkinson's disease patients with freezing of gait (FOG+), without freezing of gait (FOG-) and control subjects. SAI was not significantly different among three groups (p= 0.207). Each column represents mean value. Error bars indicate standard deviations.

B. SAI at single ISIs in FOG+, FOG- and controls. The horizontal axis shows ISI values (the time between the peripheral stimulation and cortical stimulation). ISIs were determined by adding 2, 4, 6, 8 ms to the latency of the N20 component. The vertical axis shows the percentage of test MEP at each ISI. Repeated-measures ANOVA showed that SAI courses were similar for all three groups (p= 0.107). For all groups, SAI responses changed significantly for ISIs +6 ms and +8 ms (p< 0.001). Error bars indicate standard deviations.



## CONCLUSION

Overall these experiments shed light on the pathophysiological characteristics of SAI.

In the first study we have investigated its cortical somatotopic feature, suggesting the existence of a centre surround organization in the motor hand area. A question that remains unclear is if SAI phenomenon is due to a cortical sensory-motor inhibition or to a pure motor inhibition of afferent sensory signal.

In the second study we have tried to understand the meaning of the SAI curve shifting from inhibition to facilitation for ISIs longer than 5 mms. Our data strongly support the potential role of cerebellum in this gradual shifting and explaining the difference of mechanism underlying PAS protocol at two different ISIs (21.5 and 25 ms). In the second part of the thesis, the third and the fourth study corroborated the role of SAI in evaluation central cholinergic system integrity. Even if there are conflicting results in literature, may be due to heterogeneity of patients recruited in the studies (i.e. different cognitive impairments, treatments, different neurodegenerative disorders), our results provided further evidence for SAI as a useful tool to discriminate cholinergic from non cholinergic forms of dementia and that MCI patients with executive dysfunction are mainly characterized by preserved in vivo central cholinergic circuitry.

Since for MCI there is still no official treatment approved from Food and Drug Admnistration (FAD) (Cooper et al. 2013), SAI might be considered as a powerful tool to identify those MCI patients that can benefit of cholinomimetic treatment.

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