RELATIONSHIP BETWEEN CAPACITY FOR MOTOR CORTICAL PLASTICITY AND LEARNING A COMPLEX PERCEPTUAL-MOTOR SKILL

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Autumn 2019
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ABSTRACT

Merikoski, V. 2019. Relationship between capacity for motor cortical plasticity and learning a complex perceptual-motor skill. Department of Biology of Physical Activity, University of Jyväskylä, Master's Thesis in Biomechanics, 87 pp., 3 Appendices.

Motor skill training and paired associative stimulation (PAS) are known to induce long-term potentiation -like plasticity in human motor cortex. Magnitude of motor skill training induced plasticity is related to skill learning results. However studies have had difficulties in finding associations between neurostimulation induced plasticity and motor skill training effects. The purpose of this study was to examine associations between PAS and motor training induced neuroplasticity and learning results of a complex perceptual-motor skill. Volunteers were recruited for a three week long study consisting of a PAS measurement session on the first week, 5-day juggling skill training intervention on the second week and a retention session on the third week. Data was analysed from 13 volunteers (men=4, women=9). PAS consisted of 200 stimulus pairs (ISI=20 ms) targeting right flexor carpi radialis muscle (FCR): first a stimulus was given to right median nerve (1.5 x MT) after which a TMS stimulus was given to FCR muscle area on contralateral primary motor cortex (120 %RMT). Juggling skill was measured as successful catches per attempt (CPA) PRE and POST each session and on retention and transfer skill tests. Reaction time was tested with simple visual reaction time test on first, fifth and retention training sessions. Neurophysiological measurements were conducted during PAS, fist motor training, fifth motor training and retention motor training sessions. Peak-to-peak MEP amplitudes were measured from FCR muscle area in left motor cortex PRE, after (POST) and 20 minutes after ($POST_{20}$) PAS and motor training with stimulus intensities 100, 110, 120, 130 and 140 % RMT. Average MEP amplitudes were calculated as mean from all intensities. Maximal M-waves were measured PRE and POST sessions. Capacity for corticospinal plasticity was measured as acute percentage change of peak-to-peak motor evoked potential (MEP) amplitude induced by PAS and first motor training session. Statistical analyses were conducted with related samples Wilcoxon signed rank test, Mann-Whitney U test and Spearman's rank-order correlation. All participants improved their juggling skill though fours participants did not reach skill acquirement criteria of CPA$\geq$4 during the five-day intervention. The gain of skill was well retained and the skill transferred to a transfer task. Visual reaction time did not improve as a group but greater improvement correlated with slower initial reaction time and slower juggling skill learning. On average peak-to-peak MEP amplitudes increased right after PAS by 18% (SD=36, n=13, p=0.28) and 20 minutes after by 16% (SD=31, n=12, p=0.07) though effects did not reach statistical significance. First, fifth and retention training sessions induced an acute suppression of MEPs that weakened in 20 minutes after the end of training. Change of MEP amplitudes PRE to $POST_{20}$ first motor training session correlated negatively with the reaction time on training day 5 (n=12, r_s=-0.62, p=0.03). Baseline MEP amplitudes did not change as a group. However an increase of baseline MEP amplitude correlated with negatively with reaction time change from training day 1 to day 5 (n=8, r_s=-0.81, p=0.01). Five participants that experienced elevated MEP sizes after 20 minutes from first training session also improved their reaction times and had fastest reaction times on day 5. MEP changes did not correlate with juggling skill development at any point during the study.

The differences in learning efficacy were not related to training induced acute or long-term changes of corticospinal excitability. This study did not find any relationship between capacity for corticospinal neuroplasticity and development of a motor skill. In most participants juggling training induced an acute suppression of MEPs similar to post-exercise depression effect that has been typically observed after a session of repetitive motor exercise with no motor learning. Results indicated though, that the first juggling training session might have induced LTP-like motor cortical plasticity in some participants who also improved their visual reaction time. Neuroplasticity may have focused on other brain areas that were not measured in this study, like areas focusing on visual processing and visuomotor planning.

Key words: juggling, motor skill learning, neuroplasticity, paired associative stimulation, reaction time

TIIVISTELMÄ

Merikoski, V. 2019. Relationship between capacity for motor cortical plasticity and learning a complex perceptual-motor skill. Liikuntatieteellinen tiedekunta, Jyväskylän yliopisto, biomekaniikan pro gradu -tutkielma, 87 s., 3 liitettä.

Taitoharjoitus ja parillinen assosiatiivinen stimulaatio (PAS) aiheuttavat long-term potentiation -kaltaista synaptista plastisuutta (LTP) primaarisella motorisella aivokuorella. Tämän tutkimuksen tarkoituksena oli tutkia PAS intervention ja motorisen taitoharjoittelun tuottaman plastisuuden yhteyttä taidon kehittymiseen. Hypoteesina oli, että suurempi PAS:n tai taitoharjoituksen aiheuttama kortikospinaalisen herkkyyden muutos olisi yhteydessä taidon oppimiseen. Tutkimukseen rekrytoitiin terveitä nuoria aikuisia, joilla ei ollut kokemusta jongleerausharjoittelusta (13 henkilöä: 4 miestä, 9 naista). Tutkimuskäynnit jakautuivat kolmelle viikolle siten, että ensimmäisellä viikolla toteutettiin PAS tutkimus, toisella viiden kerran mittainen jongleerausharjoitusjakso ja kolmannella retentioharjoitus. PAS interventioon kuului 200 stimulusparia: sähköstimulus (1,5 x MT) annettiin oikeanpuoleiseen keskihermoon kyynärtaipeen kohdalta ja 20 ms sen jälkeen TMS stimulus (120 %RMT) flexor carpi radialis (FCR) lihaksen alueelle vasemman puoleiselle motoriselle aivokuorelle. Jongleeraustaitoa mitattiin kunkin harjoituksen alussa ja lopussa onnistuneina kiinniottoina per jongleerausyritys (CPA). Yksinkertainen visuaalinen reaktioaikatesti toteutettiin ensimmäisen, viidennen ja retentiomittauskerran alussa. PAS tutkimuskerralla sekä ensimmäisellä, viidennellä ja retentiomittauskerralla tehtiin lisäksi neurofysiologisia mittauksia, jotka kohdistettiin FCR lihakseen. Motorinen herätevaste (MEP) mittaus tehtiin transkraniaalisella magneettistimulaatiolla (TMS) aina ennen (PRE) PAS interventiota tai motorista harjoitusta, sen jälkeen (POST) ja 20 minuuttia sen jälkeen ($POST_{20}$). TMS:llä mitattiin keskimääräinen huipusta huippuun MEP amplitudi 100, 110, 120, 130 and 140 % RMT stimulointivoimakkuudella. Motorisen aivokuoren plastisuuden kapasiteetin mittareina käytettiin PAS intervention ja ensimmäisen harjoituksen aiheuttamaa MEP amplitudin muutosta. Maksimaaliset M-aallot mitattiin aina ennen (PRE) ja jälkeen (POST) intervention. Tilastollisissa analyyseissä käytettiin Wilcoxon merkittyjen sijalukujen testiä, Mann-Whitney U –testiä ja Spearmanin järjestyskorrelaatiota. Kaikki 13 tutkittavaa kehittyivät jongleerauksessa. Heistä yhdeksän saavutti viiden harjoituksen aikana CPA $\geq$ 4 taitotason, matalimman suoritustason, jota voidaan kutsua jongleeraukseksi. Saavutettu taitotaso säilyi kuuden päivän tauon aikana ja taitotaso siirtyi myös siirtovaikutustestiin, jossa jongleerattiin samaa cascadi-kuviota, mutta eripainoisilla palloilla. Visuaalinen reaktioaika ei parantunut tilastollisesti merkitsevästi ryhmänä, mutta yksilötasolla kehitystä tapahtui osalla. Reaktioajan nopeutuminen oli yhteydessä hitaampaan reaktioaikaan alkutesteissä sekä hitaampaan jongleerauksen oppimiseen. MEP amplitudit kasvoivat PAS interventiossa 18% (SD=36, n=13, p=0.28) POST ja 16% (SD=31, n=12, p=0.07) $POST_{20}$, mutta tulokset eivät olleet tilastollisesti merkitseviä. MEP amplitudit olivat kunkin jongleerausharjoituksen jälkeen pienemmät kuin ennen harjoitusta, mutta palautuivat lähelle lähtötilannetta seuraavan 20 minuutin aikana. MEP amplitudin muutos ensimmäisenä harjoituskertana korreloi negatiivisella kertoimella viidennen harjoituskerran reaktioajan kanssa (n=12, rs=-0.62, p=0.03). Reaktioajan muutos ensimmäisen ja viidennen harjoituskerran välillä korreloi saman aikavälin PRE MEP/M_{max} amplitudimuutoksen kanssa (n=12, r_s=-0.81, p=0.01). Viidellä henkilöllä MEP amplitudi kasvoi poikkeuksellisesti ensimmäisen harjoituksen jälkeen. Lisäksi heillä reaktioajat nopeutuivat harjoitusviikolla ja he myös omasivat nopeimmat reaktioajat viidennellä harjoituspäivänä.

Tutkimuksessa ei havaittu yhteyttä PAS:n tai harjoittelun aiheuttaman aivojen plastisuuden ja motorisen taidon oppimisen välillä. Reaktioajan kehittyminen sen sijaan oli yhteydessä lyhyen ja pitkän aikavälin kortikospinaalisen herkkyyden muutoksiin. Kukin harjoitus aiheutti akuutin kortikospinaalisen herkkyyden pienenemisen, jolla oli yhtäläisyyksiä post-exercise depression -vaikutuksen kanssa. Ensimmäinen jongleerausharjoitus saattoi kuitenkin tuottaa LTP -kaltaista motorisen aivokuoren plastisuutta eräillä tutkittavilla, joiden visuaalinen reaktioaika parani harjoittelun seurauksena. Jongleerausharjoittelu saattoi aiheuttaa neuroplastisuutta aivoalueilla, mitä ei tässä tutkimuksessa mitattu.

Asiasanat: plastisuus, jongleeraus, motorinen oppiminen, parillinen assosiatiivinen stimulaatio

ABBREVIATIONS

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BDNF	brain-derived neurotrophic factor
CONSΔ%MT	relative skill consolidation
CPA	catches per attempt
DEX	activity level of participating to activities employing manual dexterity
FCR	flexor carpi radialis
GABA	gamma-Aminobutyric acid
LICI	long-interval intracortical inhibition
LTD	long-term depression
LTP	long-term potentiation
MAPK	mitogen-activated protein kinase
MEP	motor evoked potential
MT	motor training
NMDA	N-methyl-D-aspartate
PAS	paired associative stimulation
PED	postexercise depression
RET	retention
RMT	resting motor threshold
RT	Reaction time
rTMS	repetitive transcranial magnetic stimulation
TBS	theta burst stimulation
SICI	short-interval intracortical inhibition
SPORTS	Activity level of sports
SPORTS&DEX	Combined activity level of sports and activities employing manual dexterity
TMS	transcranial magnetic stimulation

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1 INTRODUCTION

Motor skill learning induces functional and structural changes in the nervous system: neuroplasticity (Dayan et al 2011). Long-term potentiation (LTP) and long-term depression (LTD) are types of synaptic plasticity in which existing synapses between neurons are either strengthened or weakened (Paulsen & Sejnowski 2000). LTP-like synaptic plasticity in motor cortical areas is an important mechanism behind early motor skill learning. Multiple motor skill training sessions may induce structural plasticity. (Rosenkranz 2007a.) Transcranial magnetic stimulation (TMS) is a measurement method that has been used for detecting motor cortical plasticity (Vallence & Ridding 2014). Juggling is a complex bimanual multi-joint perceptual motor skill. TMS has not been before used for measuring motor cortical effects of learning such complex motor skills.

Non-invasive neurostimulation methods are used to induce and measure neuroplasticity in humans (Vallence & Ridding 2014). Paired associative stimulation (PAS) is used for inducing neuroplasticity in the cortical areas of the brain and is widely used as a method for brain research. (Stefan et al 2000.) Main effects of PAS are LTP- and LTD-like synaptic plasticity (Stefan et al 2002). PAS induced plasticity is also similar to motor skill training session induced plasticity (Rosenkranz et al 2007a; Ziemann et al 2004).

This work focuses on neuroplasticity induced by motor learning and PAS and on their relationship with motor learning results. Earlier research implicates that the magnitude of motor training induced plasticity is related with motor learning results (Hirano et al 2018; Jensen et al 2005; Smyth et al 2010). There is also evidence that skill trained persons have greater capacity for neurostimulation-induced plasticity (Kumpulainen et al 2014; Rosenkranz et al 2007b) and that history of prior motor training enhances motor learning (Pereira et al 2013). These findings would suggest that greater capacity for neurostimulation-induced plasticity would be related to enhanced motor skill learning. However the few studies focusing on the matter have had difficulties in finding such relationship (López-Alonso et al 2015; Vallence et al 2013) and clearly the topic deserves further research.

2 MOTOR CONTROL OF VOLUNTARY MOVEMENTS

The nervous system directs functions of an organism and generates different types of behaviour (Nienstedt et al 2009, pp. 516–518). Motor systems generate voluntary, reflexive and rhythmic movements (Kandel et al 2000, pp. 654). Voluntary movements are induced to accomplish a goal and they require co-operation of all parts of motor system and sensory systems (Kandel et al 2000, pp. 347). Juggling is a complex perceptual motor skill. Control of juggling is based on processing and integrating visual, haptic and proprioceptive information with coordinated movements. (Sánchez García et al 2013.) This chapter focuses on describing the organization and the function of the motor and sensory systems involved in the motor control of voluntary movements, with special interest in sensorimotor function involving somatosensory and visual information.

2.1 Organization of motor and sensory systems

Nervous system is specialized in relaying information swiftly and precisely through neural networks that consist of neurons and their synaptic connections (Nienstedt et al 2009, pp. 72, 516–518). Typical description of a neuron contains dendrites, a soma, an axon and axon terminals (Enoka 2008, pp. 182–183; Kandel et al 2000, pp. 86). A neuron conveys information by transmitting an action potential through it´s axon into synapses triggering neurotransmission. An action potential is a wave of depolarization and subsequent repolarization and hyperpolarization. Action potentials are generated at the axon hillock if post-synaptic potentials depolarize the neuron over an action potential threshold. Wave of depolarization is caused by a flow of positive ions (Na^+ and Ca^{2+}) into the cell through voltage-gated ion channels. Generated wave of depolarization travels through axon membrane until reaching the axon terminal. The depolarization wave is followed by a wave of repolarization, which is caused by an outflow of K^+ ions. (Enoka 2008, pp. 186–187; Kandel et al 2000, pp. 169.) Neurons are connected to one another and to target organs through synapses. An action potential triggers synaptic transmission from pre- to postsynaptic neuron. Synaptic transmission depolarises or hyperpolarises the postsynaptic neuron depending on whether the synapse is inhibitory or excitatory type. The integration of synaptic potentials

dictates whether an action potential is generated in a neuron. (Enoka 2008, pp. 192–193; Kandel et al 2000, pp. 207-2012)

Motor systems. The motor systems are arranged hierarchically to three levels: cortical motor areas, brains stem and spinal cord. Each level has neural circuits for purposes of processing sensory information and modulating and producing movement commands. Higher motor systems also modulate the function of lower motor systems (Kandel et al 2000, pp. 663–671). Cortical motor systems specialize in voluntary movements and include premotor areas and primary motor cortex. Cortical motor systems project to motor neurons of spinal cord and brain stem (Kandel et al 2000, pp. 663). Corticomotoneuronal system comprises of descending axons of corticospinal tract that originate mostly from primary motor cortex and project monosynapticallly to spinal alpha-motor neurons (Squire 2009, pp. 197–198). Brain stem motor areas include medial descending systems that are involved in postural control and lateral descending systems that have supporting role in the control of distal limb movements. (Kandel et al 2000, pp. 663.) For example reticulospinal system originating from brain stem is involved in cooridation of locomotion and feed forward motor control of skilled voluntary movements (Squire 2009, pp. 154–157). Motor systems of spinal cord consist of neural networks that produce reflexes and rhythmical movement patterns (Kandel et al 2000, pp. 663; Squire 2009 pp. 73–79). Cerebellum and basal ganglia also have important roles in the control of movement and they affect the function of other motor systems (Kandel et al 2000, pp. 347, 663; Nienstedt et al 2009, pp. 558). Motor systems are also affected by sensory systems and brain´s non-motor modulatory systems (Kandel et al 2000, pp. 656–657, 333–334).

Organization of cortical motor areas. Primary motor cortex (M1) contains motor maps for muscles and is organized somatotopically. Any individual muscle has controlling sites in the motor cortex that are distributed in wide area. Different muscle areas overlap and form maps for movements. A motor map may activate multiple muscles to move a body part towards a direction. Therefore stimulus to one point may activate several muscles and one muscle can be activated from a wide area. (Kandel et al 2000, pp. 758–759; Latash 2012, pp. 193.) Neural coding of the M1 is complex and includes different movement variables, like movement direction, joint movements and load. Coding of neurons is also task dependent and flexible.

(Squire 2009 pp. 105–111.) Premotor areas are involved in programming the planned voluntary movement. Neurons of premotor areas are coded to represent goals of movements, for example spatial goals. (Squire 2009 pp. 111.) Premotor areas activate before the initiation of movement and project to primary motor cortex and spinal neurons. Different parts of premotor areas have different roles in movement planning. Supplementary motor areas have a role in planning for movement sequences and in learning movement sequences. Lateral premotor areas plan the movement according to sensory information and have a role in associative motor learning. (Kandel et al 2000, pp. 770–777.)

Organization of sensory areas. Sensory information is generated in sensory organs and conveyed into different processing areas through neural pathways (Kandel et al 2000 pp. 338). Some processing of peripheral sensory information already occurs at spinal cord. (Kandel et al 2000, pp. 663; Nienstedt et al 2009, pp. 546–547). Thalamus is the first processing area for sensory information in the brain and it relays information into different parts of nervous system (Kandel et al 2000, pp. 341–344; Nienstedt et al 2009, pp. 478). Highest level of sensory processing occurs in cerebral cortex. Primary sensory areas receive information from neural pathways originating from sensory organs and begin the cortical processing of sensory information. (Kandel et al 2000, 344–345; Nienstedt et al 2009, pp. 479–480). Association areas of the cerebral cortex integrate information from different sources and generate the understanding of the state of oneself and surroundings. (Nienstedt et al 2009, pp. 560). Unimodal association areas integrate of sensory information from one sensory system and are located next to the primary sensory area (Kandel et al 2000, pp. 350–351; Nienstedt et al 2009, pp. 479–480). Multimodal association areas integrate information from multiple brain areas. (Kandel et al 2000, pp. 350–351.)

2.2 **Voluntary movement**

Planning and execution of voluntary movement. Voluntary movements are prepared in the association areas of the cerebral cortex. Posterior association areas integrate sensory information and project to the anterior association areas. Anterior association areas are responsible for outlining behaviour and project to motor association areas (Kandel et al 2000,

pp. 350, 355–356). Premotor areas in the motor association cortex prepare motor commands and motor programs. (Kandel et al 2000, pp. 760–761.) Primary motor cortex is the final processing stage of voluntary movement. Motor commands are generated in the pyramidal neurons of fifth layer of motor cortex from where the signal descends via corticospinal axons to spinal motor neurons and muscles through the corticospinal tract. (Kandel et al 2000, pp. 347–348; Komi 2011, pp. 1, 118.)

Sensorimotor integration. Sensory information is utilized in motor planning. Sensory processing occurs simultaneously at multiple different levels. Information travels sequentially from primary to unimodal to multimodal sensory association areas and from there to motor association areas. Simultaneously primary sensory areas project to motor areas and other brain areas. Multimodal motor association areas combine sensory information with motor planning and sent output to primary motor areas. (Kandel et al 2000, pp. 353–356.)

Feed forward and Feedback control. Sensory information is utilized as anticipatory information and feedback information for movement execution. In feed forward motor control sensory information is utilized in movement planning before the movement. Feed back control refers to the control of movement from moment-to-moment as the movement progresses. The processing of sensory information has a phase lag, which means that the feedback control can be used only in slow movements. (Kandel et al 2000, pp. 656–657; Latash 2012, pp. 114–117) Optimal feedback model comprises of controllers that utilize sensory information as well as information about motor output and movement goals and computes movement trajectories continuously (Figure 1) (Squire 2009, pp. 114).

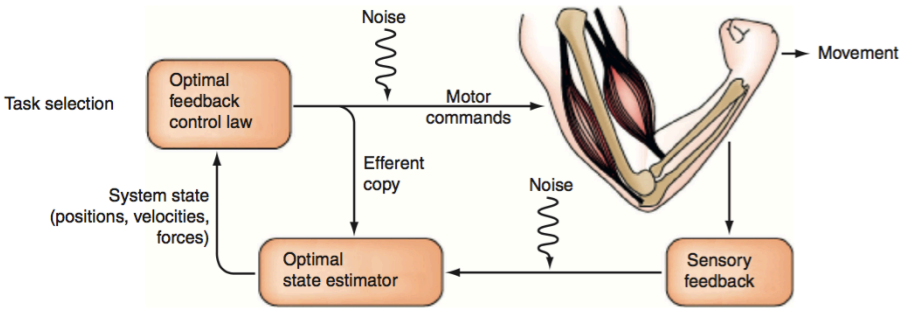

FIGURE 1. Representation of principles of optimal feedback control theory (Squire 2009, pp. 114)

3 NEUROPLASTICITY

Neuroplasticity refers to both acute and long-lasting functional and anatomical changes in the nervous system and is a key mechanism in learning and memory (Kandel et al 2000, pp. 34). Memories and learning can be crudely categorized to explicit and implicit type. Explicit memory refers to memory of facts and experiences. Implicit learning and memory involves changes in perceptual, motor and emotional circuits that happen unconsciously. Skills and behavioral responses are examples of implicit memory. It is common that a learning situation induces the formation of both implicit and explicit memories. (Kandel et al 2000, pp. 1228–1230; Sweatt 2010, pp. 4–7) Also learning may involve a conversion from conscious (explicit) processing to unconscious (implicit) processing. (Kandel et al 2000, pp. 1243–1244.) Different types of learning however share a lot of similar cellular events and mechanisms (Kandel et al 2000, pp. 1272–1274; Klintsova & Greenough 1999; Sweatt 2010, pp. 152).

3.1 Synaptic plasticity

Synaptic plasticity refers to the modulation of the strength of existing synaptic connections between neurons (Paulsen & Sejnowski 2000). A neuron relays information to other neurons via synapses. Most synapses in human nervous systems are chemical synapses that utilize neurotransmitters as mediators of synaptic transmission. An action potential causes a synaptic vesicle being released from the synaptic cleft of the presynaptic neurons. Neurotransmitter receptors at the postsynaptic neuron bind the neurotransmitter molecules causing the opening of ion channels of postsynaptic membrane, which induces either excitatory or inhibitory postsynaptic potentials at postsynaptic neuron. The integration of synaptic potentials dictates whether the postsynaptic neuron fires. (Kandel et al 2000, pp. 207-2012; Enoka 2008, pp. 192–193)

Synaptic plasticity has homo- and heterosynaptic forms. Homosynaptic plasticity refers to plastic changes in the synapses that were activated during plasticity inducing event.

Heterosynaptic plasticity accompanies homosynaptic plasticity in the surrounding synapses that were not activated during plasticity inducing event. Heterosynaptic long-term depression (LTD) often accompanies homosynaptic long-term potentiation (LTP) and heterosynaptic LTP often accompanies homosynaptic LTD. It has been theorized that heterosynaptic plasticity is needed to maintain balance of synaptic weights. (Chistiakova et al 2014.)

Synaptic plasticity has short-term forms that last from milliseconds to few minutes and long-term forms that last for hours (Catterall & Few 2008). Short-term plasticity modulates synaptic efficacy in a timeframe of milliseconds after a triggering event. Short-term plasticity involves mainly presynaptic changes that modulate the probability of neurotransmitter release from presynaptic neuron. (Fortune & Rose 2001.) Short-term synaptic facilitation and depression are mechanisms behind some types of short-term learning but they also operate in basic function of sensory processing (Fortune & Rose 2001). Long-term synaptic plasticity is a crucial mechanism in memory formation and storage in many types of learning (Klintsova & Greenough 1999; Sweatt 2010, pp. 152).

3.1.1 Long-term synaptic plasticity

Long-term potentiation (LTP) and long-term depression (LTD) are types of synaptic plasticity that are mechanisms behind many types of learning and memory. Learning related LTP has been observed in hippocampus, amygdala, cerebellum and cerebral cortex (Klintsova & Greenough 1999; Sweatt 2010, pp. 152). There is evidence that motor cortical LTP-like plasticity is a mechanism behind motor skill learning of in humans and other mammals (Squire 2009, pp. 732–733).

Hebbian type rules govern homosynaptic associative types of synaptic plasticity (Chistiakova et al 2014). According to Hebb´s law, a long-lasting change of synaptic efficacy may occur when presynaptic and postsynaptic neurons are activated sequentially. Asymmetric Hebbian learning rule states that in LTP presynaptic neuron must fire prior to postsynaptic neuron. Timing is crucial and involves backpropagating action potential in postsynaptic neuron fast after presynaptic neuron activation. Long-term depression (LTD) occurs if the activation

pattern is reversed and post-synaptic neuron fires before presynaptic neuron. LTP and LTD types of synaptic plasticity is an indicator that an activation pattern has been learned which increases the probability of the activation pattern occurring in the future. (Paulsen & Sejnowski 2000.)

Early and late versions of LTP. LTP involves different processes and phases that occur in different timelines. LTP phases have been extensively studied in hippocampal neurons. Short-term potentiation (STP), also sometimes called t-LTP has been considered to be an initial phase of LTP though it could also a distinctive type of short-term synaptic plasticity. It is likely that STP works through presynaptic mechanisms. This type of plasticity has been reported to last from 30 minutes up to 6 hours after it´s induction. (Lauri et al 2007). Early LTP occurs during the first 1–3 hours after it´s induction. Early LTP involves a functional change in the synapse that increases the chance that a neurotransmitter vesicle is released into the synaptic cleft. Late LTP persists over 24 hours and requires several trains of stimuli to transpire. Late LTP involves protein synthesis and even synaptogenesis. (Kandel et al 2000, pp. 1262–1264.)

3.1.2 Cellular mechanisms of long-term synaptic plasticity

It is known that there are many types of LTP that have similar effects on synaptic activity but involve different mechanisms. Mechanisms that are involved in LTP differ in different types of learning though there are also similarities (Thomas & Huganir 2004.) Mechanisms of long-term synaptic plasticity in hippocampal pyramidal neurons have been researched extensively (Sweatt 2010, pp. 153). The literature sited in this chapter has focused mainly on hippocampal neurons. Characteristics of cortical synaptic plasticity are discussed more on the next chapter.

Postsynaptic component of synaptic plasticity. AMPA receptors are neurotransmitter receptors for glutamate that mediate neural transmission in glutamaergic synapses (Sweatt 2010, pp. 153). In active synapses AMPA receptors are clustered at the postsynaptic membrane of the postsynaptic neuron. In a silent synapse there are no AMPA receptors at the postsynaptic membrane. Trafficking AMPA receptors into or out of synaptic membrane

modifies the activity of a synapse and is a mechanism in synaptic plasticity. LTD involves endocytosis of AMPA receptors from synaptic membrane. (Malinow & Malenka 2002.) LTP involves exocytosis of AMPA receptors to the synaptic membrane (Malinow & Malenka 2002) and synthesis of new AMPA receptors (Klintsova & Greenough 1999). Different signaling pathways can trigger LTP. NMDA receptors are voltage-depended glutamate receptors that act as calcium ion channels. NMDA receptors detect pairings of synapse activation combined with depolarization of postsynaptic neuron. (Sweatt 2010, pp. 161–162.) They have a role in controlling many types of LTP. NMDA receptor activation leads to an influx of calcium ions into postsynaptic cell, which in turn triggers MAPK cascade, a chain of events that leads to protein synthesis. (Thomas & Huganir 2004.) In addition to enhancing the efficacy of active synapses, LTP may involve also activation of silent synapses, which has been proposed to work by AMPA receptor exocytosis. (Klintsova & Greenough 1999).

Presynaptic module of LTP. There is evidence that at least some types of LTP involve presynaptic components that enhance neurotransmitter release or circulation. (Kandel et al 2000, pp. 1260–1261; Zakharenko et al 2003). Non-associative LTP depends on calcium ion influx into presynaptic cell. In associative LTP presynaptic neurotransmitter release is enhanced by retrograde signal from postsynaptic neuron. (Kandel et al 2000, pp. 1260–1261.) Brain-derived neurotrophic factor functions as a signaling substance for presynaptic component of LTP but is not required for postsynaptic module of LTP. In addition the presynaptic module of LTP requires activation of postsynaptic L-type voltage-gated calcium ion channels (Figure 2). Presynaptic module of LTP is likely independent of postsynaptic module. (Zakharenko et al 2003.)

Heterosynaptic plasticity. Heterosynaptic LTP and LTD on the other hand do not necessitate prior presynaptic activity (Chistiakova et al 2014). LTP and LTD involve presynaptic changes at the axon terminal. Retrograde signaling systems are involved in presynaptic component of heterosynaptic plasticity. Postsynaptic component in heterosynaptic plasticity is initiated by rise in intracellular calcium ion concentration that is caused by back-propagating action potentials of post-synaptic neuron. The rise of intracellular calcium can induce either heterosynaptic LTP or LTD depending on the priming of the synapse. (Chistiakova et al 2014.)

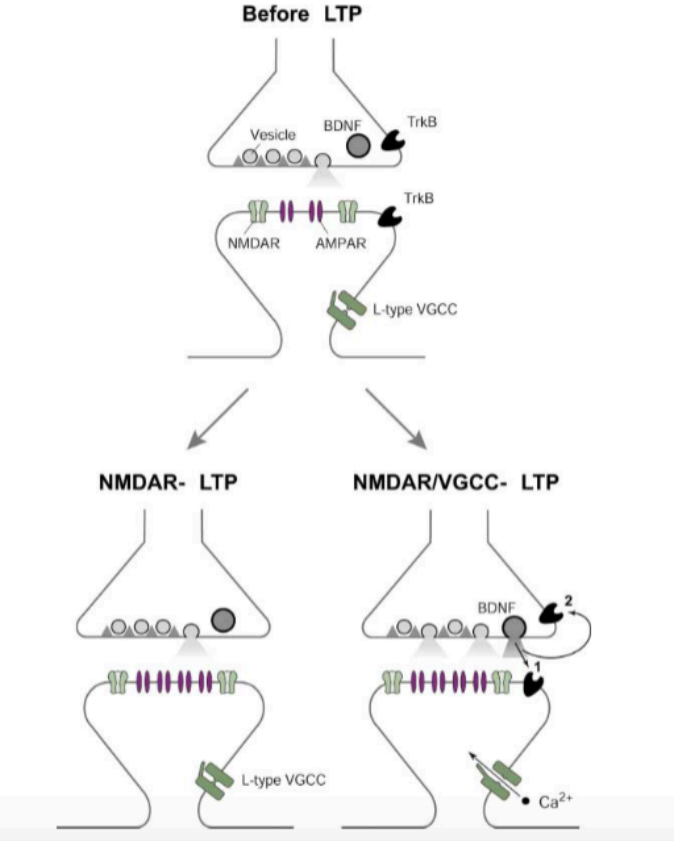

FIGURE 2. Representation of two different types of LTP. NMDAR dependent LTP induces AMPA receptor trafficking into postsynaptic membrane but no changes in presynaptic neuron. NMDAR/VGCC –LTP induces both pre- and postsynaptic changes and is dependent on postsynaptic L-type voltage gated calcium ion channels and presynaptic brain-derived neurotrophic factor (BDNF). (Zakharenko et al 2003.)

3.1.3 Synaptic plasticity in cerebral cortex

Both LTP and LTD types of synaptic plasticity have been observed in excitatory synapses cerebral cortex. Associative NMDA receptor dependent LTP is a principal type of LTP in cerebral cortex. (Squire 2009, pp. 186–187). Cortical long-term synaptic plasticity has been observed in synapses located in II/III and V layer (Squire 2009, pp. 731). Layer II contains granule cells and layer III external pyramidal cells. Layers II/III also contain dendrites from layer V neurons and synapses that give output to layer V neurons. Layer V contains synapses from cortico-cortical and thalamo-cortical afferents and pyramidal neuron somas. (Kandel et

al 2000, pp. 327–329). Layer V corticospinal pyramidal neurons in the primary motor cortex produce and mediate movement orders to spinal motor neurons (Kandel et al 2000, pp. 347). In addition to NMDA receptor dependency, acetylcholine receptors also have a role in cortical synaptic plasticity. Like in hippocampal neurons, cortical LTP induces changes in AMPA receptor phosphorylation. (Squire 2009, pp. 731–733).

Characteristics of synaptic plasticity in sensory cortices. Roelfsema & Holtmaat (2018) proposed that synaptic plasticity in sensory cortices is gated by feedback signals and steered by neuromodulatory systems. Their hypothesis states that a plasticity-inducing event induces tagging of the synapses for plasticity. Tagged synapses would go through plastic changes if tagging is followed by a stronger event in the other synapses of the same neuron. They proposed that the tagging is mediated by cortico-cortical connections and/or thalamic connections. Cortical neuromodulatory systems that are involved in modulation of cortical plasticity include dopaminergic, cholinergic, serotonergic and noradrenergic pathways. Modulatory systems can affect both the size and direction of plastic changes and are the proposed systems to steer plasticity. (Roelfsema & Holtmaat 2018.)

Synaptic plasticity in adult human motor cortex. Scientific research suggests that LTP and LTD types of synaptic plasticity occurs in human cortices. In humans synaptic plasticity is referred as LTP-like and LTD-like plasticity as the evidence is indirect but suggests strongly on similar mechanism as in other mammals. (Delvendahl et al 2012.) LTP-like plasticity in human motor cortex is NMDA receptor dependent (Bütefisch et al 2000). Reduction in GABA, a type of inhibitory neurotransmitter substance, is also involved in LTP-like plasticity during motor learning (Bütefisch et al 2000; Floyer-Lea et al 2006). LTP-like plasticity in motor cortex involves also protein synthesis and brain-derived neurotrophic factor (Dayan et al 2011).

3.2 **Synaptogenesis**

Synaptogenesis refers to the formation of new synapses. Synaptogenesis is involved in long-term forms of both implicit and explicit learning. (Kandel et al 2000, 1254–1265.) Long-

lasting motor cortical reorganization involves synaptogenesis and occurs during late phases of motor skill learning in rats (Kleim et al 2004; Squire 2009, pp. 187). Likely synaptogenesis is a mechanism behind long-term motor learning also in humans (Rozenkranz et al 2007 a).

The formation of a synapse in cultured hippocampal neurons begins with neuritogenesis, which involves extension of axon, axonal branches and dendrites of the cultured neurons (Figure 3). The growing axons are capable of secreting synaptic vesicles and as such the axon can start interacting with postsynaptic neuron as soon as they make contact. As two neurons make contact they may begin to form synaptic connection. Synaptic vesicles cluster in presynaptic membrane whereas post-synaptic cell membrane experiences localization of glutamate receptors and glutamate carriers. Scaffolding proteins are involved both in formation and maturation of synapses. (Verderio et al 1999.)

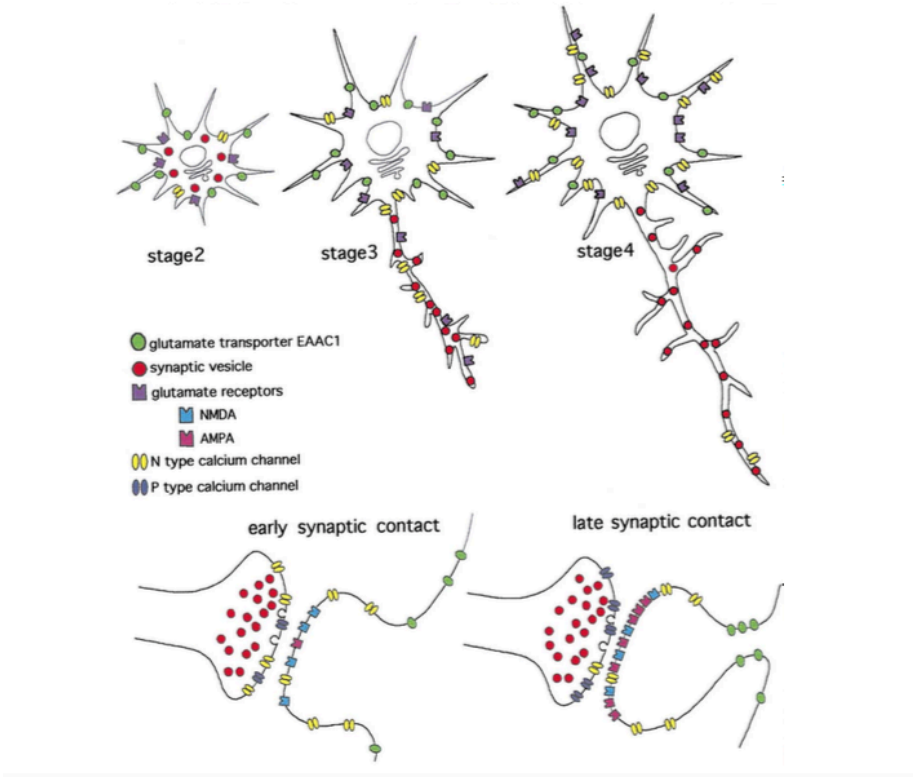

FIGURE 3. Model of neuritogenesis and synaptogenesis in cultured hippocampal neurons (Verderio et al 1999).

3.3 **Neurogenesis**

Neurogenesis is a process where new neurons are formed from stem cells. Neurogenesis is characteristic to a developing nervous system but has been observed also in adult mammals (Genin et al 2014). In adult humans markers of neurogenesis has been observed in dentate gyrus of hippocampus (Eriksson 1998; Spalding et al 2013) and in striatum (Ernst et al 2014). Evidence of adult human neurogenesis comes from birthdating neurons and observations of neurogenic stem cells and markers that indicate the presence of precursor cells, progenitor cells and immature neurons (neuroblasts). Evidence suggests that excitatory granule cells form in hippocampus from dividing progenitor cells. (Eriksson et al 1998.) Carbon dating of neurons indicates that new hippocampal neurons form throughout life (Figure 3). Every day around 700 new hippocampal neurons are formed in dentate gyrus. (Spalding et al 2013.)

Adult human neurogenesis appears to be limited to hippocampus and striatum, whereas neocortex functions without neurogenesis. Hippocampus is important for learning and memory of explicit knowledge. The new neurons are theorised to contextualise new information relative to existing and have a role in forgetting. Neurogenesis may not be necessary in learning but new neurons have higher aptitude for synaptic plasticity, as they are not as heavily inhibited by interneurons as mature neurons in hippocampus are. (Kempermann et al 2018.) In the basis of scientific literature it is not likely that motor skill learning involves cortical neurogenesis.

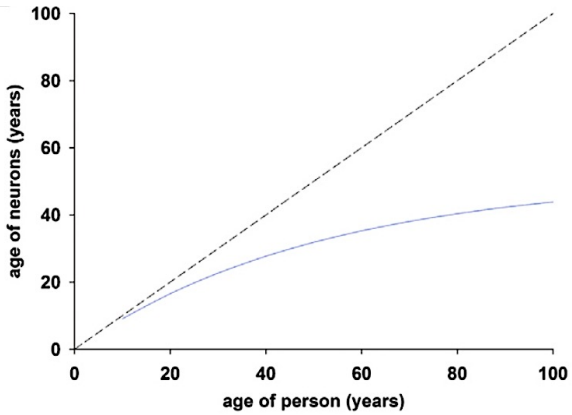

FIGURE 3. Neuron turnover in human hippocampus. Dashed line represents neurone age without neuronal turnover. Curve represents measured average age of neurons. (Spalding et al 2013.)

4 NON-INVASIVE NEUROSTIMULATION

Neurostimulation methods are useful in scientific research and medical treatments for their capability of inducing neuroplasticity. Non-invasive procedures are widely used for their relative safety and easy application. Typical non-invasive procedures used in human cotex are paired associative stimulation (PAS), repetitive transcranial magnetic stimulation (rTMS), theta burst stimulation (-TBS) and transcranial direct current stimulation (tDCS). (Vallence & Ridding 2014.)

4.1 Transcranial magnetic stimulation

TMS is a non-invasive and safe method for stimulating cortical areas of the brain. TMS generates a magnetic field, which induces an intra-cortical electric field in the brain that can depolarize neurons directly below the TMS coil. (Komi 2011, pp. 115–116; Rossi et al 2009.) TMS stimulus over motor cortex may activate corticospinal pyramidal neurons, which induces a movement command that travels through corticospinal tract to spinal motor neurons and finally muscle (Rossi et al 2009.) TMS stimulus excites pyramidal neurons indirectly by stimulating axon collaterals of other neurons that make synapses with pyramidal neurons. TMS is not able to excite axons of corticospinal neurons directly with an exception for stimulation of hand muscles with high stimulus intensities. (Komi 2011, pp. 118–120) TMS induced muscle activation, motor evoked potential (MEP), can be measured with electromyography (Rossi et al 2009.)

Common TMS applications include single-pulse TMS, paired-pulse TMS and repetitive TMS (Table 1). In the study of motor function TMS is used to research the structure and function of motor systems. TMS is a versatile method for studying neuronal interactions that drive the function and adaptation. Repetitive TMS and some paired TMS applications are used to induce neuroplasticity. (Rossi et al 2009.) Plasticity inducing protocols are useful tools in research of motor skill learning as they both induce LTP-like plasticity (e.g. Rosenkranz et al 2007a) and reorganization of motor cortical mapping (McKay et al 2002).

TABLE 1. TMS applications and research themes described by Rossi et al (2009).

TMS	Applications	Research themes
Single-pulse TMS		Cortical/corticospinal excitability
		Cortical mapping
		Neural conduction speed
Paired-pulse TMS	Single coil	Intracortical fasilitation
		Intracortical inhibition (SICI, LICI)
	Two coils	Cortico-cortical interactions
	TMS + other (e.g. PAS)	Neuroplasticity
Conventional rTMS	High frequency (> 1 Hz)	Neuroplasticity
	Low frequency (≤ 1 Hz)	Neuroplasticity
Patterned rTMS	TBS: cTBS, iTBS	Neuroplasticity

TMS measurements: excitatory and inhibitory cortical circuits. Some paired pulse measurements are used in order to measure the function of excitatory and inhibitory cortical circuits. Suprathreshold TMS pulse paired with prior subthreshold pulse may invoke cortical inhibition or facilitation depending on the interstimulus interval and stimulus intensity. (Chen 2004; Komi 2011, pp. 125) Conditioned MEP is normalized to unconditioned MEP to reveal the magnitude of facilitation or inhibition. A change in inhibition or facilitation is thought to represent a change in the excitability of the measured inhibitory or excitatory neural network. (Chen 2004.) Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter that acts in many different types of neurons in brain (Kandel et al 2000, pp. 285). Different GABAergic inhibitory circuits mediate short-interval intracortical inhibition (SICI) and long-interval intracortical inhibition (LICI) (Chen 2004). SICI is likely mediated by $GABA_A$ receptors and LICI by $GABA_B$ receptors (Chen 2004; Komi 2011, pp. 125). Intracortical facilitation is likely mediated by glutamate (Chen 2004). Multiple neural circuits have been identified that mediate interhemispheric facilitation and inhibition. (Komi 2011, pp. 127–128.). Inhibitory and excitatory neural circuits also interact with one another (Chen 2004).

4.2 **Paired associative stimulation**

Stefan et al (2000) showed that paired associative stimulation (PAS) is capable of inducing associative plasticity in the human motor cortex. Their PAS protocol consisted of 90 stimulus pairs of peripheral nerve stimulation and TMS stimulation. First a stimulus was given to medial nerve which was followed a second stimulus of TMS to motor cortex on the motor area of abductor pollicis brevis muscle on contralateral side. Stimulus pairs were given at 0.05 Hz over 30 minutes. Different interstimulus intervals were tested of which 25 ms was effective in inducing associative plasticity (Figure 4). (Stefan et al 2000.) PAS induced MEP amplitude changes likely represent LTP-like and LTD-like synaptic plasticity (Delvendahl et al 2012.)

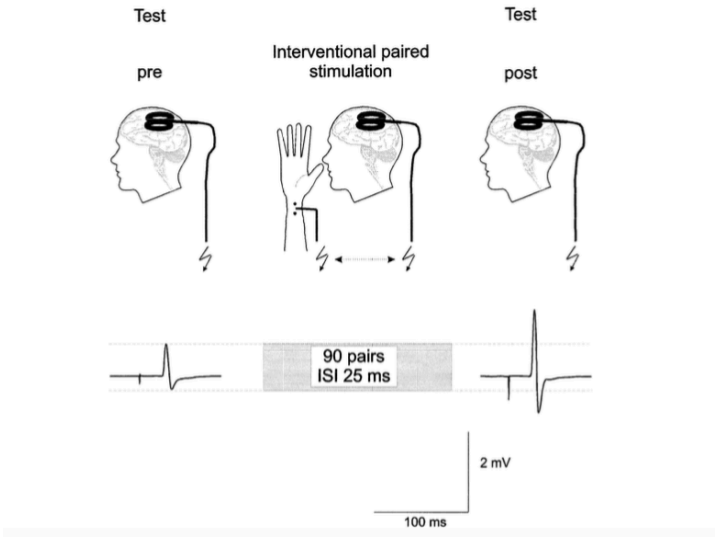

FIGURE 4. PAS stimulation protocol used by Stefan et al (2000).

Long-term potentiation (LTP). PAS may induce LTP-like plasticity if the peripheral afferent feedback from peripheral nerve stimulation arrives to the motor cortex at the same time as TMS stimulus is given (Stefan et al 2000; Wolters et al 2003). When aiming for LTP induction, interstimulus interval is to be set so that afferent signal has just enough time to travel through somatosensory tract into somatosensory cortex and from there to motor cortex (Stefan et al 2000). Interstimulusinterval is chosen according to the target muscle (Table 2).

TABLE 2. Examples of studies with LTP-like PAS effect.

Study	Area	Interstimulus interval	Effect
Stefan et al 2000	Hand	25 ms	MEP ↑
Rosenkranz et al 2007a	Hand	25 ms	MEP ↑
Lamy et al 2010	Forearm	20 ms	MEP ↑
Meunier et al 2007	Forearm	20 ms	MEP ↑
Kumpulainen et al 2012	Lower leg	Individual: somatosensory evoked potential + 18 ms	MEP ↑

Long-term depression (LTD). Wolters et al 2003 found that PAS is capable of inducing LTD-like plasticity in the human motor cortex. PAS conducted with interstimulus interval of 10 ms induced a decrease in MEP sizes in ABP muscle that remained for approximately 90 minutes. (Wolters et al 2003.) Later PAS induced LTD-like MEP suppression has been observed also in lower limbs (Alder et al 2019). LTD is induced by PAS if ISI is set so that the afferent signal from peripheral signal reaches M1 after TMS stimulus. (Wolters et al 2003.)

Spinal modulation. Although PAS induces plasticity mainly at cortical level, there is some evidence of concurrent changes in spinal modulation (Meunier et al 2007, Lamy et al 2010). Changes in spinal excitability have been researched with F-wave and H-reflex measurements. In F-wave studies F-wave was measured by stimulating median nerve supramaximally, which induced a second wave of activity. F-wave amplitude changes reflect alpha motor neuron excitability without supraspinal influence. Spinal excitability changes have not been observed when measured with F-wave. (Stefan et al 2000; Nishihira et al 2006.) Some studies have reported an increase of H-reflex amplitudes after PAS targeting flexor carpi radialis muscle (Meunier et al 2007, Lamy et al 2010). H-reflex is an artificial reflex that is induced by electrically stimulating Ia-afferents that bring input monosynaptically from muscle spindles to alfa-motor neurons. H-reflex measures the excitability of alfa-motor neurons but is also affected by presynaptic modulation. (Komi 2011, pp. 233–234) Lamy et al (2010) found that PAS reduces the presynaptic inhibition of Ia terminals between Ia-afferent fibers and alpha-

motor neurons. As supraspinal areas modulate presynaptic Ia-inhibition (Kandel et al 2000, pp. 724), a PAS induced cortical changes might also affect presynaptic inhibition and thus H-reflex amplitude.

Relation to motor learning process. PAS induced LTP-like plasticity is similar to that observed during the early phase of motor skill learning (Rosenkranz et al 2007a; Ziemann et al 2004). Rosenkranz et al (2007a) demonstrated that motor skill training interferes with PAS effect at the early phase but not in later phases of motor skill learning. Ziemann et al (2004) supported the finding as they found that only motor action that resulted in skill learning occluded the PAS effects. However studies have failed to find any relationship between the magnitude of PAS and motor skill training induced motor cortical neuroplasticity (López-Alonso et al 2015; Vallence et al 2013).

PAS induced plasticity is influenced by several factors (Table 3). For example prior physical activity may enhance or occlude PAS effects. Aerobic exercise has been shown to enhance responses to PAS (Mang et al 2014) whereas participation to prior motor skill training session occludes LTP-like effect (Stefan et al 2006, Rosenkranz et al 2007a). PAS effects also show a great inter- and intra-individual variability. High intra-individual variation across different measurement sessions indicates against comparing PAS effects in same subjects. Group averages of PAS effects are however reproducible. (Fratello et al 2006.)

TABLE 3. Factors that are known to influence PAS effect.

Reference	Factors that influence PAS effect
Stefan et al 2000	Interstimulus interval
Stefan et al 2004	Attention during PAS
Sale et al 2007	Time of the day for PAS
Mang et al 2014; Rosenkranz et al 2007a; Stefan et al 2006	Motor activity prior PAS
Concerto et al 2017	Stress level of the participant
Sale et al 2008	Hormonal levels

5 MOTOR SKILL LEARNING

Motor skill learning refers to practice induced improvements in spatial and temporal accuracy of a motor task (Willingham 1998). Motor skill learning involves functional and structural changes occurring at many locations in the nervous system. Willingham et al (1998) reviewed the literature and composed a theory that motor skill learning is caused by repetitive use of motor control processes and that the learning tunes those processes for the trained task. This work focuses especially on characteristics of the motor skill learning and the role of the motor cortical areas in the learning. Changes in other brain areas are also mentioned briefly.

5.1 Learning phases

Karni et al (1998) proposed that the process of learning new motor skills involves changes in cortical representation and that these changes occur in different stages. Fast learning occurs during first minutes of first training session. Slow learning accounts for slower improvement in skill that starts to cumulate after high number of repetition. Consolidation phase means periods between training sessions that are also essential in motor skill development. Classification of motor skill learning process to a fast learning phase and a slow learning phase was suggested by Karni et al (1998) and has since been commonly used in scientific literature (e.g. Dayan et al 2011).

The fast phase is brief and involves a switch in M1 activation. First repetitions are associated with a habituation like response, where the activation on M1 decreases. As the number of repetitions builds up, a switch in ordering effect is seen and the activation of M1 begins to increase. (Karni et al 1995.) The fast phase is thought to involve experimenting with existing movement strategies. The performance improves as the suitable strategy takes shape. (Hirano et al 2015; Karni et al 1998; Korman et al 2003).

The slow learning phase consists of lots of repetitions compared to the fast learning phase. The slow improvement of skill associated is gained as task specific connections in the brain are strengthened and created. (Hirano et al 2015.) The slow learning phase is also associated

with between session gains of skill. Learning that occurs between sessions is referred consolidation or off-line learning. The skill gain is also well retained after the training has ended. (Hirano et al 2015; Karni et al 1998; Korman et al, 2003.) Interestingly the gains of performance associated with the first training session can transfer to untrained side of the body whereas gains of skill induced by further training session do not transfer (Korman et al 2003). Slow learning phase is associated with functional and structural plasticity in different parts of motor systems.

5.2 **Brain activity**

Brain activity in motor learning has been studied with fMRI and PET scanning. Fast motor learning phase is associated with increased activity in contralateral side of trained muscle in supplementary motor areas, premotor cortex, dorsomedial striatum and posterior parietal cortex. The activity of primary motor cortex, dorsolateral prefrontal cortex and presupplementary motor areas on the contralateral side of trained muscle decreases during fast motor learning phase. Activity of cerebellum increases in both sides. In ipsilateral hemisphere the activity of supplementary motor areas and premotor cortex increases and the activity of presupplementary motor areas and dorsolateral prefrontal cortex decreases during slow motor learning phase. (Dayan et al 2011.)

Slow motor skill learning phase is associated with increase in activity of contralateral primary motor cortex, primary sensory cortex, supplementary motor areas and dorsolateral striatum with decrease of cerebellar activity. Activity of dorsolateral striatum increases also in ipsilateral hemisphere. (Dayan et al 2011.)

5.3 **Changes in cortical and corticospinal excitability and cortical representation**

A single motor skill training session induces an acute increase of corticospinal excitability that is often accompanied by reduced SICI (Table 4). Hirano et al (2015) proposed that the enhancement of M1 excitability requires a large amount of repetition that is achieved after the switch from fast to slow learning phase. After multiple training sessions a single session no

longer induces acute changes to corticospinal excitability or SICI. Instead multisession training is associated with slower increase of baseline motor evoked potentials. (Jensen et al 2005; Rosenkranz et al 2007a.) Studies demonstrate that the mechanism behind training induced acute corticospinal excitability changes is LTP-like plasticity in motor cortex (Rosenkranz et al 2007a; Ziemann et al 2004), but other mechanisms, like synaptogenesis are likely responsible for long-term improvements (Rosenkranz et al 2007a). Long-term training and expertise is associated with higher baseline corticospinal excitability of trained muscle area (Hirano et al 2014).

TABLE 4. Examples of motor skill learning tasks reported to induce LTP-like effect after single training session.

Study	Task	Muscle group	Effect
Rosenkranz et al 2007a	Rapid finger tapping	Thumb	MEP ↑ SICI ↓ PAS25 effect reversal
Garry et al 2004	Pegboard manipulation	Hand muscles	MEP ↑ SICI ↓ after right hand training, no change after left hand training
Cirillo et al 2011	Visual tracking	Fingers	MEP ↑ SICI ↓
Jensen et al 2005	Visual tracking	Biceps	MEP ↑
Hirano et al 2015	Visual tracking	Ankle flexors	MEP ↑ that was associated with slow-learning stage

Relationship between magnitude of LTP-like plasticity and learning. Many studies have found an association between motor learning outcomes and magnitude LTP-like effect (Garry et al 2004; Hirano et al 2018; Jensen et al 2005). Majority of the studies focused on single session training adaptations. Gary et al (2004) found a relationship between learning results and corticospinal excitability in finger muscles essential for the task and only on the preferred hand although both were trained. Hirano et al (2018) demonstrated a difference in skill

development that depended on training induced development of corticospinal excitability during single training session. Participants whose I/O slope peaked faster and higher also learned the task faster. Their I/O slope peaked at the middle of the training session and after the session I/O slope had already decreased to near PRE values. Slower increase and peaking of the slope was associated with slower improvement in performance. Participants whose I/O slope did not change did also not improve their performance. (Hirano et al 2018.) Jensen et al (2005) reported statistically significant correlations between long-term gains of skill and long-term changes in corticospinal excitability.

Cortical representation. During the slow learning phase the motor areas of the brain go through reorganization that strengthens the cortical representation for the task in the brain (e.g. Hirano et al 2015; Karni et al 1995; Pascual-Leone et al 1995). Motor representation of the trained motor task expands over time as a result of motor skill training (Karni et al 1995, Karni et al 1998). The areas where a TMS stimulus can induce a movement for the finger flexors and extensors expand gradually over the days when training a piano playing sequence (Pascual-Leone 1995). Slow cortical reorganization likely involves synaptogenesis (Dayan et al 2011). A fast and transient type of cortical reorganization on the other hand is involved in fast learning phase (Kleim et al 2004).

5.4 **Structural plasticity**

Motor skill training induces changes in brain matter structure that are detectable with MRI. An increase of motor cortical thickness has been observed as soon as an hour after balance skill training (Taubert et al 2016). In longitudinal studies grey matter volume increases have been observed in following brain areas in humans: parietal areas, frontal areas, cortical areas involved visual and visuo-motor processing, hippocampus and nucleus accumbens. In other mammals structural plasticity has been found also on pyramidal neurons of the motor cortex. Cross-sectional studies also indicate that motor skill training induces structural grey matter changes in different brain areas that depend on the qualities of the trained skill. The experience of training the skill affects the magnitude of structural plasticity. (Dayan et al 2011.) It is likely that the task specificity of plasticity explains why some studies have found motor cortical plasticity and other not. Animal studies indicate that grey matter changes

reflect many different types of structural plasticity: neuritogenesis, synaptogenesis, structural plasticity in existing synapses and glial hypertrophy (Dayan et al 2011).

Longitudinal studies have found evidence of motor skill training induced white matter structural changes in frontal and parietal areas that seem to occur in parallel with grey matter changes. Cross-sectional studies also indicate that white matter is modified during motor learning. White matter structural changes are proposed to enhance conduction properties of axons. (Dayan et al 2011.)

5.5 **Difference of motor skill training to other forms of exercise**

Motor skill training has differential effects to the corticospinal tract compared to other types of motor exercise like strength and endurance training. Postexercise depression of MEPs (PED) has been reported to occur after sufficient duration of fatigue inducing exercise (Brasil-Neto et al 1993; Samii et al 1997) and repetitive non-fatiguing exercise (Bonato et al 2002).

Fatiguing exercise. Fatiguing exercise may induce a brief post-exercise facilitation and subsequent post-exercise depression. Lenz & Nielsen (2002) observed post-exercise facilitation right after exercise that declined in approximately 25 seconds. In addition with increase of MEP sizes, maximal M-waves were also elevated during post-exercise facilitation. (Lenz & Nielsen 2002.) Post-exercise depression (PED) after fatiguing contractions has been reported to last from few minutes up to 30 minutes (Kotan et al 2015; Maruyama et al 2006; Samii et al 1997). Maruyama et al (2006) observed reduced SICI that accompanied PED but recovered faster than MEPs (5 min vs. >15 min). Post-exercise facilitation and depression are thought to have cortical and peripheral components (Lenz & Nielsen 2002.).

Repetitive non-fatiguing exercise and PED. Miyaguchi et al (2017) reported a decrease of short latency afferent inhibition and an unchanged SICI during PED induced by non-fatiguing exercise. They concluded that exercise induced PED is likely to involve suppression of cholinergic inhibitory circuit activity (Miyaguchi et al 2017). Miyaguchi et al (2016) observed that PED was greater after repetitive isotonic muscle contractions than isometric and that

increased level of contraction increased PED. Interestingly imagined sustained handgrip too induces PED (Kluger et al 2012). Feeling of high effort during the task and general fatigue after the task is a typical finding in PED studies assessing non-fatiguing exercise (Avanzino et al 2011, Kluger et al 2012). These findings raise a question whether general fatigue could be involved in PED after non-fatiguing protocols. Teo et al (2012) however observed that PED can be induced also by less demanding exercise and proposed that PED effect is an aftereffect of voluntary movement that is not depended on fatigue.

Strength training. A recent meta-analysis showed that a single session of strength training often increases the excitability of corticospinal pathway at cortical and spinal levels (Mason et al 2018). Meta-analysis from Kidgell et al (2017) indicated that several weeks of strength training might decrease cortical silent period and SICI with no change in motor threshold and only weak indications towards increase of corticospinal excitability. Kiedgell et al (2017) observed inconsistency in the effect direction and magnitude on corticospinal excitability in the reviewed literature that may result from differences in the used strength training protocols. Chronic strength training might reduce inhibition in cortical circuits (Lahouti et al 2019). In the study of Lahouti et al (2019) a background of chronic strength training was associated with reduced SICI and reduced active motor threshold compared to control group. Literature suggests that single strength training does not affect intracortical inhibitory circuits (Lahouti et al 2019; Mason et al 2018), whereas short-term training interventions and chronic strength training may decrease the excitability of intracortical inhibitory circuits (Kidgell et al 2017)

Endurance training. Endurance training induces angiogenesis and of the trained areas in the motor cortex and increases cerebral blood flow but does alter cortical circuitry (Swain et al 2003). A bout of aerobic exercise promotes neuroplasticity in motor cortex of untrained muscles without changes in cortical excitability (MCDonnel et al 2013). McDonnel et al (2013) reported enhanced neurostimulation induced plasticity after light aerobic training. Smith et al (2014) reported reduced SICI in hand areas after aerobic exercise.

6 THE SKILL OF JUGGLING

Juggling is a skill that requires accuracy in throwing and catching and also a sense of rhythm. In juggling one rhythmically repeats tossing and catching a number of objects. There are countless known juggling patterns of which the most researched is the cascade pattern with three balls. This work will from now on focus solely on the three-ball cascade pattern.

6.1 The three-ball cascade pattern

In cascade pattern one throws objects from hand to hand one at a time. Left and right-handed tosses follow each other (Figure 5; Figure 6). The cycle is symmetrical in both sides. Hand movements follow a 1:2 frequency locking and the phase lag between balls (phase locking) is $2\pi/3$ (Post et al 2000).

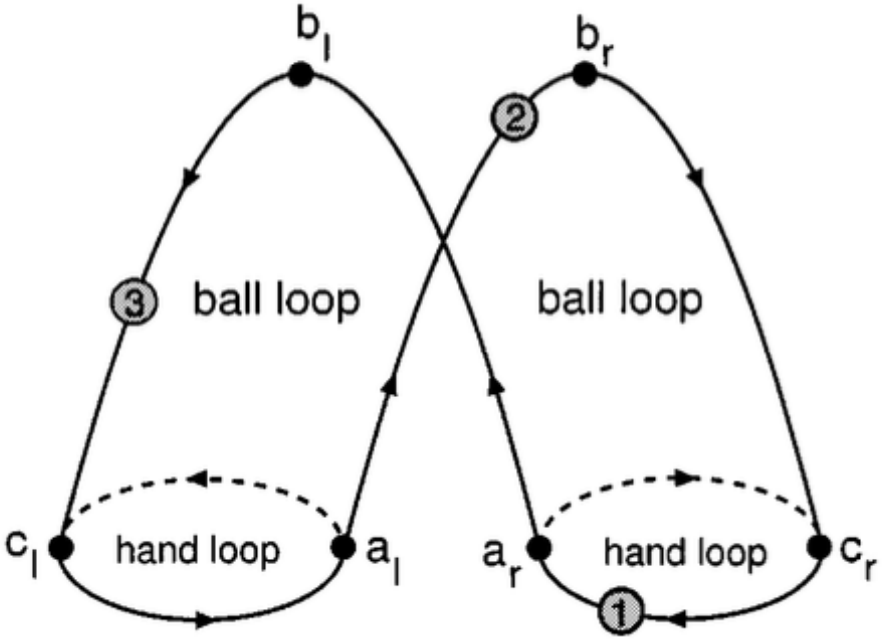

FIGURE 5. Illustration of hand and ball loops the 3-ball cascade; a=tossing point, b=highest point of the flight, c=Catching point; l and r refer to left and right side (Post et al 2000).

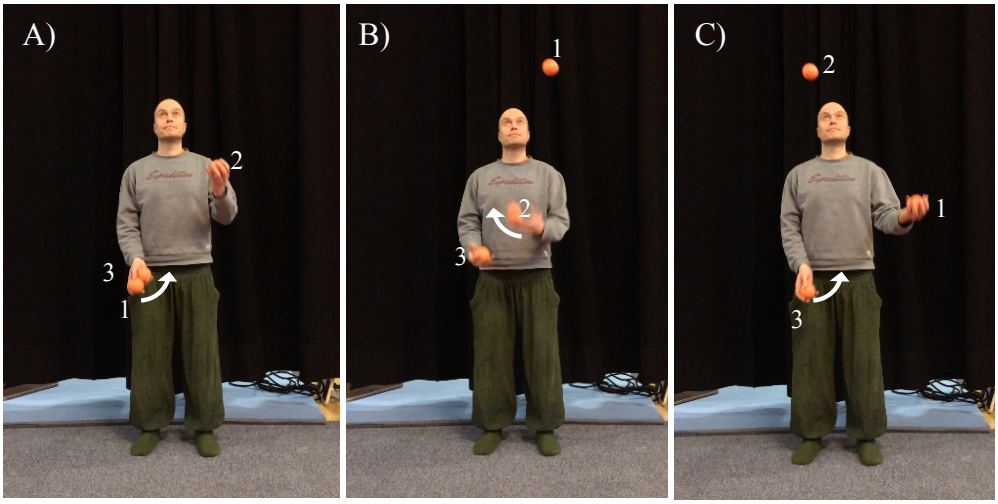

FIGURE 6. Demonstration of the three-ball cascade. Numbers represent different balls in tossing order. The arrow marks the direction of the following toss. A) First toss. B) Second toss. C) Third toss.

Temporal characteristics of juggling pattern. Juggling frequency has large effects on biomechanical aspects of the skill. Three-ball cascade juggling at a self-chosen speed can be presented mathematically in four dimensions whereas in fast cascade juggling dimensions needed for representation increase to six (3 balls x 2 dimensions). During faster juggling the temporal variance of catch-catch cycle is higher with also a larger spatial variance of juggling; the juggler is required to make larger corrections in order to keep the rhythm. (Post et al 2000). Mean self-chosen speed of juggling three balls is approximately 1.4 Hz in experts and faster in less experienced jugglers (Mapelli et al 2012). The juggling frequency is controlled by the height of each throw and experts are able to successfully control the temporal variables (Huys & Beek 2002).

Temporal and spatial control of juggling pattern. As other natural movements, the execution of juggling is prone to fluctuations. Because of fluctuations, the temporal integrity of the pattern needs sustained with corrections during juggling. It is suspected that reported variability in spatial and temporal aspects of catching is a control mechanism to sustain the juggling pattern. (Post et al 2000) In experienced jugglers left and right hands seem to have different roles in control strategy. In vertical direction the spatial movement pattern of hands is nearly symmetrical whereas in anterior-posterior and left to right directions hand movements are more variable and there is more tendency for asymmetry of hands. (Mapelli et al 2012).

Perception during juggling. Jugglers use visual, proprioceptive and haptic information in controlling the juggling pattern (Sánchez García et al 2013). It is assumed that more experienced jugglers need to rely less on the foveal vision and instead utilize the peripheral vision and proprioceptive and haptic information (Huys & Beek 2002). In a study that used different ball weights confirmed that even intermediate jugglers are able to some extent adjust their juggling according to information from proprioception (Sánchez García et al 2013).

Visual control strategies. The most preferred visual reference comes from the ball at its zenith though the visual reference from other phases suffices. The gaze does not need to be locked at the ball, as it is enough if the juggler sees the ball in peripheral vision. Less experienced jugglers tend to point their gaze on individual balls and the amplitude of their gaze movements are relatively large. Experienced jugglers are able to sift between gaze through and visual tracking strategy though and they use smaller eye movements in tracking (Huys & Beek 2002). Interestingly experienced jugglers benefit from an external fixation point, which might result from improved movement planning and attention (Dessing et al 2012). Developing a gaze locking strategy is however essential to juggling skill learning (Huys et al 2004)

6.2 **Characteristics of juggling skill development**

Juggling can be separated into sub-systems that develop at different time scales during the learning of juggling. During the learning process the degree of frequency locking improves faster that the degree of phase locking. These adaptations occur quite rapidly in the early training process. The performance outcome is the last variable to improve and the performance is gained exponentially after acquiring the basics. The skill continues to improve over the years a juggler spends gaining expertise. Ability to juggle in different tempos fluently develops slowly and is one sign of expertise. Frequency locking also continues to strengthen during the advancement of skill from intermediate to expert. (Huys et al 2004.)

Development of spatial and temporal characteristics. The spatial and temporal variability of ball movements decrease during learning. The throwing accuracy improves which is likely a key cause in the decreased variability. The variability of hand movements is larger in juggling and does not change during learning. During the training the juggling pattern also stabilizes

towards one plane of movement, as horizontal and sagittal components of spatial trajectory decreases and vertical component increases. (Huys et al 2004.)

Development of visual strategies. Learning to tract the balls visually is likely to be an essential element in learning the juggling skill. Gaze of novice jugglers tend to fluctuate in different directions. A vertical 3:2 frequency locking develops usually early in the training process. Developing a 3:1 horizontal gaze-locking strategy is especially important in order to improve the skill. When experience accumulates the template of exploitable eye movement strategies grows (gaze through/ fixed point strategy). (Huys et al 2004.)

6.3 **Training induced changes in the brain**

Changes in brain. Juggling training induces changes in brain matter volume that can be seen during and after the training period. Most notable change is a bilateral increase in grey matter volume in visual and parietal cortex that does not interact with training volume of juggling performance (Boyke et al 2008; Draganski et al 2004; Scholz et al 2009). Scholz et al (2009) found evidence of a change in white matter structure underlying intraparietal sulcus after 6 weeks of training but Sampaio-Baptista et al (2014) were not able to reproduce the finding. In elderly juggling training was also associated with grey matter increase in the right side of hippocampus and bilateral change in nucleus accumbens (Boyke et al 2008).

Relationship between juggling skill and brain changes. Some brain changes due to training do interact with training results. Better juggling performance was associated with a post-training grey matter volume increase in primary motor cortex and dorsal parietal cortex in the study of Sampaio-Baptista et al (2014). They also found that training intensity interacts with some skill related brain changes. Better performance can be associated with either grey matter increase or decrease in dorsolateral prefrontal cortex depending on training intensity so that higher intensity training was related to increased grey matter volume and low intensity to decreased gray matter volume. (Sampaio-Baptista et al 2014).

7 PURPOSE OF THE STUDY

There is evidence that the magnitude of motor skill training induced plasticity interacts with magnitude of motor training results on a single training session (Hirano et al 2018; Smyth et al 2010). Similar relationship has been observed between long lasting plasticity generated in the course of several weeks and long-term learning results (Jensen et al 2005). It is possible that the baseline capacity for plasticity might be related with motor skill learning results still after multiple sessions. Motor skill training induced corticospinal excitability changes have mostly been studied with simple motor tasks. Juggling is a complex perceptual motor skill. Effects of juggling training on corticospinal excitability on motor area of upper extremities have not been researched before.

Mechanisms in paired associative stimulation (PAS) and motor skill training induced cortical plasticity overlap (Rosenkranz et al 2007a; Zieman et al 2004). PAS induces higher magnitude of corticospinal plasticity in skill-trained groups than non-skill trained groups (Kumpulainen et al 2014; Rosenkranz et al 2007b). There are few studies that have aimed to demonstrate a relationship between non-invasive neurostimulation induced motor cortical plasticity and motor learning results (López-Alonso et al 2015; Vallence et al 2013). There is some data indicating that at least reaction time development during motor training may be associated with plasticity induced by some non-invasive stimulation protocols (López-Alonso et al 2015). However neither of the two studies found any relationship between PAS induced neuroplasticity and motor learning during single training session (López-Alonso et al 2015; Vallence et al 2013).

The purpose of this study is to examine the relationship between capacity for plastic changes in the motor cortex and learning of a complex motor task. Capacity for plasticity is examined from the magnitude of corticospinal excitability changes after PAS and after first motor training session. It is hypothesised that greater magnitude of plastic changes may be associated with faster learning of motor skill. This study should bring new information on the role of motor cortex in learning process of three-ball juggling, a complex perceptual-motor task.

8 METHODS

8.1 Participants

Volunteers for the study were searched for from the students and staff of the local university and local circus school. Recruitment notifications were distributed via email and paper posters. Inclusion criteria for the research project were: A) No background of juggling training, B) age between 18-30 years, C) no health problems or medications that could affect juggling training or TMS measurements and D) no irremovable metallic objects on or near the head. For this study an additional pre-requirement was to have no background of intensive skill training of hand area. 19 individuals volunteered to participate for the study. Six volunteers either discontinued the study or were dropped from the final analysis. Reasons included: personal reasons (3), adverse effects during the study (1), too prominent skill training background (1) and feeling unwell during a session for unrelated reasons (1). One participant discontinued the study for having a brief epileptic like seizure during paired associative stimulation.

Data from 13 participants aged 18–24 was analysed for the present study (4 men, 9 women). All participants were right handed. All of the participants were novices in juggling and none participated in goal-oriented motor skill training of the hand area. All the volunteers read information leaflet and had a chance to ask questions before writing a written consent for participating the study. Volunteers were informed that they could quit the study any time for any reason. Before measurement the volunteers also filled in a questionnaire that screened for contraindications of the study. The study was approved by the ethic committee of the University of Jyväskylä and was conducted in accordance with the Declaration of Helsinki.

8.2 Preliminary questionnaires

Preliminary questionnaires assessed contra-indications for participation to the study as well as other factors that might influence the results of the study including health status and recreational activities. Recreational activities were asked with open questions about A) what

activities one participated in regularly, B) how long one has participated in the said activity and C) how much time one spends doing the said activity weekly. First these questions were asked about participation in sports and exercise. Then the same questions were asked about participation in activities employing manual dexterity like handcrafts, instrument playing and racket games. Handedness was questioned in another sheet. The handedness questionnaire was established according to Cohen (2008), a handedness test based on Olfield (1971).

8.3 **Experimental design**

Experimental protocol consisted of 7 measurement sessions spread over 3 weeks (Table 5). Appointments involved neurophysiological testing and a motor skill training intervention (MT). Main purpose of the first appointment was to measure changes in corticospinal excitability induced by paired associative stimulation intervention. Then motor skill training intervention was scheduled to begin on week 2, after at least 5 days had passed from PAS intervention. Motor skill training intervention consisted of five 3-ball-juggling training sessions on 5 consecutive days (henceforth MT1, MT2, MT3, MT4 and MT5). Retention skill training session (henceforth MTRET) took place on third week after six days from MT5. MT1, MT5 and MTRET included also neurophysiological testing and reaction time measurement.

Neurophysiological testing on each day had similar formula and focused on flexor carpi radialis muscle (FCR) of the right wrist. During the testing the participants were seated with their right arm supported and relaxed approximately at 110° elbow angle and secured to the armrest at the wrist (Figure 7). Prior to measurements EMG electrodes were placed on skin. Hot spot for transcranial magnetic stimulation and optimal electrical stimulation site was searched for and marked with a marker pen. Electrodes for electrical stimulation were attached. TMS was used to measure input-output curve before (PRE), right after (POST) and 20 minutes after ($POST_{20}$) PAS or motor training. Maximal M-wave was measured prior to PRE measurement of TMS and between POST measurements of TMS. Muscle EMG was recorded during juggling for each participant on MT5 or retention session to ensure that the FCR muscle was rhythmically active during juggling (Appendix 1).

TABLE 5. Experimental design. Protocol started with PAS intervention on week 1. Week 2 consisted of 5 motor skill training session on consecutive days. Retention skill test and skill training session took place six days after MT5 on week 3.

WEEK 1	WEEK 2			WEEK 3
PAS	MT1	MT2, MT3, MT4	MT5	MTRET
Questionnaires	RT	MT	RT	RT
M_{max} PRE	M_{max} PRE		M_{max} PRE	M_{max} PRE
TMS PRE	TMS PRE		TMS PRE	TMS PRE
PAS	Juggling tutorial		MT	Retention test
TMS POST	PRE test		TMS POST	MT
M_{max} POST	MT		M_{max} POST	TMS POST
$TMS\ POST_{20}$	TMS POST		$TMS\ POST_{20}$	M_{max} POST
	M_{max} POST		Juggling EMG	$TMS\ POST_{20}$
	$TMS\ POST_{20}$			Juggling EMG
				Transfer test

PAS= paired associative stimulation, RT=reaction time test, TMS=transcranial magnetic stimulation, PRE test, retention test, transfer test= juggling skill tests. MT= motor skill training, Juggling EMG= EMG measurement of FCR muscle during juggling.

FIGURE 7. The arm dynamometer chair used in the study.

8.4 **Neurophysiological tests**

8.4.1 EMG

Electromyographic (EMG) activity of the flexor carpi radialis muscle was recorded from the right forearm with disposable bipolar surface electrodes (Blue Sensor N, Ag/AgCl, 0.28 cm2). Location of FCR muscle belly was palpated and skin was prepared by scrapping the skin with sandpaper and cleaning with antiseptic. Electrodes were placed over FCR muscle belly and a ground electrode was placed on elbow (Figure 8). Jaberzadeh et al. (2004) and Stowe et al (2008) were used as a reference for electrode placement. Correct electrode placement and good data quality was verified from the EMG signal at rest and during movement by asking the subject to flex their wrist and fingers. Electrodes for EMG and electrical stimulation were not removed during the session.

EMG signal was amplified (1 000 x) and high-pass filtered (10 Hz) by preamplifier (NL824, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK). Then the signal was band-bass filtered (10 Hz to 1 000 Hz) by another amplifier (NL900D/NL820A Digitimer Ltd., Hertfordshire, UK). The signal was sampled and imported to computer by an A/D converter (CED power 1401, Cambridge Electronics Design Limited, Cambridge, UK). Signal was recorded and analysed with Spike2 software (Cambridge Electronics Design, Cambridge, UK).

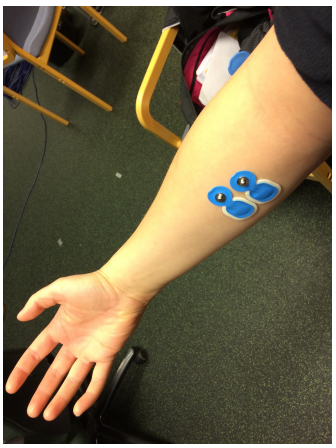

FIGURE 8. EMG electrode placements over the FCR muscle belly.

8.4.2 Peripheral nerve stimulation

Peripheral nerve stimulation was performed using a Digitimer stimulator (DS7A, Digitimer Ltd, Hertfordshire, UK). A single-use WhiteSensor 4500M (d=6.35 cm) electrode was used as cathode and a multiuse V–trodes (d=3.18 cm) electrode was used as anode for the stimulation. The ipsilateral median nerve was stimulated at inside of right elbow. The whiteSensor electrode was placed on skin inside the elbow over cubital fossa as in Green et al (2015). V–trodes electrode was placed on skin medial to biceps as in Stowe et al (2008), few centimeters proximal and medial from the other electrode. Before the single-use cathode electrode was attached, an optimal stimulating site was searched moving a different cathode on the skin inside and over the elbow and giving single stimuli.

Motor threshold was evaluated by giving single stimuli with different intensities until the smallest intensity inducing a visible twitch of 2^{nd} and 3^{rd} finger and wrist. Then a Maximal M-wave was searched by gradually increasing stimulus intensity from submaximal intensities to supramaximal until M-wave amplitude reached a plateau.

8.4.3 Transcranial magnetic stimulation

TMS was performed with Magstim 2002 stimulator (Magstim, Whitland, UK). An 8-shaped coil (70mm) was used to stimulate right hand FCR muscle motor area. The coil was held over left hemisphere tangential to the scalp with the handle pointing backwards and to the left at 45° angle to the sagittal plane. One researcher was tasked with holding the coil at the right position with help of a custom made support attached to the chair and markings on the head (Figure 9). The hot spot for FCR muscle was searched for starting from 5.3 cm to the left side from Cz and 1.4 cm anterior to Cz as recommended by Wassermann ym. (1992). Cz is a point in skull that is located on sagittal plane on the midpoint between nasion and inion. Resting motor threshold (rMT) was defined as the lowest intensity that induced at least three visible MEPs out of five stimuli (Komi. 2011, pp. 120). Participants were instructed to sit still but relaxed on the chair and count from one to 200 in their mind during the stimulation.

Motor evoked potentials were measured PRE, POST and 20 min POST intervention. I/O curve was measured with stimulus intensities of 100, 110, 120, 130 and 140 % of the RMT so that for each of the intensities, ten consecutive stimuli (Komi 2011, pp.120) were given with randomized interval of 5–9 s between each stimulus. Disturbances during a set of stimuli were noted and an extra stimulus was given per disturbance for replacement. Order of the stimulus sets of different intensities was randomized for each subject before first measurement day and the order stayed for the same subject through the study.

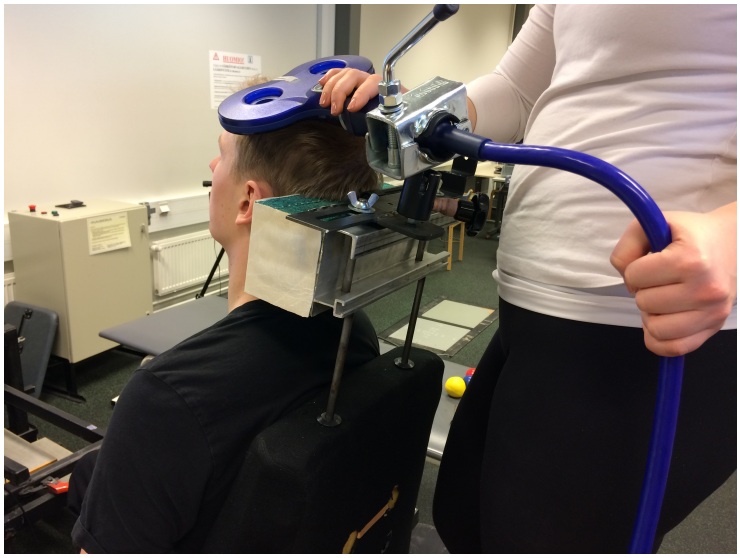

FIGURE 9. A custom made support was used to help keep TMS coil on the wanted position. One researcher was tasked to hold the handle on place.

8.4.4 Paired associative stimulation

PAS consisted of 200 stimulus pairs of peripheral nerve stimulation to the right the ipsilateral median nerve and TMS targeting the FCR muscle of right hand. Interstimulus interval between stimuli was set at 20 ms (Lamy et al 2010), with stimulus intensity of 1,5 x MT for peripheral nerve stimulation and 120 % rMT for TMS. Participants were asked to count from 1 to 200 in their minds and then start over, while focusing on the feeling of the hand being stimulated.

8.5 Motor skill training and testing

8.5.1 Juggling skill training and testing

Participants trained three-ball cascade juggling for five days (MT1–MT5) during week 2. Retention and transfer of the skill was tested six days after MT5 during retention training session (MTRET). A training session consisted of a warm up and a 30-minute long 3-ball-cascade juggling training session (Figure 10). MT1 and MTRET included also additional skill testing. Before the first session participants watched a tutorial video about the juggling technique. The tutorial was filmed with the help of a local juggler and juggling teacher and was only used for the purpose of this study. Training was conducted with beanbag juggling balls (130 g, 66 mm, colours: yellow, red and blue).

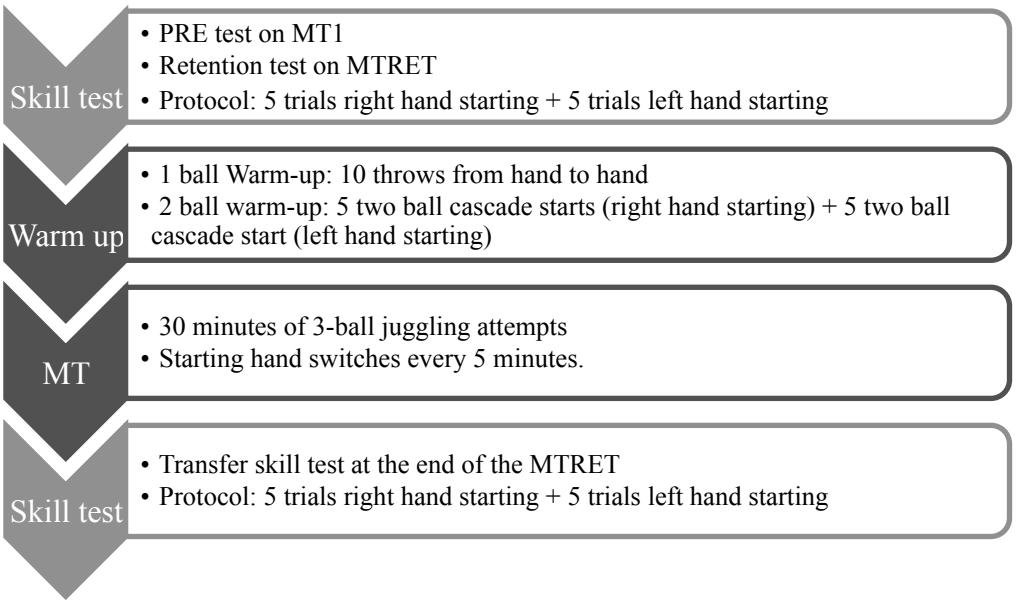

FIGURE 10. Design of motor skill training sessions. First session and retention session started with skill tests. Training started with 1-ball and 2-ball warm up cascade throws. Warm up was followed by 30 minutes long 3-ball cascade juggling training session.

Sessions started with warm-up throws: first 10 throws from one hand to another with one ball and 10 cycles of 2-ball-cascade with two balls. After warm-up the 3-ball juggling session

consisted of 30 minutes of 3 ball cascade attempts with the starting hand switching every 5 minutes. One researcher supervised and timed the training. Researcher did not give any feedback or advice about the juggling technique during the training but made sure that the participants remembered the task correctly and encouraged if needed.

After watching the juggling tutorial during the first training session the participants performed juggling skill-test that consisted of ten three-ball-juggling attempts, five starting with right hand and another five starting with left. On the retention day the juggling session started with a similar 10 test of ten attempts. The duration of the retention test was reduced from the training time so that the 3-ball training and the test together lasted 30 minutes. At the end of the retention appointment participants performed one last transfer test with three balls of different sizes and weights (50 g, 200 g and 300 g) (Custom made for the study).

Juggling skill was monitored during training sessions and additional skill tests (MT1 and retention). First and last ten minutes of the 3-ball-cascade training were recorded on a video camera. Pre skill test, retention test and transfer test were recorded in full.

8.5.2 Reaction time testing

A simple reaction time test was designed to test reaction time (RT) and its development during the study. The test was remodelled after Taimela (1991) study protocol. Testing took place at the beginning of the first, fifth and retention motor skill training sessions before neurophysiological testing. During the test the participants were seated on the arm dynamometer chair with the right arm secured from wrist on the armrest approximately at a 110° elbow angle. The force dynamometer was connected to computer via A/D converter. Participants were instructed to correspond to flashing light by flexing arm and wrist against the wrist strap briefly as fast as possible. An investigator held the light approximately 1.5 meters from the participant at the eyelevel. Test consisted of 10 visual stimuli given randomly with inter-trial interval ranging between 1–5 s. Preceding the test the participants practised the event with 3 warm up trials. Hearing protectors were used to ensue that participants reacted to light and not clicking sounds coming from the equipment.

8.6 **Data analysis**

Activity levels of recreational activities were graded as 0=low activity (< 1 session / week), 1 = amateur (1-2 sessions / week), 2 = active (3-5 sessions / week) and 3= high activity (>5 sessions / week). Activity level was determined for participation to sports and exercise (henceforth SPORTS), for participation to activities employing manual dexterity (henceforth DEX) and for combined activity of sports and manual skill (henceforth SPORTS&DEX). Handedness was calculated according to Cohen 2008. As all participants were right handed the handedness rating was not used in further analysis.

Successful catches per attempt (CPA) was used as a measure of juggling skill. CPA was calculated from video clips recorded during motor skill training sessions. The first 10 attempts of the 3-ball juggling on the first and last five minutes of each session were analysed. For each juggling attempt the consecutive catches were summed up until a ball was dropped. Ten consecutive attempts were analysed and CPA was calculated as an average of trials. Skill development was calculated as change in successful catches per juggling attempt (ΔCPA) between points of interest. ΔCPA was determined from PRE and POST measurements of every motor skill training session. Also ΔCPA was defined for change of juggling skill from starting skill test (PRE) to $MT1_{POST}$, $MT5_{POST}$ and retention test. Skill acquisition day was determined to be the training day when CPA ≥ 4 was achieved and from when onward the average CPA≥ 4 was well maintained as in the protocol of Bebko et al (2003). Problems with video recording caused missing data for two participants on MT4.

Consolidation of the skill was tested to see how well the participants retained the gained skill level after break from training. Consolidation after MT1 ($CONS_{MT1}\Delta\%$) was measured as percentage change of CPA from $MT1_{POST}$ to $MT2_{PRE}$. Similarly percentage retention (RETΔ%) and transfer (TRANSFΔ%) of the skill was analysed as percentage change: RETΔ% as a percentage change of CPA from $MT5_{POST}$ to Retention test and TRANSFΔ% as percentage change of CPA from $MTRET_{POST}$ to Transfer test.

Reaction time data was analysed with Spike2 software. RT was analysed as the time between marker for stimulus and the start of force production. Reaction time for MT1 (henceforth RT1), for MT5 (henceforth RT5) and retention session (henceforth RTRET) was defined as an average of ten trials. The change of reaction time was reported as difference between reaction times. RT5 is missing in one participant. Otherwise a complete data set was acquired.

Maximal peak-to-peak M-wave amplitude was analysed from the raw data to get a PRE and POST value for each session with Spike2 software. Dysfunction of EMG pre-amplifier caused some Mmax measurements to fail. Out of 13 participants the number of participants that had missing data point either or both PRE and POST measurement on each session was: PAS=1, MT1=2, MT5=3 and MTRET=2. Eight participants had a complete data set for M_{max} PRE measurements from every session.

Peak-to-peak MEP amplitudes were analysed from the raw data with Spike2 software. Raw EMG data was scanned visually to screen out poor quality MEPs. Then MEP amplitude was determined for each successful trial. Average MEP amplitudes were calculated for each of stimulus intensities PRE intervention, POST intervention and 20 minutes POST intervention (POST20). Then the average MEP size of all the intensities was calculated. The change of MEP amplitudes during the intervention was reported as percentage change. The change from PRE intervention to POST intervention is henceforth referred as $\Delta\%_{POST}$ and the change from PRE intervention to POST20 as $\Delta\%_{POST20}$. Absolute MEP amplitude values were used for acute MEP change comparisons of single sessions because of the high number of failed M-wave measurements. Normalized values (MEP/M_{max}) were used for comparison of baseline MEPs from different days. Percentage change of baseline MEP/M_{max} was calculated between PAS and MT1, MT1 and MT5 and between MT1 and RET. This comparison was calculated only for participants that had complete MEP/M_{max} data from PRE measurement of each day (n=8).

8.7 **Statistical analyses**

For each parameter, mean and standard deviation (SD) were analysed. Non-parametric tests were used for statistical analysis. Statistical analysis was performed with SPSS. Related samples Wilcoxon signed rank test was used to search for statistically significant differences and Spearman's rank-order correlation was used for correlational analyses. Statistical significance was set at $p < 0.05$ and $p < 0.1$ was set for trends.

Juggling. Wilcoxon signed rank test was used to search for statistically significant differences between CPA PRE and POST of every motor training session. Significance of the development of skill during 5-day training period was analysed by comparing each MT_{POST} result to the MT_{POST} of the next day. Consolidation of skill was tested comparing CPAs of MT_{POST} and $MT+1_{PRE}$. Retention was tested comparing of $MT5_{POST}$ to retention test and transfer was tested comparing $MTRET_{POST}$ to transfer test. Motor skill training sessions MT1, MT5 and MTRET were selected as points of interest and all further statistical analyses focused on those. Spearman's rank-order correlation was analysed between PRE test result and later skill learning results. Results of relative skill consolidation, relative retention, relative transfer and their relationship to each other and other skill learning results was also analysed with Spearman's rank-order correlation.

Reaction time (RT). Statistically significant differences were searched in reaction time results RT1, RT5 and RTRET with Wilcoxon signed rank test. Spearman's rank-order correlation between reaction time results and between reaction time and juggling skill results was analysed.

Neurophysiological tests. Wilcoxon signed rank test was used to test the difference between MEP amplitudes from PRE to POST and from PRE to $POST_{20}$ of every TMS measurement session. Difference between M_{maxPRE} and $M_{maxPOST}$ was tested similarly at every TMS measurement session. In-session comparisons were conducted with all available data points. Multisession baseline MEP/M_{max} differences between PAS and MT1, MT1 and MT5 and between MT1 and MTRET were tested for statistical significance for the group of 8

participants that had complete MEP/M_{max} data set for baseline measurements. Spearman's rank-order correlation was analysed between different combinations of in-session and multisession MEP changes with the focus on correlation between MEP changes on first sessions (PAS and MT1) and later in-session or multi-session MEP changes. Spearman's rank-order correlation was analysed between MEP changes (in-session and multisession) and motor performance parameters of juggling and reaction time.

Recreational activity. Spearman's rank-order correlation was also analysed between recreational activity levels and results of MEP changes, juggling and reaction time. The purpose was to check whether activity level interacted with juggling performance, reaction time or plasticity.

9 RESULTS

9.1 Juggling skill and reaction time

9.1.1 Juggling performance

Participants advanced in juggling skill during the intervention (Figure 11). Average change of juggling skill was at PRE–$MT1_{POST}$ 1.8 (SD=1.5, n=13), at PRE–$MT5_{POST}$ 18.6 (SD=30.2, n=13) and at PRE–retention 10.6 (SD=13.8, n=13) catches per attempt (CPA). PRE test result correlated positively with later juggling performance: $CPAMT1_{POST}$ (n=13, r_s=0.78, p=0.002), $CPAMT5_{POST}$ (n=13, r_s=0.87, p=0.000) and retention test (n=13, r_s=0.88, p=0.000). Nine participants reached the skill acquisition criteria (CPA ≥ 4) during the five-day training period (Figure 12).

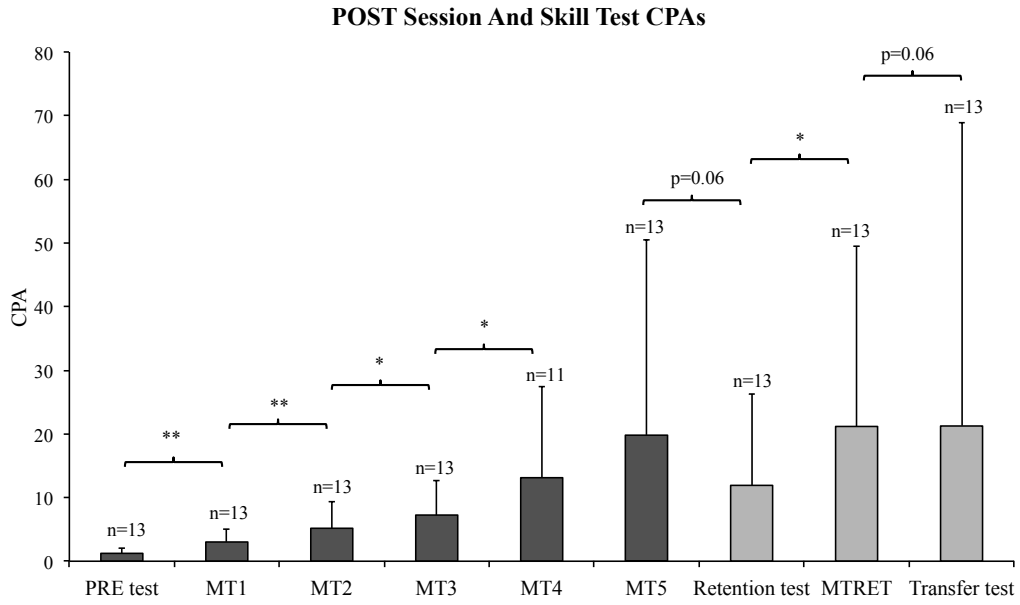

FIGURE 11. Catches per attempt of juggling (CPA) after each motor skill training session MT1-MTRET and at skill tests (PRE, Retention and Transfer). Statistically significant differences between consecutive points of time are flagged: *=p<0.05, **=p<0.01.

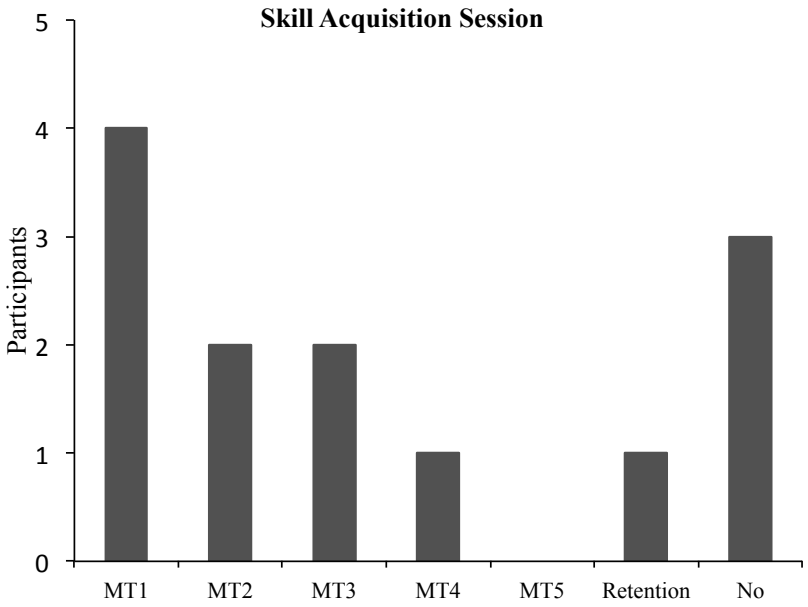

FIGURE 12. Histogram of skill acquisition session. Skill acquisition session was defined as the session when a participant reached juggling skill level of CPA ≥ 4.

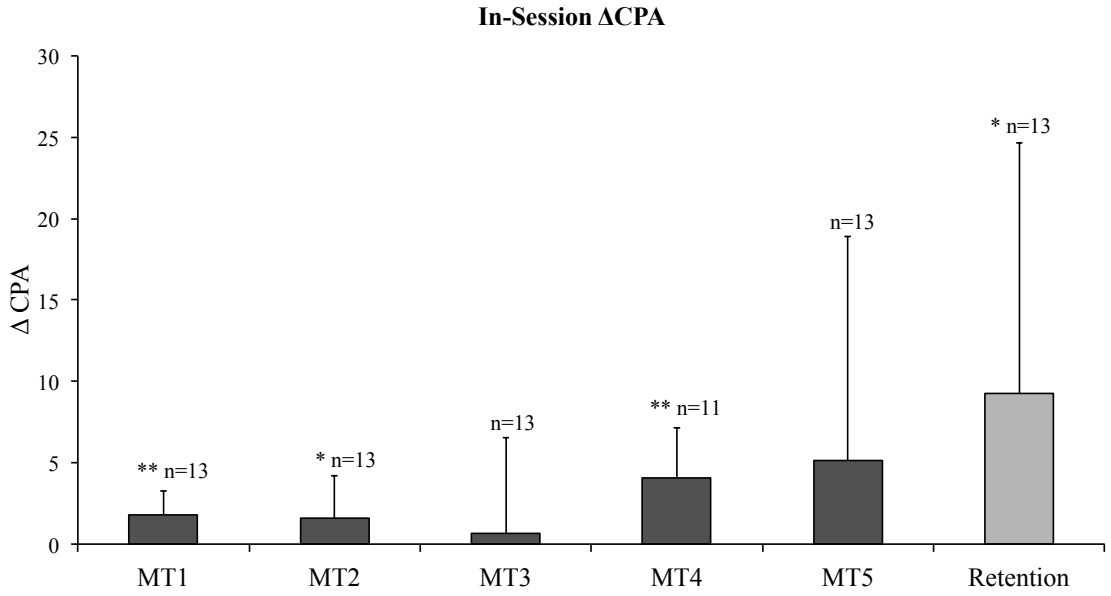

FIGURE 13. Change of average catches per attempt of juggling (CPA) during single motor training sessions (MT1–MT5) and retention session. *=p<0.05; **=p<0.01 =statistically significant difference between PRE and POST MT.

In-session juggling skill development. Development of skill during single session was statistically significant on MT1, MT2, MT3, MT4 and MTRET ($p<0.05$ or $p<0.01$) (Figure 13). There were no statistically significant changes in CPA during consolidation periods of approximately 24 hours. On average CPA increased by 27 % (SD=77, n=13) during the consolidation period between MT1 and MT2. Relative consolidation of MT1 did not correlate with CPA or changes in CPA during the study but correlated with relative retention of juggling skill (n=13, r_s=0.56, p=0.05).

Retention. CPA decreased during the break between MT5 and Retention session for 10/13 participants (Wilcoxon p=0.06). Average change of CPA was -7.9 catches (SD=16.9, n=13) and as percentage -16% (SD=38, n=13). Relative retention correlated negatively and statistically significantly with following juggling results: $CPAMT1_{POST}$ (n=13, r_s=-0.64, p=0.02), $CPAMT5_{POST}$ (n=13, r_s=-0.61, p=0.03), ΔCPAMT1 (n=13, r_s=-0.77, p=0.002), and $\Delta CPAPRE–MT5_{POST}$ (n=13, r_s=-0.62, p=0.02).

Transfer. Transfer test result was smaller compared to $MTRET_{POST}$ for 10/13 participants (Wilcoxon p=0.06) with average change of the whole group: ΔCPA=0.1 (SD=22,3, n=13) and Δ%CPA=-24% (SD=37, n=13). The difference between RET_{POST} and Transfer test did not reach statistical significance (p=0.06). Relative transfer did not correlate with CPA or change of CPA at any point during the study. During the transfer test one participant gained CPA by 70 from the RET_{POST}, which accounted for the small average change of CPA. This participant had acquired juggling skill fast and reported boredom during retention session. All participants that had acquired the skill (CPA ≥ 4) also maintained skill level that was above acquisition criteria at transfer test.

9.1.2 Reaction time

Average reaction time (RT) was 216 ms (SD=0.048, n=13) at baseline and did not change statistically significantly as a group during the intervention (Figure 14). Average change of reaction time from first to fifth training session was -2 ms (SD=21, n=12, p=0.64) and from first to retention training session -4 ms (SD=34, n=13, p=0.46). RT1 correlated significantly

with RTRET (r_s=0.61, p=0.03) but not with RT5 (r_s=0.36, p=0.26). Reaction time on first session correlated negatively and statistically significantly with change in reaction time ΔRT1–RT5 (n=12, r_s=-0.68, p=0.02) and with a trend with ΔRT1–RTRET (n=13, r_s=-0.48, p=0.09). Reaction time change ΔRT1–RT5 correlated with ΔRT1–RTRET (n=12, r_s=0.685, p=0.01).

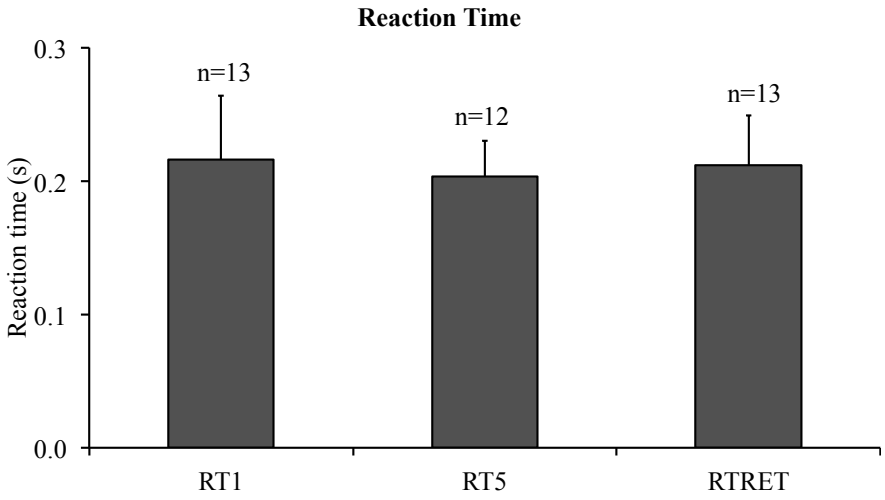

FIGURE 14. Reaction time at the start of the first (RT1), fifth (RT5) and retention (RTRET) training sessions.

Correlation between reaction time and juggling skill. Baseline reaction time RT1 correlated significantly with the baseline juggling test result (n=13, r_s=-0.59, p=0.03) and the transfer skill test (n=13, r_s=-0.59, p=0.04). The change in reaction time from RT1 to RTRET correlated significantly with change of CPA during the same timeline (r_s=0.66, p=0.01). The same was not true for the development of the parameters from day 1 to day 5 (n=12, r_s=-0.07, p=0.83). Skill acquisition day correlated almost significantly with reaction time change from RT1 to RTRET (n=13, r_s=-0.55, p=0.053). Reaction time or reaction time changes did not correlate with relative consolidation POST MT1, relative retention or relative transfer.

9.2 **TMS**

9.2.1 MEP amplitudes and MEP changes

PAS session. Average MEP amplitudes increased compared to PRE value in 8 of 13 participants right after PAS and in 8 of 12 participants 20 minutes after PAS. For six participants MEP amplitudes were elevated both right after PAS and 20 minutes after. On average MEP amplitudes increased PRE to POST by 18 % (SD=36, n=13) and PRE to $POST_{20}$ by 16 % (SD=31, n=12). Differences between MEP amplitudes were not significant PRE to POST (p=0.28, n=13) or PRE to $POST_{20}$ (p=0.07, n=12) (Figure 15 A). No statistically significant differences were seen between MEP amplitudes at any single stimulus intensities from PRE to POST values or PRE to $POST_{20}$ values (Figure 15 B). Maximal M-wave amplitude was 9.10 mV (SD=4.62, n=12) PRE and 9.23 mV (SD=4.78, n=12) POST and there was no statistically significant change (p=0.75, n=12).

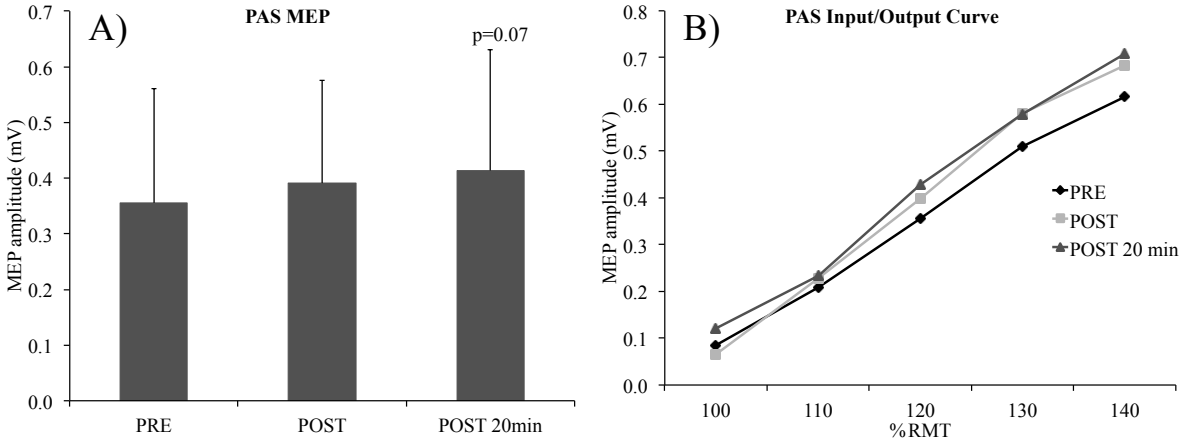

FIGURE 15. Average MEP amplitudes before (PRE, n=13), right after (POST n=13) and 20 minutes after (POST 20 min, n=12) PAS. There were no statistically significant differences between PRE and POST or POST 20 min MEP amplitudes. Figure A) Shows MEP amplitude averages from all stimulus intensities. There was a trend for increase of average MEP PRE to POST20 (p=0.07). Figure B) shows the Input/Output curve.

MT1. MEP sizes decreased right after first juggling training session for 10 participants and increased for 3 (n=13). After 20 minutes from juggling MEP sizes decreased for 8 participants and increased for 5 participants (n=13) compared to PRE. Average DELTA% right after training was -25 % (SD=32) and -14 % (SD=32) after 20 minutes (n=13). Average MEP size differences were not statistically significant between PRE and POST (n=13, p=0.08) or PRE and $POST_{20}$ (n=13, p=0.13) (Figure 16 A). When looking at single intensities, the change in MEP sizes was significant with intensities of 110, 120 and 140 %RMT right after MT1 and with intensities of 120 and 140 %RMT 20 min after MT1 (Figure 16 B). Maximal M-wave amplitude was 9.04 mV (SD=3.38, n=11) PRE and 9.02 mV (SD=3.57, n=11) POST and there was no statistically significant change (p=1.00, n=11).

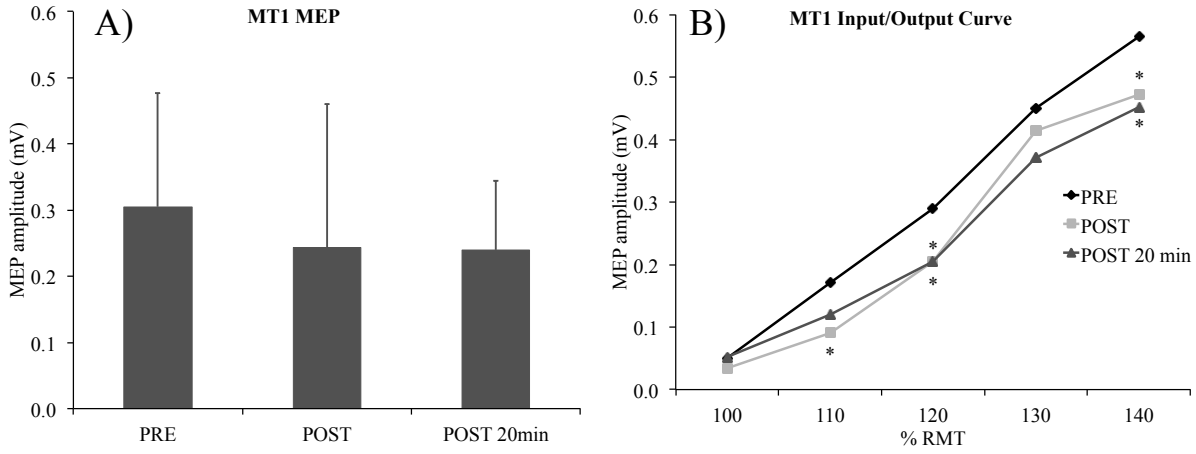

FIGURE 16. Average MEP amplitudes before (PRE), right after (POST) and 20 minutes after (POST 20 min) first motor skill training session (n=13). Figure A) Shows MEP amplitude averages from all stimulus intensities. Figure B) shows Input/Output curve, *=statistically significant difference ($p<0.05$) compared to PRE value.

MT5. MEPs at fifth juggling session decreased right after training for 10 participants and 20 min after for 8 participants (n=13). Average DELTA% was -12% (SD=39) right after and 4 % (SD=49) twenty minutes after compared to PRE (n=13). Average MEP change from PRE to POST was statistically significant (n=13, p=0.05) but the same was not true from PRE to $POST_{20}$ (n=13, p=0.55) (Figure 17 A). At single intensities a statistically significant

difference in MEP sizes was only seen right after juggling training at intensity of 140 %RMT (Figure 17 B). Maximal M-wave amplitude was 8.48 mV (SD=2.66, n=10) PRE and 9.15 mV (SD=3.30, n=10) POST and there was no statistically significant change (p=0.11, n=10).

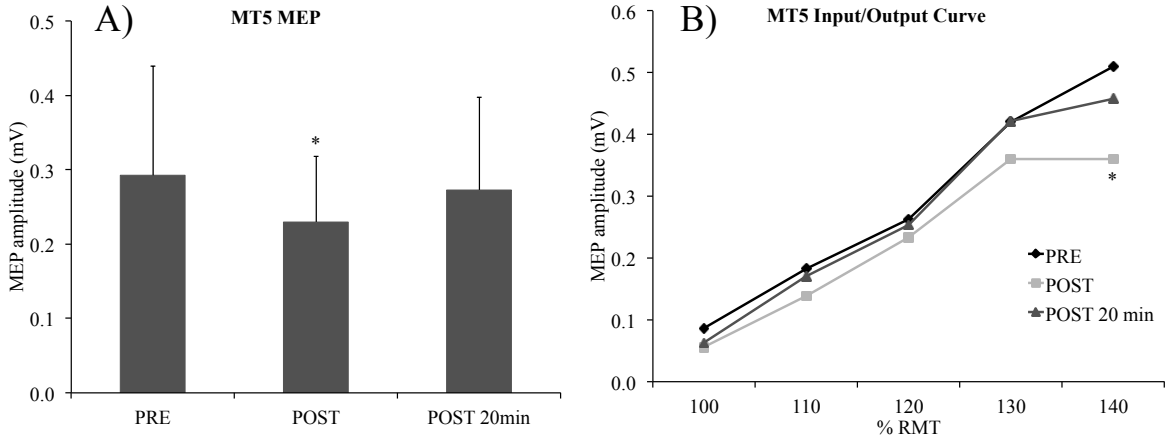

FIGURE 17. Average MEP amplitudes before (PRE), right after (POST) and 20 minutes after (POST 20 min) fifth motor skill training session (n=13). Figure A) Shows MEP amplitude averages from all stimulus intensities. Figure B) shows Input/Output curve. *=statistically significant difference ($p<0.05$) compared to PRE value.

Retention Session. MEP amplitudes decreased right after retention training session for 10 participants and 20 min after for 8 participants (n=13). On average MEP sizes changed -25 % (SD=39) right after juggling and -8 % (SD=36) 20 minutes after juggling compared to PRE (n=13). MEP change was statistically significant from PRE to POST (n=13, p=0.03*) but not PRE to $POST_{20}$ (n=13, p=0.50) (Figure 18 A). At different stimulus intensities a statistically significant difference was found at 110 and 140 %RMT PRE to POST and at 140 %RMT PRE to $POST_{20}$ (Figure 18 B). Maximal M-wave amplitude was 9.34 mV (SD=3.04, n=11) PRE and 8.99 mV (SD=3.22, n=10) POST and there was no statistically significant change (p=0.37, n=11).

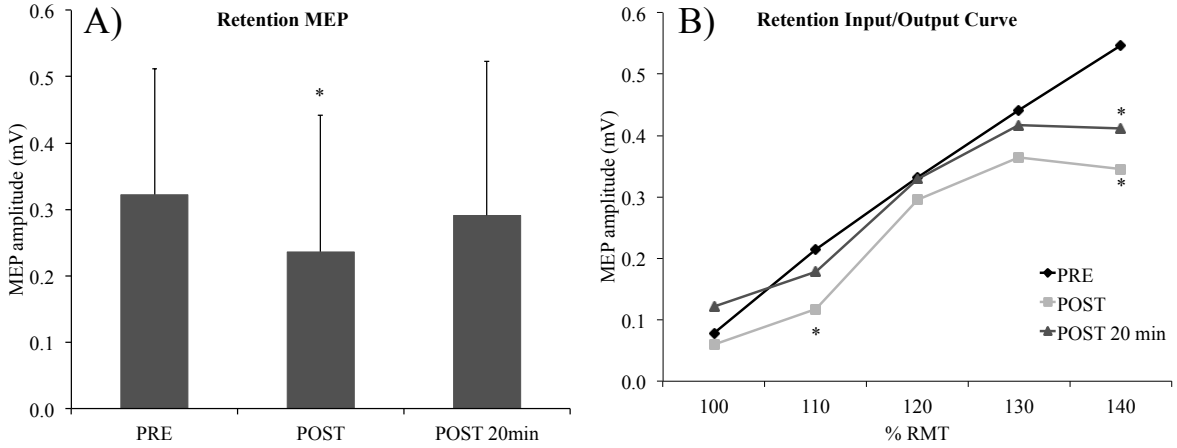

FIGURE 18. Average MEP amplitudes before (PRE), right after (POST) and 20 minutes after (POST 20 min) retention motor skill training session (n=13). Figure A) Shows MEP amplitude averages from all stimulus intensities. Figure B) shows Input/Output curve. *=statistically significant difference ($p<0.05$) compared to PRE value.

Multi-session baseline MEP amplitude development. Average MEP_{PRE}/M_{max} from different days were similar sized and did not change statistically significantly at any point during the training (n=8) (Figure 19). Table shows average changes and standard deviations of baseline MEP changes from PAS to MT1, from MT1 to MT5 and from MT1 to MTRET (Table 6).

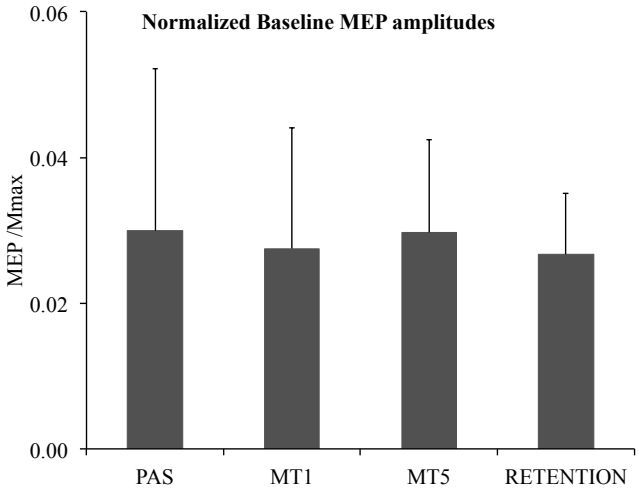

FIGURE 19. Normalized baseline MEP amplitudes on PAS, MT1, MT5 and retention sessions (n=8).

TABLE 6. The percentage change in normalized baseline MEP amplitudes during the study (n=8).

	Change in MEP_{PRE}/M_{max} (Δ%)		
	M	SD	p
PAS_{PRE}–$MT1_{PRE}$	19	55	0.58
$MT1_{PRE}$–$MT5_{PRE}$	18	49	0.58
$MT1_{PRE}$–RET_{PRE}	10	33	0.40

9.2.2 Correlations between MEP amplitude changes

In-session correlations. In-session MEP changes Δ%POST and $\Delta\%POST_{20}$ correlated statistically significantly on PAS session (n=12, r_s=0.75, p=0.005). In-session MEP changes on MT1 correlated but not statistically significantly (n=13, r_s=0.52, p=0.07). In-session MEP changes on MT5 correlated statistically significantly (n=13, r=0.70, p=0.008). In-session MEP changes on retention session did not correlate statistically significantly (n=13, r_s=0.48, p=0.10).

Correlation between MEP changes on PAS and MEP changes on later sessions. $\Delta\%PAS_{POST}$ correlated with $\Delta\%RET_{POST20}$ (n=13, r_s=-0.59, p=0.04). $\Delta\%PAS_{POST}$ also correlated, though not statistically significantly with $\Delta\%MT5_{POST20}$ (n=13, r_s=0.43, p=0.14) and $\Delta\%RET_{POST}$ (n=13, r_s=-0.45, p=0.12). Other correlations between $\Delta\%PAS_{POST}$ and MEP changes on other sessions were small and not significant. $\Delta\%PAS_{POST20}$ did not correlate with MEP changes during MT1, MT5 or MTRET.

Correlation between MEP changes on MT1 and later session. $\Delta\%MT1_{POST}$ correlated negatively and significantly with $\Delta\%MT5_{POST}$ (n=13, r_s=-0.63, p=0.02) and near significantly wit $\Delta\%MT5_{POST20}$ (n=13, r_s=-0.54, p=0.06). There was a negative trend for correlation between $\Delta\%MT1_{POST20}$ and $\Delta\%RET_{POST20}$ (n=13, r_s=-0.51, p=0.08). No other notable correlations were observed between MEP amplitude changes from different sessions.

Correlation between in-session MEP changes and multi-session baseline MEP amplitude changes. In-session MEP change PRE to $POST_{20}$ PAS correlated with baseline MEP

amplitude change from PAS to MT1 (Figure 20) but otherwise in-session changes during PAS did not correlate with baseline MEP changes. In-session MEP change $\Delta\%MT1_{POST}$ correlated statistically significantly with baseline MEP change from PAS to MT1 (r_s=-0.81 p=0.02, n=8) and not significantly with baseline MEP changes from MT1 to MT5 (r_s=0.62, p=0.10, n=8). In-session MEP change $\Delta\%MT1_{POST20}$ did not correlate with baseline MEP amplitude changes other than with baseline MEP change from PAS to MT1 (r_s=-0.71, p=0.05, n=8). Correlation between in-session MEP changes on later sessions (MT5 and MTRET) and baseline MEP changes were not tested.

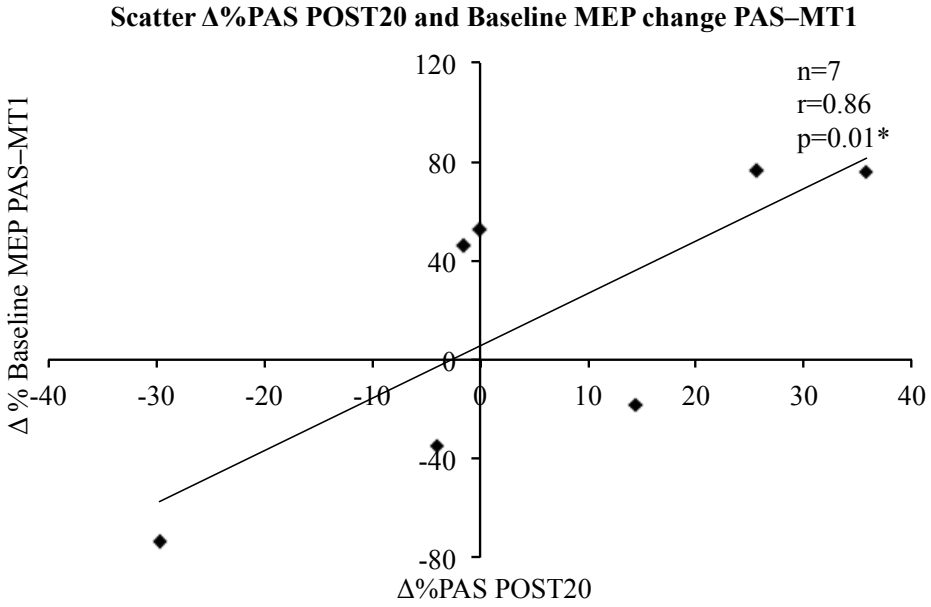

FIGURE 20. Scatter diagram of acute MEP amplitude change PRE to 20 min POST PAS and baseline MEP amplitude change from PAS to MT1.

9.3 **Correlations between MEP changes and results of juggling and reaction time**

Correlations between MEP changes on PAS and motor learning parameters. Change in MEP size from PRE to POST PAS correlated with negatively and statistically significantly with percentage transfer (Figure 21 A), but correlation was no longer significant at $\Delta\%POST_{20}$ (Figure 21 B). Otherwise MEP changes did not correlate with juggling results (Table 7). A statistically significant correlation was found between reaction time at the retention and

$\Delta\%PAS_{POST20}$ (n=12, r_s=0.58, p=0.05). Otherwise Δ%PAS and RT results did not correlate statistically significantly.

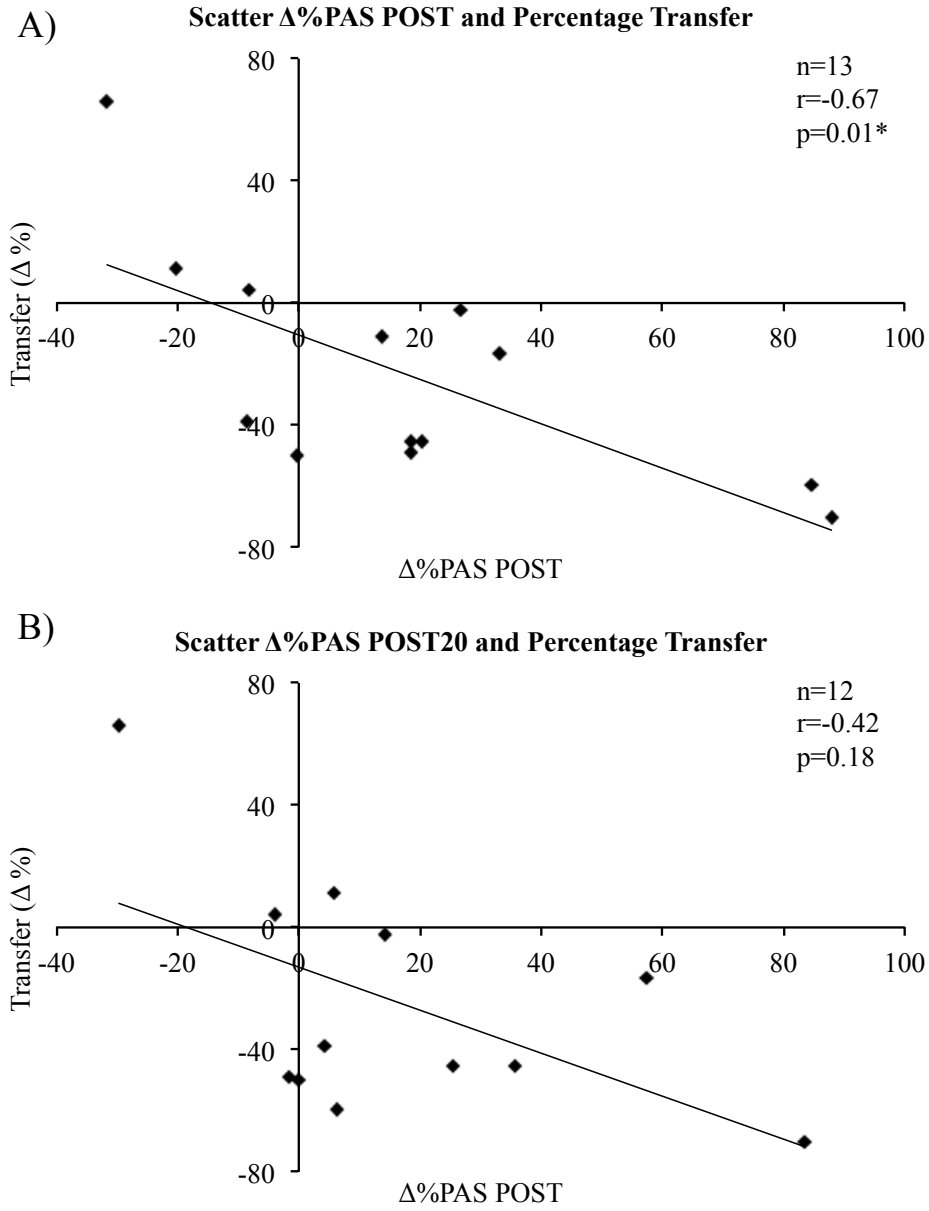

FIGURE 21. Scatter diagram of MEP changes on PAS and relative skill transfer (Δ%). A) MEP change from PRE to POST, and B) MEP change from PRE to 20 min POST. r=Spearman's rho correlation coefficient.

TABLE 7. Correlation between MEP change during PAS and results of juggling skill and reaction time. Percentage change of MEPs was analysed from PRE to POST ($\Delta\%PAS_{POST}$) and from PRE to 20 minutes POST PAS ($\Delta\%PAS_{POST20}$).

	$\Delta\%PAS_{POST}$			$\Delta\%PAS_{POST20}$		
	r_s	p	n	r_s	p	n
CPAPRE	0.11	0.72	13	0.06	0.85	12
$CPAMT1_{POST}$	0.07	0.82	13	-0.02	0.94	12
$CPAMT5_{POST}$	0.05	0.86	13	0.00	1.00	12
CPARET	-0.15	0.62	13	-0.24	0.46	12
CPA Transfer	-0.06	0.84	13	-0.14	0.66	12
ΔCPAMT1	0.01	0.99	13	-0.13	0.70	12
$\Delta CPAPRE\text{-}MT5_{POST}$	0.04	0.89	13	0.00	1.00	12
ΔCPAPRE-RET	-0.16	0.60	13	-0.24	0.46	12
Skill acquisition	-0.16	0.60	13	-0.17	0.59	12
CONSΔ%MT1	-0.27	0.36	13	0.02	0.95	12
RETΔ%	-0.28	0.36	13	-0.13	0.70	12
TRANSFΔ%	**-0.67**	**0.01***	**13**	-0.42	0.18	12
RT1	0.19	0.54	13	0.22	0.48	12
RT5	-0.01	0.98	12	0.08	0.81	11
RTRET	0.19	0.54	13	**0.58**	**0.05***	**12**
ΔRT1–RT5	-0.22	0.48	12	-0.10	0.77	11
ΔRT1–RTRET	-0.26	0.39	13	0.09	0.78	12

Abbreviations: CPA= Catches per attempt of juggling, ΔCPA= Change of juggling skill, Skill acquisition= session when CPA≥4, $CONS_{MT1}$=percentage consolidation of skill after MT1 (Δ%), RETΔ%= percentage retention of skill (Δ%), TRANSF= percentage transfer of skill (Δ%), RT= reaction time (s), ΔRT=reaction time change (s).

r_s= Spearman´s Rho correlation coefficient

*= $p<0.05$; **=$p<0.01$

Correlations between MEP changes on MT1 and motor learning parameters. A trend of correlation was found between $\Delta\%MT1_{POST}$ and percentage transfer (Δ%) (n=13, r_s=0.54, p=0.06), but otherwise MEP changes on first training session did not correlate with juggling skill results (Table 8). MEP change from PRE to $POST_{20}$ correlated statistically significantly with RT5 (Figure 22 A) and near significantly with RTRET (n=13, r_s=-0.53, p=0.06).

Participants that increased MEP amplitudes PRE to $POST_{20}$ also improved their reaction times from RT1 to RT5, but correlation between MEP change and reaction time change was not statistically significant (Figure 22 B).

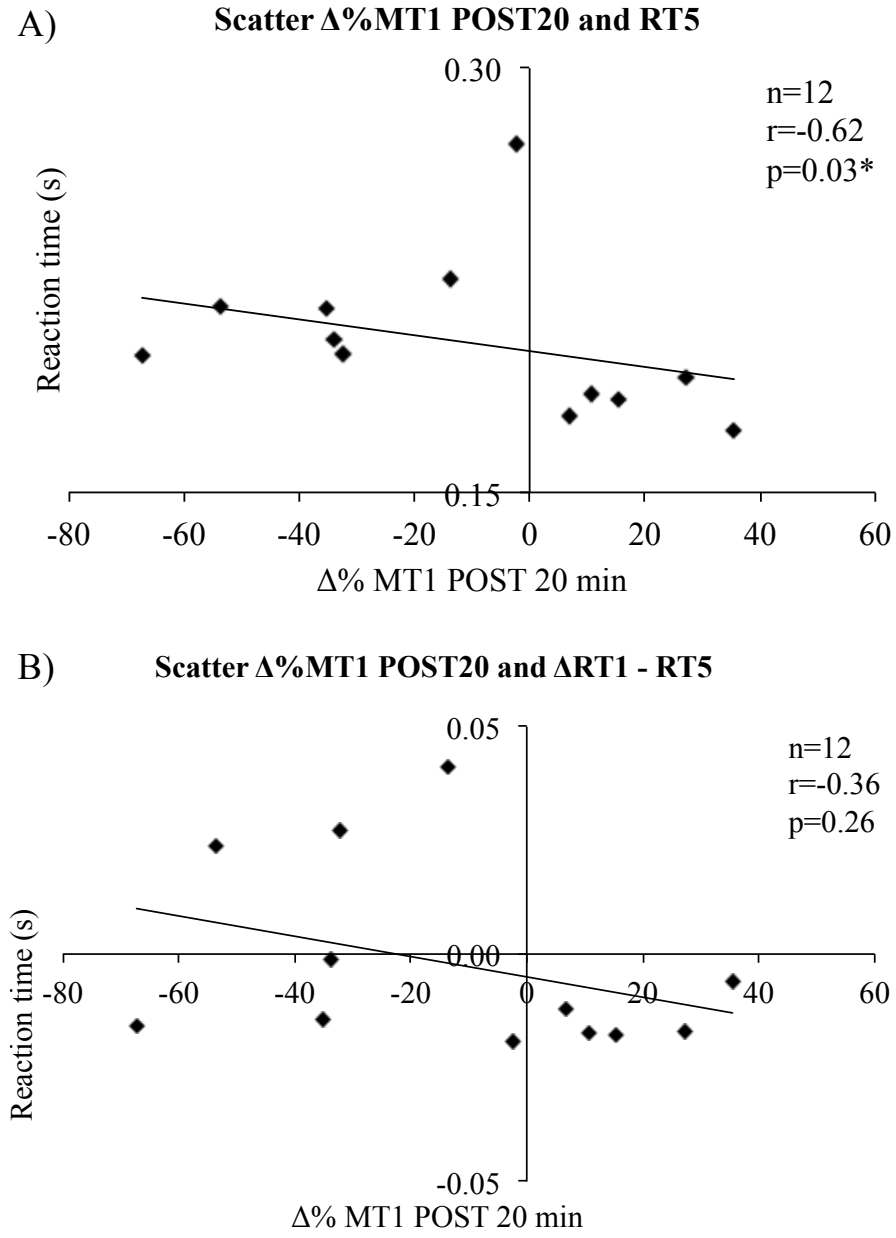

FIGURE 22. Correlation between MEP change from PRE to 20 minutes POST during first training session and A) reaction time on MT5 and B) reaction time change from first training day to fifth. r=Spearman's rho correlation coefficient, n=12

TABLE 8. Correlation between MEP change during first training session and results of juggling skill and reaction time. Percentage change of MEPs was analysed from PRE to POST ($\Delta\%MT1_{POST}$) and from PRE to 20 minutes POST ($\Delta\%MT1_{POST20}$).

	$\Delta\%MT1_{POST}$			$\Delta\%MT1_{POST20}$		
	r	p	n	r	p	n
CPAPRE	-0.17	0.57	13	-0.13	0.68	13
$CPAMT1_{POST}$	0.06	0.84	13	0.02	0.94	13
$CPAMT5_{POST}$	-0.05	0.87	13	-0.06	0.84	13
CPARET	-0.18	0.57	13	-0.16	0.59	13
CPA Transfer	-0.08	0.79	13	-0.10	0.75	13
ΔCPAMT1	0.14	0.65	13	0.18	0.55	13
$\Delta CPAPRE–MT5_{POST}$	-0.03	0.91	13	-0.07	0.83	13
ΔCPAPRE–RET	-0.17	0.58	13	-0.17	0.58	13
Skill acquisition	-0.11	0.76	13	-0.09	0.76	13
CONSΔ%MT1	0.15	0.63	13	-0.04	0.90	13
RETΔ%	-0.07	0.82	13	-0.26	0.38	13
TRANSFΔ%	0.54	0.06	13	0.19	0.53	13
RT1	-0.08	0.79	13	-0.36	0.23	13
RT5	-0.45	0.14	12	**-0.62**	**0.03***	**12**
RTRET	-0.01	0.97	13	-0.53	0.06	13
ΔRT1–RT5	-0.17	0.60	12	-0.36	0.26	12
ΔRT1–RTRET	-0.02	0.94	13	-0.31	0.30	13

Abbreviations: CPA= Catches per attempt of juggling, ΔCPA= Change of juggling skill, Skill acquisition = session when CPA≥4, $CONS_{MT1}$=percentage consolidation of skill after MT1 (Δ%), RETΔ%= percentage retention of skill (Δ%), TRANSF= percentage transfer of skill (Δ%), RT= reaction time (s), ΔRT=reaction time change (s).

r_s= Spearman´s Rho correlation coefficient

*= $p<0.05$; **=$p<0.01$

Correlations between MEP changes on later training sessions and motor learning parameters. MEP changes on MT5 did not correlate either with juggling results or reaction time results (Appendix 2). $\Delta\%RET_{POST}$ correlated, though not statistically significantly with reaction time at retention (n=13, r=0.51, p=0.07), but otherwise MEP changes did not correlate with juggling or reaction time results on retention session (Appendix 3).

Correlations between multi-session baseline MEP amplitude changes and motor learning parameters. Percentage changes of baseline MEP/M_{max} did not correlate statistically significantly with juggling skill results (Table 9). Percentage change of baseline MEP/M_{max} MT1–MT5 correlated positively with reaction time RT1 (Figure 23 A) and negatively with change of reaction time ΔRT1–RT5 (Figure 23 B).

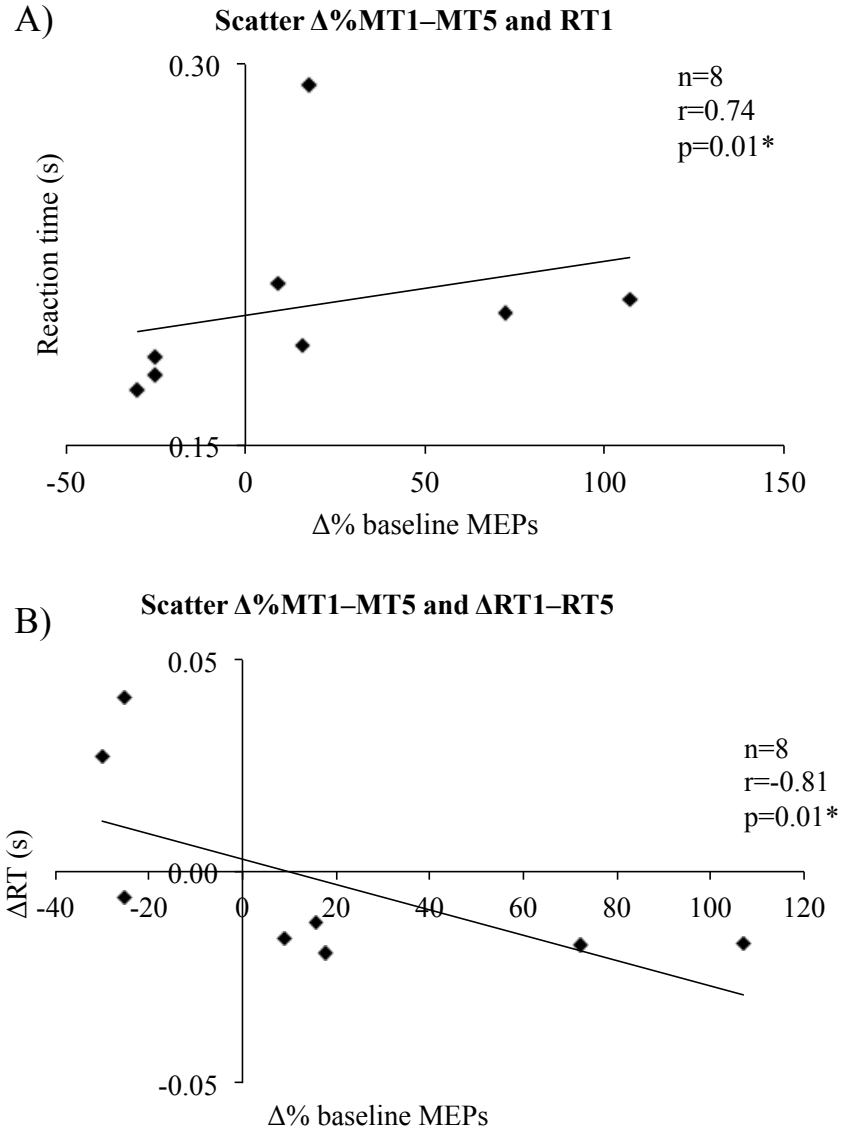

FIGURE 23. Relationship between multi-session change of baseline MEPs and A) baseline reaction time, B) change of reaction time from first to fifth training session. r=Spearman's rho correlation coefficient, n=8.

TABLE 9. Correlation between multi-session baseline MEP change and results of juggling skill and reaction time. Percentage change of baseline MEPs was calculated from MT1 to MT5 and from MT1 to MTRET. n=8.

n=8	Δ% baseline MEP/M_{max} MT1–MT5		Δ% baseline MEP/M_{max} MT1– RET	
	r	p	r	p
CPAPRE	-0.29	0.49	-0.24	0.57
$CPAMT1_{POST}$	-0.24	0.57	-0.32	0.43
$CPAMT5_{PRE}$	-0.02	0.96	-0.43	0.29
CPARET	-0.10	0.82	-0.50	0.21
CPA Transfer	-0.24	0.57	-0.38	0.35
ΔCPAMT1	0.25	0.55	0.11	0.80
$\Delta CPAPRE\text{-}MT5_{POST}$	0.12	0.78	-0.38	0.35
ΔCPAPRE–RET	-0.02	0.96	-0.48	0.23
Skill acquision	0.03	0.95	0.28	0.51
$CONS_{MT1}$	-0.05	0.91	-0.36	0.39
RETΔ%	-0.57	0.14	-0.14	0.74
TRANSFΔ%	0.45	0.26	-0.14	0.74
RT1	**0.74**	**0.04***	0.33	0.42
RT5	-0.05	0.91	0.33	0.42
RTRET	0.29	0.49	0.55	0.16
ΔRT1–RT5	**-0.81**	**0.01***	-0.05	0.91
ΔRT1–RTRET	-0.36	0.38	-0.10	0.82

Abbreviations: CPA= Catches per attempt of juggling, ΔCPA= Change of juggling skill, Skill acquisition = session when CPA≥4, $CONS_{MT1}$=percentage consolidation of skill after MT1 (Δ%), RETΔ%= percentage retention of skill (Δ%), TRANSF= percentage transfer of skill (Δ%), RT= reaction time (s), ΔRT=reaction time change (s).

r_s= Spearman´s Rho correlation coefficient

*= $p<0.05$; **=$p<0.01$

9.4 **Effect of recreational activity**

Five participants participated in some activities that exploit novel motor control of hand area including handcrafts (F=1), guitar playing (F=2) and piano playing (F=2). All of them

reported that they where not training actively at the time of the study. All but one participant participated in regular sports or exercise activities (Table 10).

TABLE 10. The number of participants in each recreational activity level category (n=13).

Recreational activity level	<1 times/wk	1-2 times/wk	3-5 times/wk	> 5 times/wk
SPORTS	1	3	6	3
DEX	8	5	0	0
SPORTS&DEX	1	3	5	4

SPORTS= Activity of sports and exercise, DEX= activity of activities employing manual dexterity, DEX= combined activity of sports, exercise and motor activities employing manual dexterity.

Activity levels and juggling skill. Sports activity level correlated with following juggling results: PRE (n=13, r_s=0.59, p=0.04), retention (n=13, r_s=0.64, p=0.02), transfer (n=13, r_s=0.57, p=0.04) and ΔCPAPRE–RET (n=13, r_s=0.64, p=0.02). Participation to activities employing manual dexterity (DEX) correlated near significantly with CPA at retention (n=13, r_s=0.55, p=0.05), and with ΔCPAPRE–RET (n=13, r_s=0.55, p=0.05), consolidation of MT1 (n=13, r_s=0.51, p=0.08) and relative transfer (n=13, r_s=0.55, p=0.05). Combined activity level SPORTS&DEX correlated statistically significantly with juggling results PRE (r_s=0.68, p=0.01), Retention (r=0.73, p=0.004) and transfer (r_s=0.64, p=0.02), ΔCPAPRE–RET (n=13, r_s=0.73, p=0.004) and near significantly with $MT5_{POST}$ (r_s=0.54, p=0.06) and ΔCPAPRE–$MT5_{POST}$ (n=13, r_s=0.54, p=0.06).

Activity levels and reaction time. Sports activity correlated with ΔRT1–RT5 (n=12, r_s=0.59, p=0.05). DEX correlated with reaction time change from PRE to Retention (n=13, r_s=0.59, p=0.03). SPORTS&DEX did not correlate with reaction time results. Otherwise activity levels did not correlate with reaction time results.

MEP changes and motor activity. DEX correlated negatively with $\Delta\%PAS_{POST}$ (r_s=-0.51, p=0.08) though not statistically significantly. Otherwise in-session or multi-session MEP amplitude changes did not correlate with recreational activity levels during any motor skill training session.

10 DISCUSSION

The purpose of this study was to examine the relationship between capacity for motor cortical plasticity and motor skill learning of a complex perceptual-motor skill. Juggling skill developed statistically significantly during the intervention whereas simple visual reaction time did not change as a group. PAS induced an increase of MEP amplitudes that did not reach a statistical significance. PAS induced increase of corticospinal excitability did not correlate with juggling skill development but was associated with slower reaction times at retention. All juggling training sessions induced an immediate suppression of MEP amplitudes that weakened over time. However, MEP amplitudes increased over baseline during 20-minute period after the end of first juggling training session for five participants who were also among those that improved their visual reaction times during the intervention. Multiple motor training sessions did not induce significant change in baseline corticospinal excitability. Improvement of reaction time from first to fifth training session was associated with increase of baseline corticospinal excitability during same timeline. Motor training induced acute or multisession corticospinal excitability changes did not correlate with juggling skill development. Acute and multisession corticospinal excitability changes induced by PAS and different juggling sessions correlated in complex ways.

10.1 **Juggling skill**

All participants got better at the juggling task during the 5-day training period but not all acquired the skill (criteria CPA ≥ 4), which was an expected result on the grounds of earlier studies (Bebko et al 2003; Laughlin et al 2015). High level of long-term retention of learned motor skills has been reported in scientific literature since the early 20th century (Adams 1987). As anticipated, in the present study the participants demonstrated a long lasting learning effect of the trained juggling skill that was still apparent 6 days after the end of the training period.

During a training period performance of motor skill develops both during the training session and consolidation periods between training sessions (Korman et al 2003). In the present study

the in-session gains of skill were statistically significant on first, second, fourth and retention skill training sessions but not on third and fifth training sessions. Gains of skill were retained after 24 h consolidation periods. Many individuals experienced gains of CPA during consolidation periods of 24 h but the gains were not statistically significant as a group. In conclusion gains of skill were attained mainly during the training sessions.

Higher gain of skill was associated with higher performance level at retention test. Greater improvement in juggling performance during the 5-day training period was associated with greater relative drop of performance during 6-day break from training. Similarly greater gain of skill during the first training session was associated with poorer percentage retention after training intervention. The finding is in line with a theory of cognitive effort: greater cognitive effort should result in either slower or normal learning rate during acquisition but better retention (Lee et al 1994). However, it should be noted, that in the present study the acquired skill level was well preserved and those that gained skill faster also had higher retention and transfer test performance levels. High variation in skill also made comparison of relative gains of performance more difficult, which is why the relative learning rate was not analysed at all. Therefore comparing relative retention according to learning speed is difficult.

Skill transferred to a modified juggling task where juggling pattern remained same but the three balls were all of different sizes. There was a trend for decrease of successful throws from post retention to transfer that was similar to the decrease of skill level reported in Laughlin et al (2015). In the present study juggling skill learning during the acquisition period did not affect how well the obtained skill level was transferred to modified task. All in all the juggling skill transferred well to a modified juggling task.

10.2 **Relationship between reaction time and juggling skill**

It is known that some types motor skill training and reaction time task training can improve reaction time and movement response time (e.g. Ando et al 2002; Ando et al 2004; Dartnall et al 2009; Proctor et al 1991). In the present study no statistically significant changes in reaction time were observed. However reaction time was more likely to improve for those that

had slower initial reaction times, which is in line with findings of previous research literature (Yotani 2011).

In the present study faster initial reaction time was associated with higher baseline juggling performance level and transfer test performance. As task response times and reduced errors go hand in hand, it could explain the finding of this study (Dartnall et al 2009). It could be that faster reaction time in itself enhances juggling performance. However history of motor activity could have been an effector as well.

Interestingly improvement in reaction time was inversely related to development of juggling skill from baseline test to retention. In other words, reaction time improved in slower learners during juggling training. Maintaining a successful juggling pattern requires ability to utilize juggling pattern specific temporal, spatial and visual control strategies (Huys et al 2004). If hypothesized that the slow learners did not develop the necessary control strategies it would make sense that the training targeted different aspects of perceptual motor function than it did for fast learners. It is speculative but catching the balls might have become a reaction time task for the slow learners, which could also explain improvement in reaction time. Also reaction time development might be associated with the development of some of the sub-skills of juggling necessary to learning. This study however only measured the acquisition of the whole skill and not the sub-skills which is why such conclusion cannot be reached in basis of this study.

10.3 **PAS induced plasticity and motor skill learning**

Paired associative stimulation was aimed to induce LTP-like plasticity of motor cortex in flexor carpi radialis (FCR) muscle motor area. A trend for increase of corticospinal excitability was found twenty minutes after PAS. Based on literature (Delvendahl et al 2012) it is likely that responders to PAS stimulation experienced LTP-like motor cortical plasticity in this study. Spinal excitability was not measured but it is possible that PAS may have induced a change in spinal reflex modulation, an effect that has been observed after PAS targeting the FCR muscle (Lamy et al 2010; Meunier et al 2007).

For this study a 20ms interstimulus interval was used as it has been reported to induce a statistically significant elevation of MEP amplitudes in flexor carpi radialis muscle (Lamy et al 2010; Meunier et al 2007). It is known that the inter-individual and intra-individual variation in PAS induced neuroplasticity is high (Fratello et al 2006). In a small sample the high inter-individual variation may prevent the results from reaching statistical significance (López-Alonzo et al 2018). In the present study one participant had to leave prematurely before $POST_{20}$ measurement of PAS session, making the dataset from PAS incomplete and further reduced the likelihood of the MEP change reaching statistical significance. A number of factors are known to influence PAS effect (e.g. Sale et al 2007; Stefan et al 2004). For example hormonal levels and attention during measurement were not measured in the present study. Also the time of the day for PAS measurement differed between participants because of limitations in availability of the laboratory room. It is possible that uncontrolled factors affected the results of PAS measurement in this study.

In the present study PAS did not induce long-term change in baseline peak-to-peak MEP/M_{max} amplitudes as a group. However the magnitude of LTP-like effect in this study was strongly associated with increase of baseline MEP amplitudes from PAS to MT1. This result indicates that PAS induced plasticity may have had an effect on corticospinal excitability still after several days from the intervention. PAS induced increase of MEP amplitudes has been reported to last from 30–60 minutes (Müller-Dahlhaus et al 2015) in hand muscles and 5–60 minutes in lower limb muscles (Alder et al 2019). On the basis of scientific literature PAS effect durations extending several days were not expected. Possibility of longer-term PAS effects was suggested by De Gennaro et al (2008), who observed a change in electroencephalogram slow-wave activity during night´s sleep after PAS intervention. Their finding however only implies that PAS may affect sleep in a way that daytime synaptic potentiation has been proposed to do (Tononi & Cirelli 2006). Although the correlation between acute and long-term MEP change was strong the sample was very small. Additionally the scatter diagram (Figure 20) was not convincing enough to make strong conclusions. As there is no prior evidence of such long lasting PAS induced plasticity the possibility remains speculative until further research.

This study, like others before, failed to prove a connection between magnitude of motor skill learning and PAS induced plasticity (Vallence et al 2013, López-Alonso et al 2015). However a higher LTP-like effect was associated with poorer percentage transfer of the juggling skill. This is the first time the relationship between skill transfer and PAS effects has been studied. Concept that greater capacity for plasticity would be detrimental to transfer of motor skills seems untenable. Could PAS induced LTD-like plasticity be related with better transfer? Indeed those that had suppressed MEP amplitudes right after PAS also had better relative transfer results. After 20 minutes from PAS however, MEP amplitudes remained prominently suppressed only for one participant. It is impossible to judge the possible relationship between LTD and relative transfer in the basis of this study. The inverse relationship between relative skill transfer and magnitude of LTP-like effect found in this study is unexpected and should be treated with caution until further research.

Reaction time has been reported to have some associations with NIBS induced plastic changes but not those induced by PAS (López-Alonzo et al 2015). However in the present study the magnitude of PAS induced LTP-like effect was associated with longer reaction times on retention session. The observed relationship in this study is somewhat hard to interpret. PAS induced corticospinal changes did not correlate with reaction times on first or fifth training session or changes of reaction time. It is likely that the correlation between PAS induced plasticity and reaction time at retention was coincidental.

PAS and first motor training session induced corticospinal plasticity did not correlate with each other, which is in line with the findings of Vallence et al (2013). Larger increase of cotricospinal excitability immediately after PAS was however statistically significantly associated with larger suppression of MEPs after MT5. However the MEP change from PRE to 20 minutes after PAS did not correlate with MEP changes later in the training, which limits making conclusions on the matter. This study did not find any clear associations between acute neurostimulation induced and motor training induced corticospinal excitability changes.

10.4 **Motor skill learning and in-session corticospinal excitability changes**

A motor skill training session is often followed by an increase in MEP amplitudes that is thought to represent LTP like plasticity of motor cortical neurons in the motor areas targeted by training (Rosenkranz et al 2007a; Ziemann et al 2004). An increase of MEP amplitudes has been reported after many different types of motor training including visuomotor tracking tasks (Cirillo et al 2011; Jensen et al 2005; Hirano et al 2015). On that basis it was hypothesized that juggling would increase MEP amplitudes of flexor carpi radialis, a wrist muscle that is rhythmically activated during juggling. Instead however, a transient MEP suppression immediately after each motor training session was observed in the present study. The result was opposite to the expected.

Although juggling task and visuomotor tracking tasks used in earlier studies both require visual processing, there are also major differences in the protocols. Visuomotor tracking tasks are typically simple: a participant tracks a virtual target line by moving a cursor by contracting extensor and or flexor muscles of a single joint. (Cirillo et al 2011; Jensen et al 2005; Hirano et al 2015). Instead of one target, jugglers track the movement of multiple objects. The movements are bimanual and involve cyclic multi-joint catching and throwing movements. The relevance a single muscle would of course be higher in a single joint task compared to complex motor tasks like juggling. In juggling the coordination of multiple joints and both upper limbs according to the visual information is essential for the performance. Corticospinal excitability of single muscle areas after motor training of comlex multi-joint bimanual movements has been little researched. Could it be that the suppression in corticospinal excitability of trained muscles is characteristic for training complex motor tasks?

An example of more complex perceptual motor task researched previously is different types of pegboard tasks. McDonnel & Ridding (2006) used an approximately 15 min long training protocol that consisted of training a grooved pegboard manipulation task. The task utilizes fine motor control of hand muscles and demands attention. Task also involves fast reaching and grasping movements when reaching for new pegs. In their study the training induced a transient suppression of MEPs both in measured trained muscle and untrained muscle.

(McDonnel & Ridding 2006.) The transient MEP suppression was similar to the MEP suppression found in the present study. Pegboard training has been reported to induce either increase (Garry et al 2004) or decrease (McDonnel & Ridding 2006) of MEPs supposedly depending on the details of the used training protocol. McDonnel & Ridding (2006) proposed that longer training duration, higher attentional demands of the task and complexity of the task compared to the training protocol of Garry et al (2004) might be reasons for MEP suppression found in their study. The juggling protocol used in the present study was certainly complex and demanding. Also the protocol was relatively long compared to protocols of both Garry et al (2004) and McDonnel & Ridding (2006). If the complexity, attentional demands and duration are truly triggers for effect reversal it could help understand why the MEPs were suppressed after a 30 min long juggling session.

Depression of motor evoked potentials after juggling session could also be explained by special characteristics of the juggling skill. Juggling is perceptual motor skill in which employment of visual, proprioceptal and haptic information is crucial (Garcia et al 2013). It has been proposed that developing specific visual strategies is essential in the acquisition of juggling skill (Huys et al 2004). The ability to rely on proprioceptal and haptic information to control the movement develops slower (Huys & Beek 2002). MRI studies confirm that juggling training induces functional changes mainly in the brain areas involved in visual processing and planning (Boyke et al 2008; Draganski et al 2004; Sampaio-Baptista et al 2014). Acquisition of juggling skill may not necessitate a modification of motor cortical representation as no statistically significant changes in primary motor cortex have been reported during juggling skill acquisition, although there is evidence of performance dependant increase of grey matter in the motor cortex in the weeks after the training phase had ended (Sampaio-Baptista et al 2014). Findings that cortical plasticity relevant to juggling performance development concentrates mainly in visual but not motor areas explain why LTP-like effect was not found, but it does not explain the mechanism in MEP suppression.

What could be the mechanism behind the transient MEP suppression observed in this study? A phenomenon that could explain the suppression of corticospinal excitability after juggling is post exercise depression (PED), which has been reported to occur after fatiguing exercise (Brasil-Neto et al 1993, Samii et al 1997) and of non-fatiguing exercise consisting of

repetitive movements (Bonato et al 2002). Juggling training did not likely induce prominent muscle fatigue that would explain PED. Fatigue has been reported to impair motor learning process (Branscheidt et al 2019), which was not seen in this study as juggling skill level increased rather than decreased during sessions. In this study force generating capability or subjective feeling of fatigue was not monitored. However the possibility of fatigue cannot be altogether ruled out without the appropriate measures.

The suppression of MEP sizes seen in the present study bears similarities with PED effect reported after non-fatiguing repetitive muscle contractions. Bonato et al (2002) proposed that PED after repetitive non-fatiguing movements is caused by cortical reorganization of nerve networks, an adaptation to motor learning (Bonato et al 2002). If PED reflects a motor learning process it would explain the findings of the present study. Even if PED has been reported to occur without decrease of force generating capability there remains the question of whether PED effect could still involve some type of fatigue. For example general feelings of fatigue and high effort has been observed accompanying PED even after non-fatiguing protocols (e.g. Avanzino et al 2011, Kluger et al 2012).

Relationship between in-session MEP changes and juggling performance. Many studies have reported an association between magnitude of training induced increase of corticospinal excitability and motor performance development (Garry et al 2004; Hirano et al 2018; Jensen et al 2005). Suppression of MEP amplitudes after motor training session found in the present study is not a typical finding. In the present study no correlation was found between MEP change and skill development. Before this study McDonnel & Ridding (2006) also reported a suppression of MEPs that did not correlate with motor skill development. The absence of observable correlation in the present study is in line with the results of McDonnel & Ridding (2006), who similarly observed a transient MEP suppression that did not correlate with learning.

Relationship between in-session MEP changes and relative skill transfer. In the present study percentage transfer of juggling skill was the only juggling performance parameter to correlate, though not statistically significantly, with in-session MEP changes during juggling

intervention. Larger drop of mean MEP amplitudes during the first juggling session was associated with poorer relative transfer (p=0.06). On retention session the association was inverted though again not statistically significant: a greater drop in MEP sizes right after retention session was associated with higher percentage transfer of the skill level (p=0.11). The twist in effect direction is not so strange when considering that the acute corticospinal excitability changes from first training day to retention were also inversely correlated though not statistically significant. These findings, although hard to interpret, reveal a possibility of a relationship between corticospinal plastic changes and ability to transfer the skill to similar tasks. The topic should be further researched.

Relationship between MEP changes on first training session and reaction time. On first motor training session MEP amplitudes rose above PRE values during 20 minutes after training in five participants. Juggling training might have induced motor cortical plasticity in those participants (Rosenkranz et al 2007 a; Ziemann et al 2004). This increase of MEP sizes during first motor training session was associated with faster reaction times on last motor training session and on retention session. Reaction time also improved from first to fifth training session in all participants that experienced an increase of MEP amplitudes 20 minutes after first training session. Correlation between MEP and reaction time changes was not statistically significant though. These findings implicate that A) juggling training induced corticospinal excitability change might predict reaction time performance later in training and B) juggling training may induce reaction time improvement that is associated with LTP-like motor cortical plasticity. The effect was only seen in MEP changes on first training session and not later sessions, which might indicate motor learning. On retention session though a trend was observed that greater magnitude of MEP suppression right after training was related to faster reaction time at retention. The finding might be coincidental or a mark of effect reversal.

The results of this study indicate that juggling training causes gains of skill that are characteristic to motor skill training but generally induce a depression of MEPs that is similar to PED effect. In some participants though, an increase of MEPs was observed that was associated with faster reaction time after training. It is possible that the increase of MEP amplitudes reflected LTP-like plasticity in those participants. From the present data it cannot

be distinguished, whether the decrease (or in few cases the increase) of corticospinal excitability was beneficial to juggling skill development. The question remains if the decrease of corticospinal excitability seen in this study reflected the motor learning or other processes in the nervous system.

10.5 **Multi-session training effects**

Rosenkranz et al (2007a) observed a difference between neuroplastic responses on first training day and last training day: LTP was only observed after first training session but not after last. Swift in corticospinal response was proposed to reflect learning. (Rosenkranz et al 2007a.) On the present study acute MEP changes did not differ between different motor training days. The results of the two studies are hard to compare, as the direction of the training induced corticospinal excitability changes were different. In the present study the corticospinal excitability decreased after every session but the magnitude of decrease was inversely related between first and later sessions. It is thought that the training induced acute changes in corticospinal excitability reflect the phase of the learning process (Hirano et al 2015). In juggling the progression of skill learning is highly variable as demonstrated by this study and many others (Bebko et al 2003; Laughlin et al 2015). Although this study was not able to demonstrate similar effect to Rosenkranz et al (2007a) there were some indications for a change in effect magnitude after multiple training session.

Five-day juggling training intervention did not produce changes in baseline MEP amplitudes as a group. Again juggling training failed to produce corticospinal excitability changes characteristic motor skill training like demonstrated in Rosenkranz et al (2007a). There was however high inter-individual variation in change of baseline corticospinal excitability and the change of corticospinal excitability on first training session correlated positively, though not statistically significantly, with the change of baseline MEPs from first to fifth training session. In a closer look, the baseline MEP amplitude changes were related to some training effects of juggling training.

How do the findings findings compare to MRI studies of juggling skill development? Sampaio-Baptista et al (2014) found that better juggling performance was associated with POST training increase of gray matter volume in primary motor cortex, but there was no statistically significant change on motor cortical gray matter volume during training. In that context it would be expected that cortical changes would become apparent only after the training had ended. An additional test was conducted to test correlation between baseline MEP change from fifth training session to retention and juggling skill level at retention (n=8). No such correlation was found.

Change of baseline MEP amplitudes from first to fifth training day was associated with reaction time but not with juggling skill or development of juggling skill. Slower baseline reaction time and greater improvement of reaction time were associated with an increase of baseline MEPs from first training session to fifth. The effect was no longer visible at retention, which could mean that the association between reaction time development and MEP changes during the training phase reflected transient changes in baseline corticospinal excitability. Multi-session increase of MEP amplitudes has been linked to learning related motor cortical plasticity (Rosenkranz et al 2007a; Ziemann et al 2004). In the present study reaction time improvement was associated with both acute and multi-session increase of MEP amplitudes. These findings suggest that juggling may have induced motor cortical plasticity that was associated with training related reaction time improvement.

10.6 **Relationship between corticospinal excitability and relative skill transfer**

Relative skill transfer was calculated as a percentage change of juggling performance from the last trials of retention training session to the transfer test. The purpose of the relative transfer was to compare how well the participants transferred the acquired skill level to a slightly more difficult juggling task where the participants juggled with three differently weighed balls. This type of transfer task has been used before in the research of juggling learning (Laughlin et al 2015).

During the study juggling performance had no association with corticospinal excitability changes except for the relationship between relative skill transfer and MEP amplitude changes. For a reminder, higher LTP-like PAS effect was associated with poorer relative transfer. Larger suppression of MEPs right after first training session had a trend of associating with poorer relative transfer. The directions of correlations were counterintuitive if assumed that the typical group response for an increase of MEPs after PAS and MEP suppression right after juggling training A) were more likely beneficial to learning than detrimental or B) had nearly no effect on juggling skill as the results indicated. The absolute transfer test performance was strongly associated with the gained skill level, whereas the relative transfer did not correlate with any juggling skill or reaction time results or recreational activity levels.

Associations between MEP changes and relative transfer could well be coincidental or they might reflect some characteristics of the transfer test. The type of juggling task used in this study as transfer test is thought to test the juggler´s ability to use peripheral somatosensory information in juggling (Sánchez García et al 2013). MRI imaging studies indicate that the brain areas focusing on visual information and planning have crucial role in the acquisition of the typical three-ball cascade (Boyke et al 2008; Draganski et al 2004; Sampaio-Baptista et al 2014). In perspective of motor control the transfer task is more difficult as force production of the throws needs to be proportioned to the ball weight (Sánchez García et al 2013). The somatosensory information is processed in different brain areas than visual information. Therefore it would make sense that studying the role of motor cortex in transfer task would show different results than when studying typical juggling task. In the sense of juggling arts the ability to manipulate multiple different sized and shaped objects is valuable and an important part of the skill. The role of the motor cortex in the transfer of juggling skill to a task that demands more processing of somatosensory information remains inconclusive. It might be interesting to research whether training juggling with three differently weighed balls would induce differential changes to motor cortex than typical juggling training.

10.7 **Effects of history of motor activity**

Effects of recreational activity were tested in this study as physical activity may have an enhancing effect on skill learning, reaction times and motor cortical plasticity (Delignières et al 1994; Kumpulainen 2014; Pereira et al 2013; Rosenkranz et al 2007b). Enhancing effect of earlier motor experiences on performance of a new motor task has been confirmed in scientific literature (e.g. Pereira et al 2013). Physically active individuals have also been reported to maintain the higher level of motor performance compared to more sedentary individuals throughout the motor skill training process (Pereira et al 2013). Influence of motor history to skill learning was present also in this study. Leisure time motor activity was associated with higher juggling performance across the intervention. High physical activity has been associated with faster reaction times (Delignières et al 1994). In the present study reaction times did not correlate with recreational activity levels though reaction time was more likely to improve for less active individuals.

Recreational activity did not have any significant effects on corticospinal excitability changes in this study. However, a negative, but not statistically significant correlation was observed between participation to activities that employed manual dexterity and corticospinal excitability change PRE to POST PAS. Result is inconsistent with earlier studies that associated skill training of experts with larger neuroplastic responses to PAS (Kumpulainen 2014; Rosenkranz et al 2007b). One of the differences in protocols was that in the present study only low levels of activity was allowed. Negative correlation observed in this study could be coincidental; especially as MEP change from PRE to 20 min POST PAS did not correlate with recreational activity levels. Recreational activity levels did not otherwise correlate with acute neuroplastic responses to PAS or motor training. A limitation of the present study was that it had small number of participants and allowed many different combinations of participation to different recreational activities and therefore the effect of single type of motor activity on neuroplasticity is hard to identify.

10.8 **Limitations of the study**

The most prominent limitation of this study was a high relative amount of missing data arising mostly from the technical difficulties. Missing Maximal M-wave data was the reason that the acute MEP amplitude changes were calculated with absolute MEP values. EMG electrodes were not changed during single sessions and there was no reason to expect a change in in-session maximal M-wave. Results from the acquired data indicate that the maximal M-waves did not change during single sessions as a group, which supported the usage of absolute MEP values. Still with the amount of missing data it is not certain that there was no maximal M-wave changes that might interact with MEP results.

Normalized MEP values were used in order to compare long-term corticospinal excitability changes and the relationship between MEP amplitudes and motor skill. The comparison of baseline MEP changes was conducted only with the eight participants with a complete dataset of baseline M-waves and MEPs. The small sample likely prevented some test results from reaching statistical significance. It is also possible that some results would have lost the significance. As a consequence all of the results that contained normalized MEP amplitudes need to be regarded with caution.

Paired associative stimulation was conducted at minimum of five days before the start of motor skill training in order to avoid interaction between PAS and motor training induced plasticity. However correlational analyses showed that actually PAS might have induced plastic effects that were still present at the start of the training, and might have interacted with motor training effects. The change of baseline corticospinal excitability correlated positively with PAS induced MEP change and negatively with motor training induced acute MEP change. Interaction between effects would tamper interpretation of the results of this study. The amount of scientific data concerning ability of PAS to affect motor learning is limited. Jung & Ziemann (2009) observed that prior PAS is able to affect effects of motor training, when both are carried out on the same session in line with the rule of homeostasis of synaptic potentiation. Night´s sleep however returns the capacity for synaptic potentiation to baseline levels, and as such, PAS induced LTP should no longer no longer affect capability for

synaptic plasticity in the days following PAS. (Tononi & Cirelli 2006.) Likely there was a correlation but no causation between PAS and training induced corticospinal excitability changes, but the methods don´t allow discrimination between them.

Motor performance data also had some missing values. The juggling data missed some values on fourth training session and reaction time data missed one value on MT5. Main analyses of juggling skill were conducted with the results from first, second, last and retention training sessions, which did not have any missing data of juggling performance. The missing RT5 data point could have affected the strength of correlations between corticospinal excitability and RT5 or reaction time change from RT1 to RT5.

The implications of history of motor activity and baseline reaction time on juggling skill and skill development observed in this study were most reliable results of this study and have strong support from the earlier scientific literature. The problems with missing data means, that although the study made some interesting observations, it would be important to repeat and confirm the findings in the future with larger sample size and intact data.

10.9 **Conclusion**

In the present study participants improved their juggling skill level statistically significantly during the five-day training intervention. Juggling skill level was well retained after a six-day break and transferred to a modified juggling task. Nine out of 13 participants reached the skill acquisition criteria during the five-day intervention, four of who reached it already during the first training session. Simple visual reaction time did not change as group. Improvement in reaction time was associated with slower juggling skill development. Paired associative stimulation induced an increase of MEP amplitudes that did not reach statistical significance. First, last and retention juggling training session all induced an acute suppression of corticospinal excitability that weakened over time. No multisession changes in baseline corticospinal excitability were observed. Interestingly few participants experienced a training induced acute increase of MEPs on first session and multisession increase of MEPs that were accompanied by faster reaction times on fifth session and improvement of reaction time from

first to fifth session. Juggling skill development did not correlate with corticospinal excitability changes at any point during the study. In conclusion, capacity for plasticity was not related with juggling skill development.

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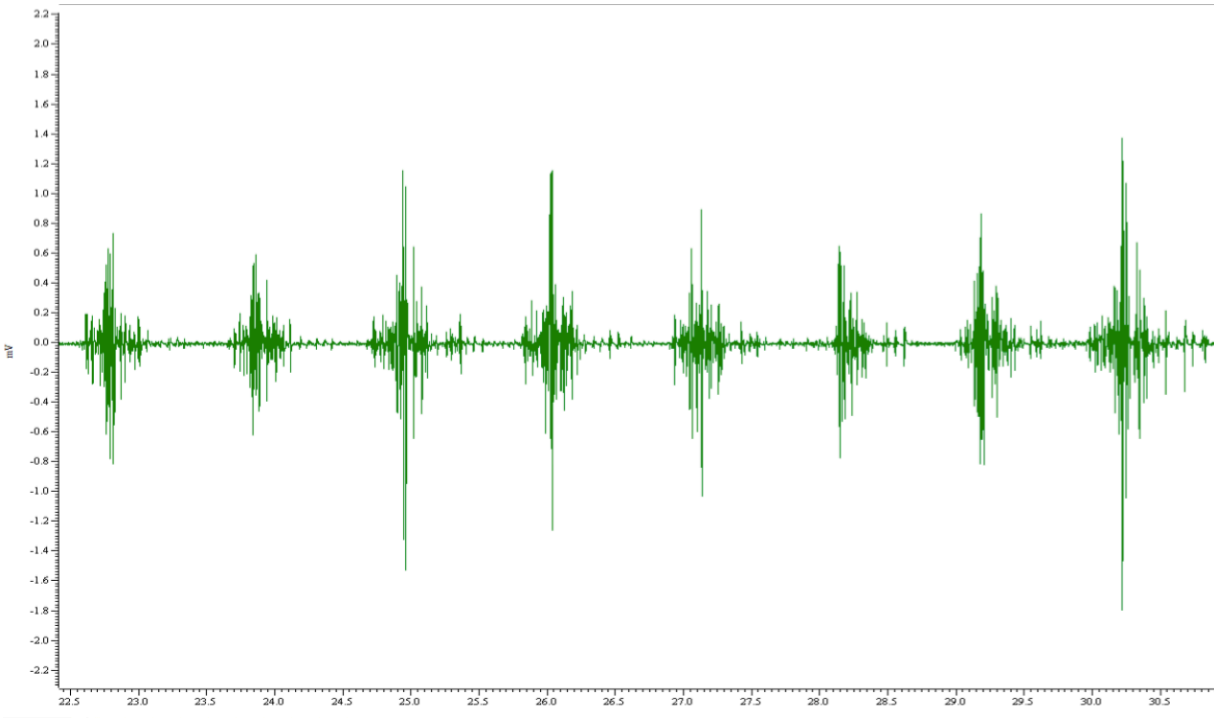
APPENDICES

APPENDIX 1. Example EMG of right flexor carpi radialis muscle during juggling after retention training session. Participant acquired the skill on first session and reached CPA>50 at retention test.



APPENDIX 2. Correlation between percentage change of MEPs during fifth training session and results of juggling and reaction time. Percentage change of MEPs was analysed from PRE to POST ($\Delta\%MT5_{POST}$) and from PRE to 20 minutes POST ($\Delta\%MT5_{POST20}$).

	Δ% MT5 POST			Δ% MT5 POST20		
	r_s	p	n	r_s	p	n
CPAPRE	0.32	0.28	13	0.19	0.53	13
$CPAMT5_{PRE}$	0.25	0.40	13	0.00	0.99	13
$CPAMT5_{POST}$	0.18	0.57	13	-0.04	0.90	13
ΔCPAMT5	0.29	0.33	13	-0.05	0.87	13
Skill acquisition	-0.01	0.96	13	0.09	0.78	13
RETΔ%	-0.26	0.39	13	-0.12	0.69	13
TRANSFΔ%	-0.13	0.67	13	-0.35	0.24	13
RT_MT1	-0.16	0.60	13	0.08	0.79	13
RT_MT5	0.13	0.68	12	0.35	0.27	12
RT_RET	-0.15	0.63	13	0.17	0.58	13
ΔRT_ MT1–MT5	0.41	0.19	12	0.29	0.35	12

Abbreviations: CPA= Catches per attempt of juggling, ΔCPA= Change of juggling skill, Skill acquisition = session when CPA≥4, RETΔ%= percentage retention of skill (Δ%), TRANSFΔ%= percentage transfer of skill (Δ%), RT= reaction time (s), ΔRT=reaction time change (s).

r_s= Spearman´s Rho correlation coefficient

*= $p<0.05$; **=$p<0.01$

APPENDIX 3. Correlation between percentage change of MEPs during retention session and results of juggling and reaction time. Percentage change of MEPs was analysed from PRE to POST and from PRE to 20 minutes POST (POST20) retention.

	Δ% RETENTION POST			Δ% RETENTION POST20		
	r_s	p	n	r_s	p	n
CPARET	-0.11	0.72	13	0.08	0.80	13
$CPARET_{POST}$	-0.35	0.25	13	-0.16	0.60	13
CPA Transfer	-0.23	0.46	13	-0.10	0.74	13
ΔCPAPRE-RET	-0.10	0.73	13	0.09	0.78	13
ΔCPARET	-0.14	0.64	13	-0.13	0.67	13
Skill acquisition	0.21	0.50	13	0.38	0.20	13
RETΔ%	0.35	0.25	13	0.43	0.14	13
TRANSFΔ%	-0.46	0.11	13	0.04	0.89	13
RT_RET	0.51	0.07	13	0.10	0.75	13
ΔRT_MT1–RET	-0.01	0.97	13	0.16	0.60	13

Abbreviations: CPA= Catches per attempt of juggling, ΔCPA= Change of juggling skill, Skill acquisition = session when CPA≥4, RETΔ%= percentage retention of skill (Δ%), TRANSFΔ%= percentage transfer of skill (Δ%), RT= reaction time (s), ΔRT=reaction time change (s).

r_s= Spearman´s Rho correlation coefficient

*= $p<0.05$; **=$p<0.01$