The BDNF Val66Met polymorphism and the effect of peripheral nerve stimulation on hand function in elderly

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Abbreviations

AMPAR (s)	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor(s)
Arc	Activity-regulated cytoskeleton-associated protein
BDNF	Brain-derived neurothropic factor
Ca2+	Calcium
CaMKII	Calmoduline- dependent protein kinase II
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
DAG	Diaglycerol
ER	Endoplasmatic reticulum
GABA	Gamma-aminobutyric acid
IP3	Inositol-1,4,5 triphosphate
JTT	Jebsen-Taylor hand function test
LTD	Long-term depression
LTP	Long-term potentiation
MAPK	Mitogen-activated protein kinase
Met	Methionine
MS	Multiple sclerose
NMDAR(s)	N-methyl-D-aspartate receptor(s)
PCR	Polymerase chain reaction
PI3K	Phosphatidylinositol-3-kinase
PIP2	Phosphatidylinositol 4,5 biphosphate
PKC	Protein kinase C
PLC- γ	Phospholipase C-γ
SWMT	Semmes-Weinstein monofilaments
SM1	Primary sensorimotor cortex
SNP	Single nucleotide polymorphism
TENS	Transcutaneous electrical nerve stimulation
TMS	Transcranial magnetic stimulation
tPA	Tissue plasminogen activator
TrkB	Tropomyosine-related kinase B receptor
Val	Valine
VAS	Visual analogue scale
VGCCs	Voltage-gated calcium channels

Abstract (a)

Achtergrond en doelstelling: Verouderen gaat gepaard met structurele veranderingen in het centraal en perifeer zenuwstelsel. Deze veranderingen hebben als gevolg dat ouderen problemen ervaren met het verrichten van dagdagelijkse taken. Studies hebben aangetoond dat perifere zenuwstimulatie (TENS) neuroplasticiteit kan induceren in de primaire sensorimotorische cortex die geassocieerd is met verbeteringen in sensorimotorische taken. Recente studies tonen aan dat het BDNF Val66Met polymorfisme geassocieerd is met een verminderd potentieel om synaptische plasticiteit te induceren. In deze studie wordt er onderzocht of korte termijn (20 min) perifere zenuwstimulatie een verbetering in de handfunctie kan teweegbrengen. Bovendien wordt er onderzocht of er een verschil is in verbetering tussen Val- en Met dragers van het BDNF gen.

Materiaal en methoden: Vijfentwintig gezonde oudere proefpersonen kregen at random zowel een TENS als SHAM interventie toegewezen. De TENS/SHAM stimulatie van de N. medianus en N. ulnaris gedurende 20 min werd gecombineerd met een sensitiviteit taak. Sensitiviteit en hand motor functie werden zowel voor als na de interventie gemeten met respectievelijk de Semmes-Weinstein monofilamenten (SWMT) test en de Jebsen-Taylor handfunctie test (JTT). Hiernaast werden er bloedstalen verzameld voor BDNF genotypering.

Resultaten: Statistische analyse toonde geen significante verbetering aan in sensitiviteit (P= 0.523) en hand motor functie (P= 0.088) na korte termijn TENS. Hiernaast werd er geen significant verschil in sensitiviteit (P= 0.338) en hand motor functie (P= 0.366) aangetoond tussen de Val groep (n=18) en de Met groep (n=5).

Conclusie: De bevindingen van deze studie tonen aan dat korte termijn TENS geen functionele verbeteringen in de dominante hand van oudere proefpersonen veroorzaakt. Bovendien was er geen verschil in verbetering meetbaar tussen de verschillende BDNF genotypes. Toch bestaat er evidentie dat TENS en BDNF een belangrijke rol vervullen in neurorehabilitatie en is het de moeite waard om verder aandacht aan te besteden aan dit onderzoek. Wanneer er effecten bekomen willen worden na een korte termijn stimulatie, zullen toekomstige experimenten zich moeten richten op de manipulatie van de parameters zoals frequentie, puslbreedte, intensiteit en stroomvorm. Deze parameters spelen een beduidende rol in de grootte en richting van het effect.

Abstract (b)

Background and purpose: It is well documented that ageing is accompanied with a decline in sensitivity and hand motor performance. Transcutaneous electrical nerve stimulation (TENS) has been proven useful to promote sensorimotor performance by inducing synaptic plasticity in the primary sensorimotor cortex. Otherwise, the BDNF Val66Met polymorphism has been associated with reduced capability to induce synaptic plasticity. The present study investigates whether short-term TENS improves sensitivity and hand motor function in elderly and to analyze whether this improvement is dependent on the BDNF genotype.

Methods: Twenty-five healthy elderly were randomly assigned to a specific intervention (TENS/SHAM) in a double-blind crossover designed study. TENS/SHAM was applied for 20 min over the median and ulnar nerve and was combined with a tactile sensitivity task. Sensitivity (touch threshold) and hand motor function were assessed before and after the intervention using respectively the Semmes-Weinstein monofilament test (SWMT) and the Jebsen-Taylor hand function test (JTT). Additionally, blood samples were collected to determine the BDNF genotype (Val or Met).

Results: No significant improvement of touch thresholds or hand function was achieved by short-term low frequency TENS. A one sample t-test revealed no significant effect of TENS on JTT performance over time relative to the SHAM condition ($P = 0.088$). A similar result was found for the touch thresholds ($P = 0.523$). Statistical analysis revealed that there was no significant difference in touch threshold ($P= 0.338$) or JTT performance ($P= 0.366$) over time for the Val group ($n=18$) versus the Met group ($n=5$).

Conclusion: The findings of this study indicate that a short-term low frequency TENS, focussing on afferent stimulation of the median and ulnar nerve, elicited no improvement in sensitivity or hand motor function in the dominant hand of elderly. Furthermore, functional performance was not influenced by the BDNF Val66Met polymorphism. However, the significance of TENS and BDNF in neurorehabilitation make them attractive targets for further investigation. Future experiments should focus on the discovery of the appropriate stimulation parameters i.e. frequency, pulse duration, intensity and waveform. These parameters seem to play a crucial role in the size and the direction of the effect.

Introduction

In elderly a decline in vision, hearing, touch and motor performance is reported [1,2]. Glasses and hearing aids became a standard support while the loss of sensitivity, necessary for coping with daily activities, is often underestimated. Because sensory inputs are crucial for fine motor performance elderly tend to reduce their daily activities [3]. However, reduced use of hand movements is associated with reduced cortical representation in the primary sensorimotor cortex (SM1) [3]. Probably, these cortical changes have further negative impact on hand sensitivity and motor function (e.g. slowing of movements and reduced coordination in grasp movements) and affect simple activities of daily life such as buttoning a shirt, tying shoes and handling small objects [2,3]. Exercise can prevent age-related sensorimotor deficits, however additional and alternative strategies such as transcutaneous electrical nerve stimulation (TENS) are successfully used in neurorehabilitation [4,5]. Recent studies [6-10] have shown that electrical stimulation of peripheral nerves can produce synaptic plasticity in SM1 by inhibition of GABAergic pathways [11]. Synaptic plasticity facilitate SM1 excitability and influences the size of cortical representation by the enhancement of synaptic connections in cortical circuits [10,12]. This phenomenon is positively correlated with sensorimotor task performance [13]. For example, a study of Cuypers et al. (2010) reported that repeated TENS sessions (3 weeks, 1 session/day) improves hand sensitivity in multiple sclerosis (MS) patients. Regarding hand motor function, Celnik et al. (2007) reported a significant improvement in a motor sequence task after long-term (2 hours) peripheral nerve stimulation in the paretic hand of cortical stroke patients.

1. Cellular mechanisms for synaptic plasticity in the central nervous system and the functional role of BDNF

Synaptic plasticity specifically refers to activity-induced modifications at pre-existing synapses. These activity dependent changes enhance the efficacy of synaptic connections and occur in all excitatory glutamergic synapses [14]. Neuronal activity induces membrane potential shifts and activates or opens presynaptic voltage-gated calcium channels (VGCC). An increase in presynaptic Ca2+ influx promotes release of excitatory neurotransmitters like glutamate [15]. Glutamate binding to its ionotropic glutamate receptors (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (AMPARs), N-methyl-D-aspartate receptors (NMDARs)) causes strong postsynaptic depolarization and induces inward postsynaptic Ca2+ currents (figure 1). Sufficient Ca2+ influx through the NMDAR initiates different signalling cascades involved in synaptic plasticity of the human sensorimotor cortex [14,16].

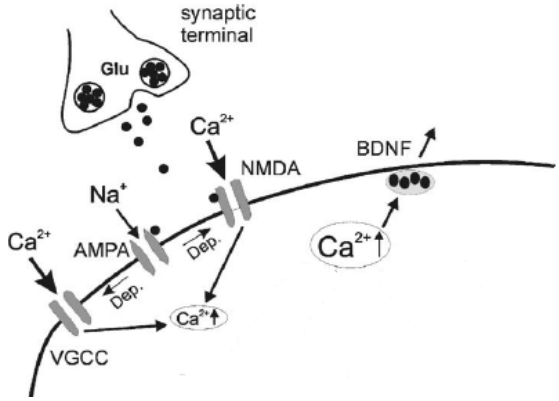

Figure 1: Neuronal activity triggering Ca2+ influx through VGCCs and NMDARs. Excitatory ionotropic glutamate receptors (AMPAR and NMDAR) are the primary cause of neuronal depolarization. Strong postsynaptic depolarization mediates Ca2+ influx by either VGCC or NMDA receptors. This Ca2+ influx triggers different signalling pathways and results in brain-derived neurothropic factor (BDNF) secretion *(figure adapted from Lessmann et al. 2003)*.

1.1. Ca2+ induced signalling cascades and early phase long term potentiation

The first stage of synaptic plasticity is triggered by rapid increases in intracellular Ca2+ through postsynaptic VGCC and NMDAR channels and the subsequent activation of calmoduline-dependent protein kinase II (CaMKII) [16]. Activated CaMKII initiates biochemical cascades that elicits insertion of AMPARs [17] and tropomyosine-related kinase B receptors (TrkB)[18].

1.1.1. Ampafication and TrkB receptor insertion

AMPARs are glutamate ionotropic receptors, which mediates fast excitatory transmission. Activated CaMKII regulates the process of AMPAR insertion, however the molecular mechanism by which CaMKII induces AMPAR delivery to the postsynaptic membrane remains to be determined [17]. Besides inducing ampafication, CaMKII phosphorylates AMPARs subunits. This results in enhanced receptor currents and strengthening of postsynaptic responses associated with synaptic plasticity [16,17]. Secondly, within minutes of depolarization, surface TrkB levels increase by nearly 4-fold [19]. This increase in the number of surface TrkB receptors of CNS neurons seems to be dependent on sufficient Ca2+ influx and CaMKII activation [18]. TrkB receptors are present in pre- and postsynaptic neurons and regulate synaptic strength [15,20].

1.1.2. Up-regulated BDNF expression and secretion

Besides receptor insertion neuronal activity up-regulates brain-derived neurothropic factor (BDNF) expression [21] and elicits BDNF secretion [18]. Neuronal activity that is accompanied with Ca2+ influx into postsynaptic cortical neurons via VGCCs and NMDARs up-regulates BDNF expression. Ca2+ signals are transduced into the nucleus and regulate the transcription factor CREB that binds to BDNF promoters and enhance BDNF gene expression [21] (figure 2).

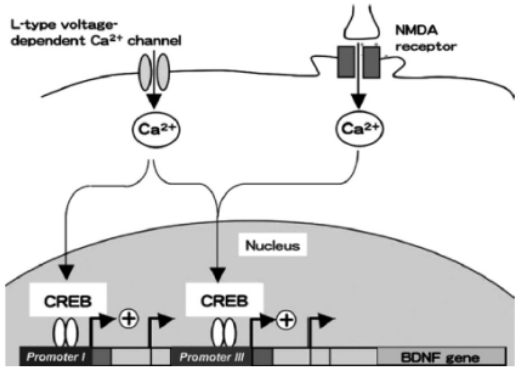

Figure 2: BDNF gene transcription by Ca2+ signals via VGCCs and NMDARs *(figure adapted from Tabuchi 2008)*.

Next the BDNF is synthesized as pro-proteins in rough endoplasmatic reticulum (ER) and is further processed in the Golgi apparatus. Secretory vesicles with pro-BDNF bud off from the trans-Golgi apparatus. Then, two types of secretory vesicles can be generated according to their mechanism of secretion. One mechanism of secretion is the constitutive pathway, another mechanism is the regulated pathway [15]. BDNF is primarily secreted from postsynaptic terminals in a regulated pathway, which is dependent on neuronal activity and Ca2+ elevations [15,22]. The majority of BDNF secreted by the regulatory pathway in central neurons is pro-BDNF and is extracellular converted to mature BDNF by tissue plasminogen factor(tPA) [20] (figure 3).

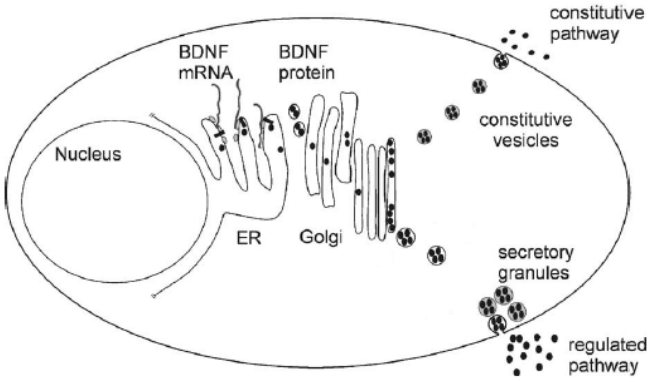

Figure 3: The route of BDNF from synthesis to secretion. BDNF is synthesized as pro-proteins in the rough ER and is further progressed in the Golgi apparatus. Two types of secretory vesicles can be generated according to their mechanism of secretion: constitutive or regulated. BDNF is primarly secreted in a regulated pathway *(figure adapted from Lessmann et al. 2003)*.

1.2. The brain-derived neurothropic factor and late-phase long term potentiation

Experiments with cultured neurons and brain slices have shown that in response to elevations of intracellular Ca2+ , pre- and postsynaptically structures release endogenously BDNF [15,23]. This endogenously secreted BDNF activates intracellular signalling pathways which play a permissive role in mediating the second stage or late-phase synaptic plasticity [15,16,20,23].

The intracellular signalling pathways that become activated are the mitogen-activated protein kinase (MAPK) pathway, the phosphatidylinositol-3-kinase pathway (PI3K) and the phospholipase C-γ pathway (PLC- γ) [18,20,24] (figure 4).

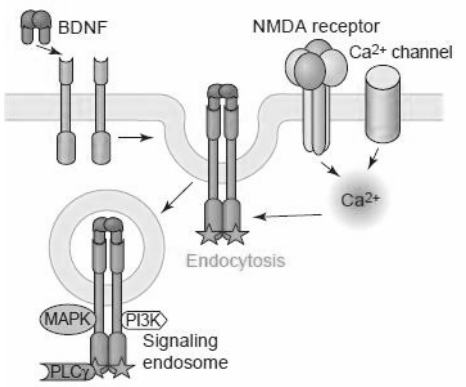

Figure 4: Postsynaptic BDNF-TrkB binding. Neuronal activity and BDNF-TrkB bindingfacilitates TrKB receptor dimerization, autophosporylation and receptor endocytosis. Adaptor proteins couple TrKB receptors to intracellular signalling cascades MAPK, PLC-γ and PI3K *(figure adapted from Nagappan and Lu 2005)*.

1.2.1. Mitogen-activated protein kinase pathway

MAPK pathway is a typical three-tiered core kinase signaling pathway (Raf-MEK1/2-ERK1/2) and is strongly activated by BDNF. Activated ERKs phosphorylate the transcription factor CREB [20,25], which mediates transcription of different genes e.g. BDNF gene [21] and activity-regulated cytoskeleton-associated protein (Arc) gene [26]. These genes cause changes in structure and function of synapses.

1.2.2. Phospholipase C-γ pathway and phosphatidylinositol-3-kinase pathway

Upon TrkB activation, PLC-γ mediates activation of protein kinase C (PKC) and causes release of intracellular Ca2+ stores [20]. PKC can phosphorylate AMPAR and NMDAR subunits. This results in enhanced receptor currents and strengthening of postsynaptic responses associated with synaptic plasticity over longer time-scales [17,20]. In addition, activation of the PI3K pathway in neurons have been implicated in the induction and maintenance of LTP at glutamergic neurons [20].

Besides facilitating neurotransmission and synaptic susceptibility at glutamergic neurons as decribed above, BDNF also plays a crucial role in LTP by depressing GABAergic neurotransmission [23]. The inhibition of GABAergic neurotransmission by BDNF is shaped in a TrkB dependent way. BDNF-TrkB binding and subsequent activation of the PKC pathway leads to internalization of postsynaptic $GABA_A$ receptors [27]. A reduction of GABA synaptic transmission leads to greater excitability, which has been linked to greater plasticity [14].

In conclusion, neuronal activity regulates BDNF expression, secretion and signalling. As a consequence synaptic connectivity and membrane excitability in glutamergic and GABAergic neurons are modified [23,27]. These modifications can influence the size of cortical representation [12], which is positively correlated with sensorimotor task performance [13] (figure 5).

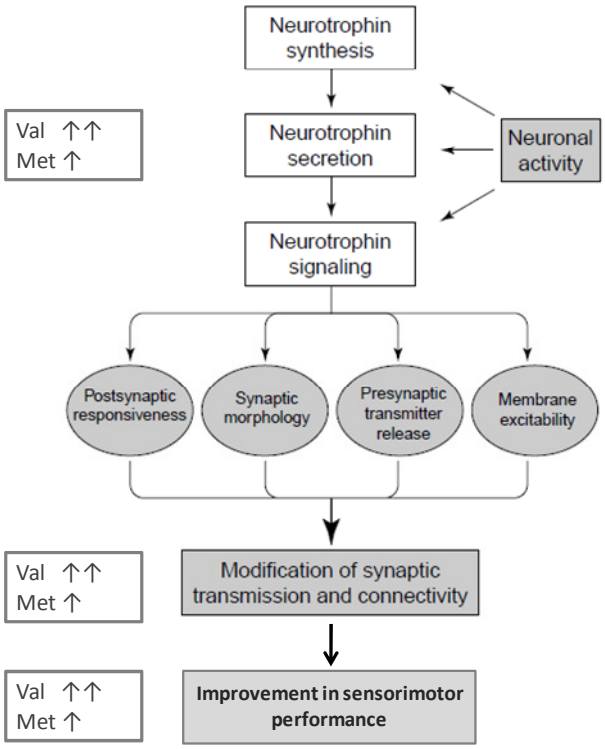

Figure 5: Neuronal activity induced synaptic plasticity. Neuronal activity regulates BDNF expression, secretion and signalling. As a consequence synaptic transmission and connectivity are modified. In contrast to Val carriers, Met carriers are associated with poorer BDNF secretion. As a result, we hypothesize that Met carriers are associated with poorer sensorimotor improvement upon TENS therapy *(figure adapted from Schinder and Poo 2000).*

However, recently a single nucleotide polymorphism (SNP) at nucleotide 196 of the BDNF gene, mapped to chromosome 11p13, has been reported (dbSNP number rs6265). This SNP produces a valine (Val) to methionine (Met) amino acid substitution at codon 66 in the 5' prodomain of the BDNF gene (BDNF Val66Met). The BDNF Val66Met polymorphism has a prevalence around 30% for heterozygotes (Val/Met) and 4% for homozygotes (Met/Met) and has been associated with diminished medial temporal lobe activity [28,29], reduced volumes of hippocampi and prefrontal cortices [30], cognitive impairments [28,31] and reduced corticospinal excitability [32] compared to Val/Val carriers. Due to the important role of BDNF in synaptic plasticity and the evidence of a functional role for this BDNF Val66Met polymorphism, makes it an attractive target for further investigation. We hypothesize that in contrast to BDNF Val carriers, BDNF Met carriers are associated with poorer capability to induce structural and/or functional neuroplasticity after TENS therapy (figure 5).

2. Objectives and experimental approach of the study

In this study we are focusing on the BDNF Val66Met polymorphism and the effect of TENS on healthy ageing. The first objective of this study is to investigate whether a short-term, low frequency (10Hz) stimulation improves sensitivity and hand motor function in healthy elderly. The second aim of this study is to analyze whether the susceptibility to plasticity is influenced by the BDNF Val66Met polymorphism. We predicted that BDNF Met carriers compared to BDNF Val carriers are associated with lower levels of sensorimotor performance. To clarify the predictions, 2 different stimulation protocols (TENS and SHAM) were tested and healthy older volunteers were genotyped for the BDNF Val66Met polymorphism. Sensitivity and hand motor function were measured using respectively the Semmes-Weinstein monofilaments test (SWMT) and the Jebsen-Taylor hand function test (JTT).

Material and Methods

1. Subjects

To evaluate the effect of TENS on sensitivity and hand motor function in healthy elderly, twenty-five volunteers (6 men and 19 women) aged 63 – 83 years (mean ± SE; 73.2 ± 0.9) participated in this study. All gave written informed consent and procedures had the approval of the ethical committee of the University of Hasselt. The Edinburgh handedness inventory was used to determine handedness [33]. All subject were right-handed (mean LQ ± SE; 95.8 ± 1.4). Subjects were screened for sensitivity using the Semmes-Weinstein monofilaments (SWMT) testing set (Smith & Nephew, Inc, Germantown, WI) [34]. The majority of participants represented the clinical categories diminished light touch (DLT) or diminished protective sensation (DPS). The Mini-Mental State examination (MMSE) [35] was used to test cognitive deficits. MMSE scores were within normal limits (>26/30 points). Subjects who were suffering from cognitive deficits (MMSE score <26/30), neurological disorders or musculoskeletal dysfunction; or with contraindications towards TENS (e.g. metallic implants or implanted electrical devices) were excluded from the study. Every participant agreed to undergo a blood sample collection for genotyping. Characteristics of the study population are presented in table 1.

2. Genetic analysis

Information about a common coding variant of the human BDNF gene (rs6265), responsible for a Val to Met change was found in the public SNP database (http://www.ncbi.nlm.nih.gov/SNP). From each subject blood samples (10 ml) were collected in EDTA tubes (Venosafe, Terumo Europe, Leuven, Belgium). DNA was extracted from these blood samples using a standard procedure (supplement 1). To check DNA concentration, the samples were electrophoresed (Model 200/2.0 Power Supply, Bio-Rad Laboratories, Nazareth, Belgium) in a 2% agarose gel (Ultra Pure Agarose, Invitrogen) at 120 V for 1 hour. All agarose gels were stained with ethidium bromide and placed in a Universal Hood imaging system (Bio-Rad laboratories, Milan, Italy). BDNF Val66Met genotype was characterized by polymerase chain reaction (PCR) and restriction fragment length polymorphism (PCR-RFLP) analysis as described previously (Cheeran 2008, Sen 2003, Kleim 2006). For PCR we used following forward primer: 5'AAAGAAGCAAACATCCGAGGACAAG3' and reverse primer: 5'ATTCCTCCAGCAGAAAGAG AAGAGG-3'. The PCR fragments were expected to result in a 274 base pair (bp) product (supplement 2). PCR was performed on a MyCycler thermal cycler (Bio-Rad Laboratories, Nazareth, Belgium). DNA amplification reactions were performed in a total volume of 25 µl, containing approximately 50ng of genomic template, 1µM of each primer (Eurogentec, Liege, Belgium), 200 µM deoxyribonucleotide triphospates (dNTP), 10x PCR reaction buffer + Mg2+ and 1 U Taq polymerase (Roche, Mannheim,

Germany). The PCR cycling conditions included an initial denaturation for 4 min at 94°C, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 45 s, followed by a final extension at 72°C for 10 min. The PCR product was sequenced with a 310 genetic analyzer (Applied Biosystems, Halle, Belgium) and checked for success on a 2% agarose gel.

Table 1: Subject characteristics included in the study

Subject	gender	age (years)	LQ (%)	MMSE	SWMT	clinical classification	BDNF genotype
1	F	63	100	30	3.61	DLT	Val/Val
2	F	65	100	30	3.61	DLT	Val/Val
3	M	65	100	30	3.61	DLT	
4	F	67	90	29	3.84	DPS	Val/Met
5	F	68	100	29	3.61	DLT	Val/Met
6	F	69	100	29	3.61	DLT	Val/Val
7	F	70	100	30	3.61	DLT	Val/Val
8	M	72	100	30	3.22	DLT	Val/Val
9	F	73	100	29	3.61	DLT	Val/Val
10	F	73	100	29	4.17	DPS	Val/Val
11	F	74	88	29	4.08	DPS	Val/Val
12	F	74	100	29	3.84	DPS	
13	F	74	100	29	3.84	DLT	Val/Val
14	F	74	100	27	3.61	DPS	Val/Val
15	M	75	100	29	3.61	DLT	Val/Met
16	F	75	88	29	3.61	DLT	Val/Met
17	F	75	100	29	3.84	DPS	Val/Val
18	M	76	70	27	3.84	DPS	Val/Val
19	M	77	88	30	3.84	DPS	Val/Val
20	F	77	100	26	4.08	DPS	Val/Val
21	F	77	100	29	3.61	DLT	Val/Val
22	F	77	76	28	2.38	N	Val/Val
23	F	77	94	29	3.61	DLT	Val/Val
24	F	82	100	29	3.84	DPS	Val/Val
25	M	83	100	30	3.61	DLT	Val/Met

Abbreviations: F= female, M=male; LQ= laterality quotient; MMSE= Mini-Mental State examination; SWMT = Semmes-Weinstein monofilament test score (expressed as the log of bending force in milligrams); N= normal, DLT= diminished light touch, DPS= diminished protective sensation; Val= valine, Met= methionine.

Next, the resulting PCR products were digested with the restriction enzyme *Hsp*92II and incubated 2 hours at 37°C. The reaction consisted of 10µl PCR product, 2 µl buffer K (10x), 1 µl *Hsp*92II, 0,1mg/ml bovine serum albumin (Promega, Madison, WI) and 6 µl distillate water. The DNA fragments were separated on a 3,5% agarose gel and visualized under ultraviolet light. When a subject carries a G nucleotide (Val carrier), *Hsp*92II digestion produced 2 products, 57 and 217 bp, whereas an A nucleotide (Met allel) produced 3 products, 57, 77 and 140 bp (supplement 2). The presence of a second Hsp92II site served as restriction digest control. Twenty-three subjects were successfully genotyped. Genotypes were coded as Val/Val and Val/Met. Val/Val homozygotes (n=18) were categorized as 'Val group'. The Val group was compared with Val/Met heterozygotes (n= 5) categorized as 'Met group'.

3. Experimental design

Twenty-five subjects participated in a double-blind sham controlled cross-over study. Subjects were invited in the laboratory one week before the start of the experiment (week 1). This was done to familiarize them with the laboratory environment and the study design. After the familiarization session, subjects returned in week 2 and 3 for an experimental session.

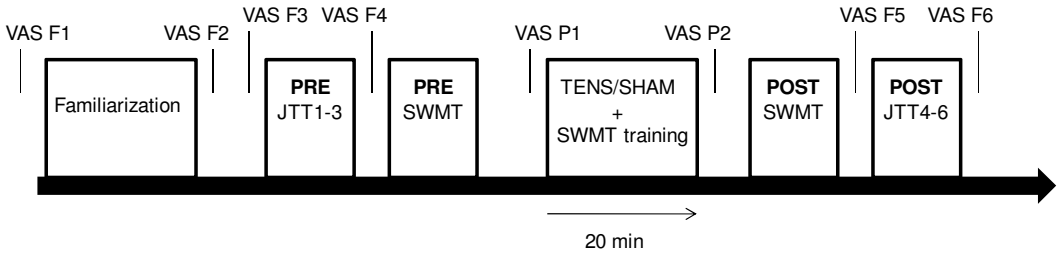

Figure 6: Experimental design of a single session. Subjects participated in 2 experimental sessions (TENS/SHAM), in a counterbalanced double-blind crossover design. All subjects practiced the JTT in a familiarization session until stable performance was reached, followed by baseline measurements of the JTT and SWMT. Subjects report their level of fatigue before (baseline) and after each JTT, using visual analogue scales (VAS F). Subject's levels of pain were obtained at the beginning (baseline) and end of each stimulation session using VAS (VAS P). Immediately after stimulation SWMT and JTT scores were determined.

All volunteers participated in two experimental sessions (TENS and SHAM). Each session was separated by an interval of 7.7 ± 0.4 (mean ± SE) days. At the start of each experimental session, all participants practiced the JTT until stable performance was reached. The performance was considered stable if total JTT time was similar for four successive trials ($P>0.05$). Subsequently, baseline measurements of SWMT and JTT (JTT 1-3) were determined. Immediately after baseline measurements subjects underwent TENS/SHAM intervention. During the intervention subjects remained seated and were trained for 15 minutes with SWMT. A new touch threshold was determined comparable to pre- and post SWMT measurements. Based on touch threshold during TENS/SHAM, five monofilaments were chosen for sensitivity training.

The threshold force, two weaker forces and two stronger forces were used for training. During this training session subjects received verbal feedback from the test administrator. The end of each intervention was followed by post measurements of SWMT and JTT (JTT 4-6). Participants report their perception of fatigue (range 0-10: 0 = no fatigue; 10 = highest level fatigue) six times in each session using a visual analogue scale (VAS). Another VAS was used to measure pain perception in the right hand/arm (range 0-10: 0 = no pain; 10 = maximal pain). This was done at the beginning and end of TENS/SHAM session (figure 6). The examiner who performed behavioural testing was blinded towards the type of intervention and the genotype.

4. Hand function measurements

4.1. *Semmes-Weinstein monofilament test*

A set of 20 Semmes-Weinstein monofilaments (Smith & Nephew, Inc, Germantown, WI) was used to asses finger sensitivity. The measurement site was the distal phalanx of the index finger. Subjects were blindfolded and received acoustic earmuffs (EM2262, North Safety Products, Montreal, QC). Subjects were seated in front of the examiner with their right hand in supination and were instructed to give a verbal response when they felt a touch. No feedback was given. The monofilaments were pressed against the skin, until the nylon hairs buckled, and were held in place for 1.5 s. A descending and ascending method was used to identify the threshold force. If there was no response the next stronger force was delivered, if there is a 'yes' response the next weaker force was delivered. The monofilament with the smallest diameter which was felt 3 out of 5 times, was recorded as the score for that fingertip. The log of force scores of the SWMT were digitized for statistical analysis.

4.2. Jebsen-Taylor hand function test

Functional hand ability was tested via the JTT (Sammons Preston, Bolingbrook, IL). This is a standardized test to assess activities of daily life and can be considered reliable and valid [36]. In this study we included 6 of 7 subtests. The subtest that were used evaluate turning cards (simulated page turning) , picking up small objects, picking up beans with a spoon (simulated feeding), stacking checkers, moving large light and heavy objects (figure 7).

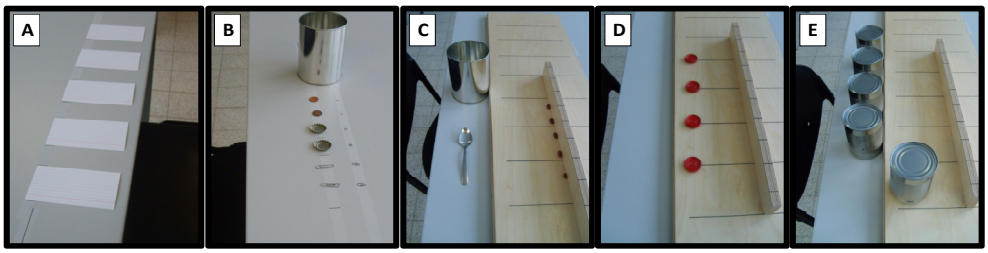

Figure 7: Subtests of the JTT. A: turn over cards, B: picking up small objects and placing them one by one in a can, C: picking up beans with a spoon and placing them one by one in a can, D: stacking checkers, E: moving large light cans and moving large heavy cans.

The subtest writing was not included as it is the only subtest with a quality assessment. Prior to testing all subjects received standardized verbal instructions. The subjects were comfortably seated and were asked to perform the tasks as rapidly and correctly as possible. When errors during JTT performance e.g. dropping of an object did not allow completion of the task, the subtest was repeated. For each subtest the time required to fulfil the task was recorded. Total JTT time was calculated as the sum of the times of each subtest. Feedback on task performance was not provided.

5. Peripheral nerve stimulation

TENS (Sonopuls 992, Enraf-Nonius, Delft) was applied for 20 minutes. Subjects remained seated and received a sensory training task by means of SWM. Self-adhesive surface electrodes (Dura-Stick II, 1,5 x 4 cm, Chattanooga Group, Hixson, TN) were simultaneously positioned over the median and ulnar nerve of the right hand. The cathode was placed proximal to the wrist, while the anode was placed distal. The perception threshold was measured three times and was then averaged. After determination of perception threshold a monophasic current with a frequency of 10 Hz and pulse duration of 800 µs was applied. The intensity of the electrical stimulus was 2,5 times perception threshold (median nerve: mean ± SE; 4.0 mA ± 0.24 / ulnar nerve: mean ± SE; 4.08 mA ± 0.18). The stimulus intensity produced a tickling sensation in the stimulated area without pain. The experimental setup for the SHAM session was identical to the TENS session. The only difference was that stimulus intensity was slowly tapered down after 30s. Prior to TENS/SHAM stimulation subjects were instructed that different waveforms are used and that the currents could be either strong or nearly noticeable. Subjects were blinded to the stimulator.

6. Data analysis

Prior to statistical analysis the data were reorganized offline. Normal distribution of all data were assessed by Kolmogorov-Smirnov tests ($P>0.05$). The JTT time (total time and subtest time) of the PRE measurements was subtracted from the time of the POST measurements. Next, the time of the SHAM condition was subtracted from the TENS condition. The same procedure was applied for the log-scores of the SWMT and for the VAS scores. A one-sample t-test with 0 as test value was performed on the difference scores. If data were not normal distributed a Wilcoxon signed rank test was used. Subsequently, subjects were divided in two groups, the Val group and Met group. In each group the total JTT time of the PRE measurements was subtracted from the time of the POST measurements. Next, the time of the SHAM condition was subtracted from the TENS condition. The same procedure was applied for the log-scores of the SWMT. To determine whether the intervention has a significant effect over time across the Val and Met group a two-sample t-test was carried out on the difference scores. Effects were considered significant if P-values were less than 0.05. All data are expressed as mean ± SE.

Results

1. Effects of peripheral nerve stimulation on sensitivity and hand motor performance

1.1. Sensitivity

To examine the possible effect of TENS on sensitivity in the index finger the SWMT was used. A one sample t-test revealed no significant effect of TENS or SHAM on sensitivity over time (P = 0.523). Within the TENS condition a one-sample t-test on the log-force score revealed no effect of time on sensitivity ($TENS_{PRE}$ = 3.62 ± 0.04 vs. $TENS_{POST}$ = 3.69 ± 0.07, P = 0.524). A similar result was found for the SHAM condition, a Wilcoxon signed ranked test revealed no effect of time on sensitivity ($SHAM_{PRE}$ = 3.69 ± 0.07 vs. $SHAM_{POST}$ = 3.70 ± 0.07, P = 0.959).

1.2. Motor performance

During the familiarization sessions total JTT time improved until stable performance was reached (for a typical example see figure 8).

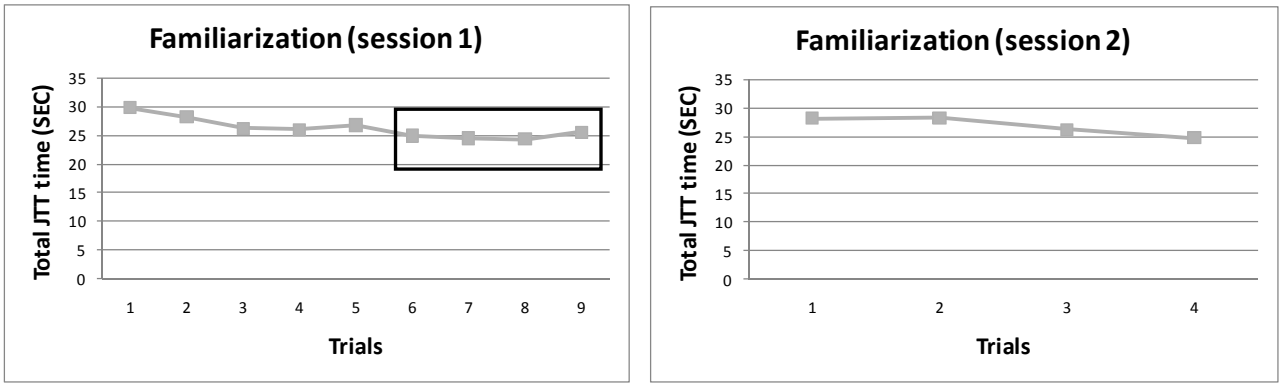

Figure 8: Familiarization plateau. Familiarization progressively reduced total JTT time until stable performance was reached (black box). In session 1, stable performance was attained after (6.4 ± 0.5; mean ± SE) times in all subjects. In session 2 all subjects reached stable performance after (4.12 ± 0.13; mean ± SE) times.

The effect of TENS on hand motor function was evaluated by the JTT. Total JTT time and partial JTT times were recorded. The mean data for total JTT time are plotted in figure 9. A one sample t-test for total JTT time revealed no significant difference between the TENS (PRE = 22.32 ± 0.59 vs. POST = 21.97 ± 0.64) as compared to the SHAM (PRE = 22.35 ± 0.52 vs. POST = 22.30 ± 0.55) condition over time (P = 0.088). Within the TENS and the SHAM condition a one sample t-test revealed no significant differences between baseline and post-measurements (TENS, P = 0.115 and SHAM, P = 0.826).

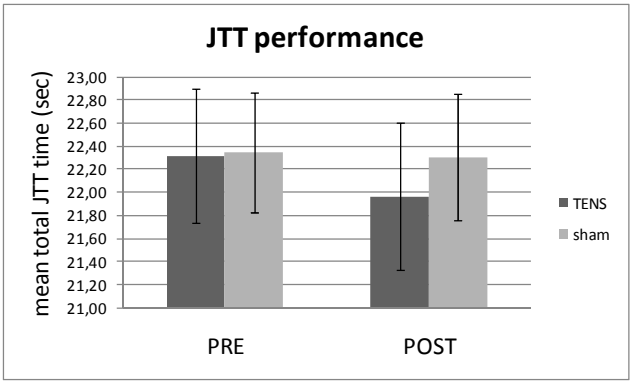

Figure 9: The mean data for total JTT time. Volunteers participated in 2 experimental sessions (TENS and SHAM). Performance improvement of total JTT time that appeared during TENS session (PRE = 22.32 ± 0.59 vs. POST = 21.97 ± 0.64; mean ± SE) was not significant.

It is reported that fine motor tasks (turning cards, picking up small objects) have a tendency to show more prominent JTT time improvement versus gross motor tasks (picking up beans, stacking checkers, moving cans) [2,37]. To evaluate possible differences between the subtests one sample t-tests were performed. The mean data for every subtest are plotted in figure 10. However, one sample t-tests for every single subtest did not reveal any significant results between TENS and SHAM over time (turning over cards: $TENS_{PRE}$ = 2.71 vs. $TENS_{POST}$ = 2.61, $SHAM_{PRE}$ = 2.71 vs. $SHAM_{POST}$ = 2.64, P = 0.560; picking up small objects and placing them in a can: $TENS_{PRE}$ = 5.33 vs. $TENS_{POST}$ = 5.43, $SHAM_{PRE}$ = 5.44 vs. $SHAM_{POST}$ = 5.43, P = 0.833; picking up small objects with a teaspoon and placing them in a can: $TENS_{PRE}$ = 5.48 vs. $TENS_{POST}$ = 5.44, $SHAM_{PRE}$ = 5.46 vs. $SHAM_{POST}$ = 5.40, P = 0.092; stacking checkers: $TENS_{PRE}$ = 3.01 vs. $TENS_{POST}$ = 3.07, $SHAM_{PRE}$ = 3.08 vs. $SHAM_{POST}$ = 3.11, P = 0.489; moving large light cans: $TENS_{PRE}$ = 2.71 vs. $TENS_{POST}$ = 2.62, $SHAM_{PRE}$ = 2.82 vs. $SHAM_{POST}$ = 2.82, P = 0.062; moving large heavy cans: $TENS_{PRE}$ = 2.79 vs. $TENS_{POST}$ = 2.64, $SHAM_{PRE}$ = 2.91 vs. $SHAM_{POST}$ = 2.86, P = 0.539). Within the TENS group, a one sample t-tests for every single subtest revealed a significant effect over time for the subtests: turning over cards (P = 0.016) and moving heavy cans (P < 0.001). A one sample t-tests for every single subtest revealed no significant effect over time for the subtests: picking up small objects and placing them in a can (P = 0.609), picking up small objects with a teaspoon and placing them in a can (P = 0.109), stacking checkers (P = 0.903), and moving large light cans (P = 0.074). Within the sham group, a one sample t-tests for every single subtest revealed a significant effect over time for the subtests: turning over cards (P = 0.044) and moving heavy cans (P = 0.025). A one sample t-tests for every single subtest revealed no significant effect over time for all subtests: picking up small objects and placing them in a can (P = 0.488), picking up small objects with a teaspoon and placing them in a can (P = 0.929), stacking checkers (P = 0.401), and moving large light cans (P = 0.924).

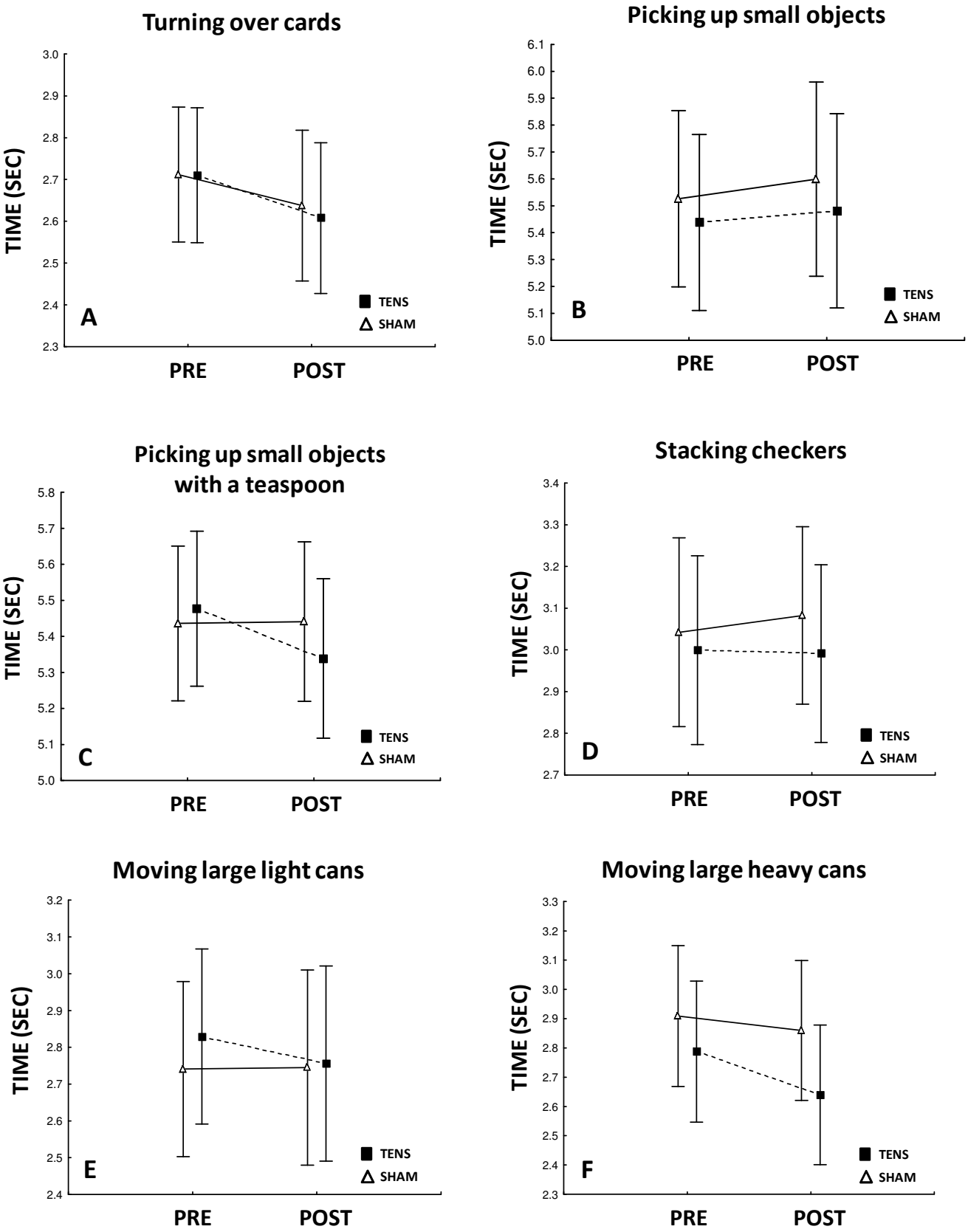

Figure 10: Mean data for partial JTT time. A: turning over cards, B: picking up small objects and placing them one by one in a can, C: picking up beans with a spoon and placing them one by one in a can, D: stacking checkers, E: moving large light cans, F: moving large heavy cans. One-sample t-tests for every single subtest did not reveal any significant results between TENS and SHAM over time.

2. The influence of the BDNF Val66Met polymorphism on sensorimotor function upon TENS therapy

Participants were divided in 2 groups (Val group and Met group) to investigate the influence of the BDNFVal66Met polymorphism on aspects of sensorimotor function upon TENS therapy.

2.1. Genetic analysis

Twenty-three subjects out of twenty-five subjects were successfully genotyped. DNA extraction failed in two subjects (table 1). Genotype frequencies were ascertained by a RFLP-PCR method. PCR products were checked for success on a 2% agarose gel. All PCR fragments resulted in the expected 274bp product (figure 11).

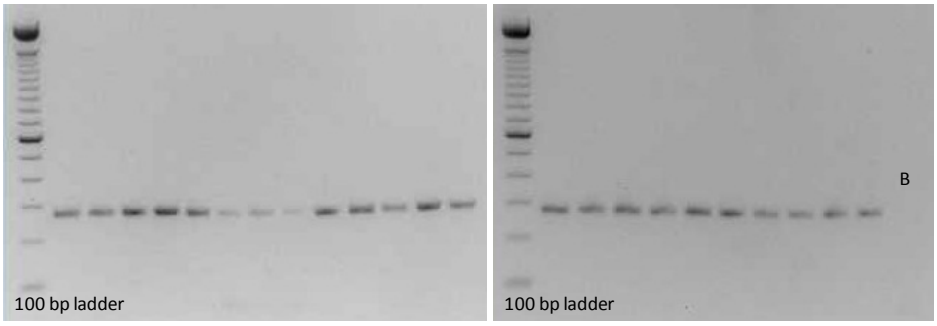

Figure 11: PCR products on a 2% agarose gel. All twenty-three PCR products resulted in a 274 bp product. No DNA was detected in the blanco sample (B).

The 3,5% agarose gels in figure 12 show the resulting restriction fragments for the subjects recruited for this study. DNA samples from Val/Val carriers show the presence of a upper (217 bp) and lower band (57bp). Val/Met carriers are distinguished from Val/Val carriers by the presence of 2 upper bands (217 and 140 bp), a 2 lower bands (77 bp and 57bp). Of the total sample eighteen subjects were Val/Val homozygotes, five were Val/Met heterozygotes and none were Met/Met homozygotes. The frequencies for the 2 BDNF genotypes were 72 % for the Val/Val group and 20 % for the Val/Met group.

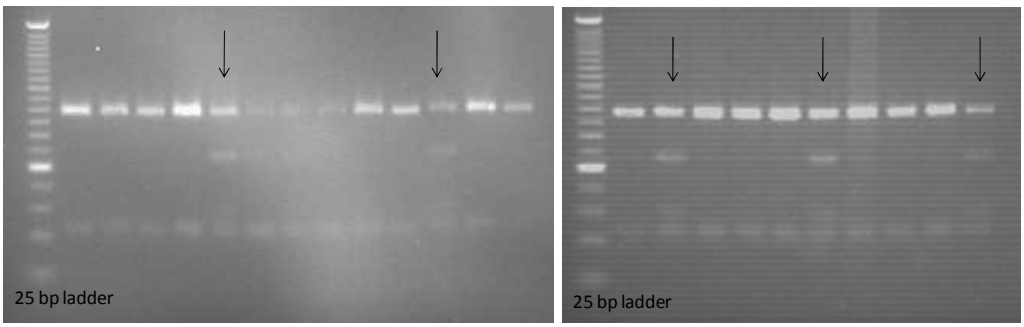

Figure 12: PCR restriction fragments on a 3,5% agarose gel. A 25 bp ladder was used to characterize the different genotypes. Of the total sample eighteen subjects were Val/Val carriers and five subjects were Val/Met carriers (indicated with the black arrows).

2.2. Sensitivity: Val group vs. Met group

To examine sensitivity in the Val and Met group touch thresholds were measured with SWMT.

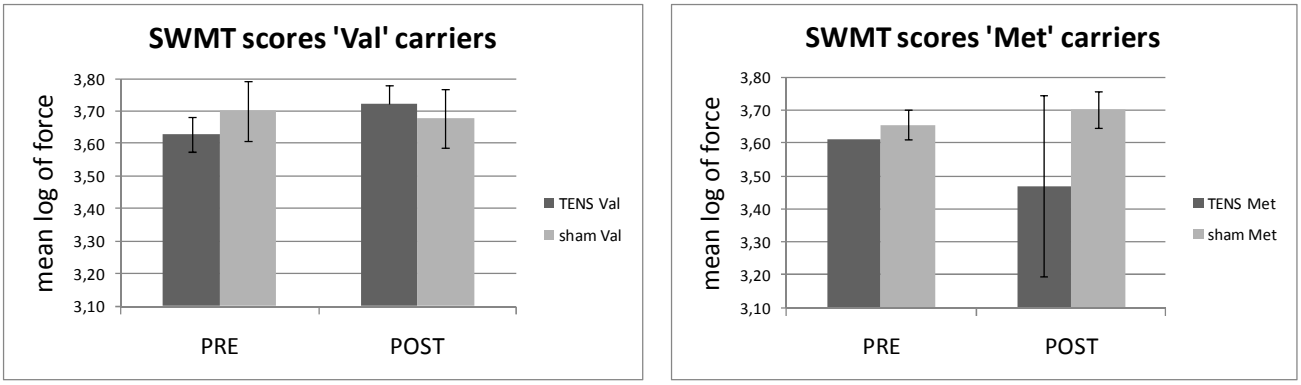

Figure 13: The mean SWMT scores (expressed as the log of force in milligrams) for the Val and Met group. Statistical analysis revealed no significant effect of TENS or SHAM on the log of force scores over time between the Val and Met group (P= 0.338).

Following results were observed in respectively the Val and Met group: $TENS_{VAL\text{-}PRE}$ = 3.63 ± 0.05 vs. $TENS_{VAL\text{-}POST}$ = 3.72 ± 0.06; $SHAM_{VAL\text{-}PRE}$ = 3.70 ± 0.09 vs. $SHAM_{VAL\text{-}POST}$ = 3.68 ± 0.09; $TENS_{MET\text{-}PRE}$ = 3.61 ± 0.00 vs. $TENS_{MET\text{-}POST}$ = 3.47 ± 0.27; $SHAM_{MET\text{-}PRE}$ = 3.66 ± 0.05 vs. $SHAM_{MET\text{-}POST}$ = 3.70 ± 0.06 (figure 13). A two sample t-test revealed no significant effect of TENS or SHAM on sensitivity over time between the Val and Met group (P = 0.338).

2.3. Motor performance: Val group vs. Met group

To investigate differences in motor performance between the Val and Met group total JTT time was recorded. The mean data for total JTT time for the Val and Met group are shown in figure 14. A two sample t-test revealed that the observed JTT times within the Val group ($TENS_{VAL\text{-}PRE}$ = 22.62 ± 0.79 vs. $TENS_{VAL\text{-}POST}$ = 22.41 ± 0.85; $SHAM_{VAL\text{-}PRE}$ = 22.67 ± 0.62 vs. $SHAM_{VAL\text{-}POST}$ = 22.73 ± 0.68) do not significantly differ from those observed within the Met group ($TENS_{MET\text{-}PRE}$ = 21.74 ± 1.05 vs. $TENS_{MET\text{-}POST}$ = 21.38 ± 1.08; $SHAM_{MET\text{-}PRE}$ = 22.13 ± 1.35 vs. $SHAM_{MET\text{-}POST}$ = 21.67 ± 1.19) (P= 0.366).

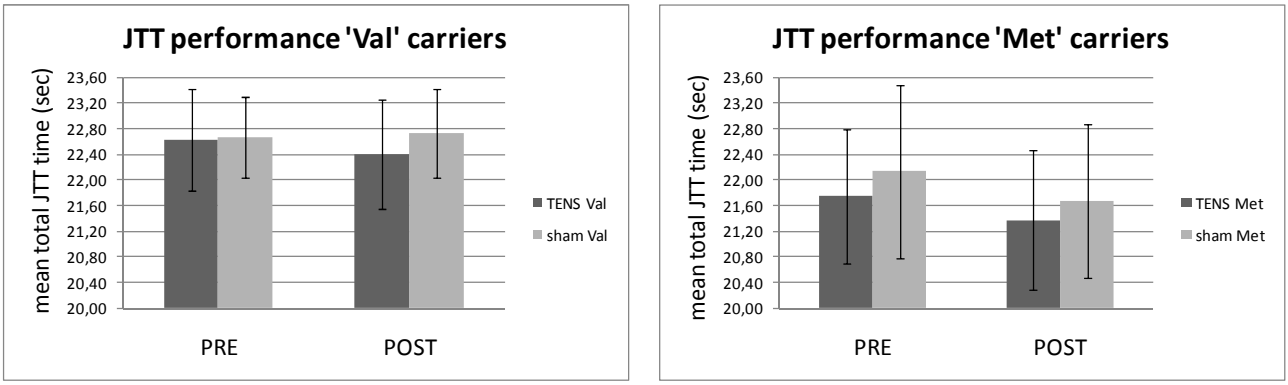

Figure 14: The mean data for total JTT time for the Val and Met group. A two-sample t-test revealed that there was no significant effect of TENS or SHAM on JTT performance over time for the Val group versus the Met group (P = 0.366).

3. Psychophysical data

TENS administration was easily tolerated by all participants. A Wilcoxon signed ranked test revealed no significant effect of TENS or SHAM on pain over time ($TENS_{PRE}$ = 1.04 vs. $TENS_{POST}$ = 0.94, $SHAM_{PRE}$ = 1.06 vs. $SHAM_{POST}$ = 0.91; P = 0.074). Pain before and after peripheral nerve stimulation was comparable.

A one sample t-test revealed only a significant effect of fatigue over time during the familiarization session ($TENS_{PRE\text{-}FAMILIARIZATION}$ = 1.16 vs. $TENS_{POST\text{-}FAMILIARISATION}$ = 1.53, P = 0.031). There was no significant effect of fatigue over time during the baseline JTT session ($TENS_{PRE\text{-}BASELINE}$ = 1.24 vs. $TENS_{POST\text{-}BASELINE}$ = 1.40, P = 0.093), the intervention ($TENS_{POST\text{-}BASELINE}$ = 1.40 vs. $TENS_{PRE\text{-}POSTintervention}$ = 1.28, P = 0.441) and post JTT session ($TENS_{PRE\text{-}POSTintervention}$ = 1.28 vs. $TENS_{POST\text{-}POSTintervention}$ = 1.32, P = 0.639). A one sample t-test revealed a significant effect of fatigue over time during the baseline JTT session ($SHAM_{PRE\text{-}BASELINE}$ = 1.17 vs. $SHAM_{POST\text{-}BASELINE}$ = 1.39, P = 0.017). According to a Wilcoxon signed ranked test, there was no significant effect of fatigue over time during the familiarization session ($SHAM_{PRE\text{-}FAMILIARIZATION}$ = 0.95 vs. $SHAM_{POST\text{-}FAMILIARISATION}$ = 1.20, P = 0.097). A one sample t-test revealed no significant effect of fatigue over time during the intervention ($SHAM_{POST\text{-}BASELINE}$ = 1.39vs. $SHAM_{PRE\text{-}POSTintervention}$ = 1.32, P = 0.748) and post JTT session ($SHAM_{PRE\text{-}POSTintervention}$ = 1.32 vs. $SHAM_{POST\text{-}POSTintervention}$ = 1.43, P = 0.240) (table 2).

Table 2: Summarized data for perceived levels of fatigue and pain during the experimental session on different time points. For timing of VAS scales see also figure 1. VAS scores are averaged for all subjects. Values are given as mean ± SE.

	TENS							
	Familiarization		Baseline		Intervention		POST intervention	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Fatigue (a)	1.16 ± 0,3	1.53 ± 0,3*	1.24 ± 0,3	1.40 ± 0,3			1.28 ± 0,3	1.32 ± 0,3
Pain (b)					1.04 ± 0,3	0.94 ± 0,2		

	SHAM							
	Familiarization		Baseline		Intervention		POST intervention	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Fatigue (a)	0.95 ± 0,2	1.20 ± 0,3	1.17 ± 0,3	1.39 ± 0,3 *			1.32 ± 0,3	1.43 ± 0,3
Pain (b)					1.06 ± 0,3	0.91 ± 0,2		

(a) Fatigue scale (0 = no fatigue; 10 = highest level of fatigue)
(b) Pain scale (0 = no pain; 10 maximal pain)

* significant

Discussion

The main finding of this study was that a 20 min low frequency TENS stimulation protocol did not reveal significant functional improvement of the dominant hand in healthy volunteers above 65 years. In addition, no differences in sensorimotor performance between the Val and Met group were observed.

It is well known that the non-pathological ageing process is associated with a general decline in sensory and motor function [1-3]. Evidence exist that changes occur in the peripheral cutaneous system. For example, the number and morphology of Meissner's and Pacinian corpuscles change in old age [3,38]. A decreased number of Meissner corpuscles in the finger is clinically associated with declines in touch thresholds [38]. The data presented in the current study confirms that participants show impairments in touch thresholds (ranging from diminished light touch to diminished protective sensation). Furthermore there is evidence that nerve conduction velocity declines and changes in morphology and excitability of the central nervous system occur [3,39,40]. Because these age-related functional and structural changes affect simple activities of daily life [2] and a rise in the number of people above 65 years is expected [41], alternative strategies for neurorehabilitation, such as TENS, are desirable.

It has been shown in human studies [6-10] that TENS can produce plastic changes in SM1. Ridding et al. (2001) reported an increased excitability in the target muscle in young (age 21-42) healthy volunteers accompanied by increased cortical map area following a long-term period (2 hours) of peripheral nerve stimulation (10Hz, 1ms, intensity above motor threshold). The authors argue that excitability changes are taking place on the cortical level because no excitability changes of spinal motoneurons were found [6,8]. However, it was not investigated whether the increased excitability was accompanied with behavioural improvements. In this respect, the present study was focusing on sensitivity and motor performance.

1. The effect of TENS on hand function in healthy elderly

The first aim of the present study was to investigate the effect of a short-term low frequency TENS (20 min, 10Hz) on hand function in healthy elderly.

Parameters and study population

A wide variety of stimulation parameters are used in TENS for clinical purposes [4,5,42,43]. Therefore, selection of the appropriate stimulation parameters (i.e. intervention time, frequency, intensity, pulse duration and waveform) is important to achieve the desired result [5,44].

In the present study a synchronous nerve stimulation of the median and ulnar nerve was applied because both nerves activate cutaneous fibers of the hand and small hand muscles of our interest. Moreover, in the past similar stimulation protocols, modulating motor performance, were used successfully [8,11,45]. The stimulation parameters applied by Ridding et al. (2001) resemble the parameters we used, with exception for time and intensity. In contrast to Riddding et al. (2001), the present study stimulated nerves with an intensity below the motor threshold. Previous studies have proven that an intensity below motor threshold for 2 hours leads to improvement in total JTT time in chronic stroke patients [46,47]. With regard to time, studies reported functional gains of hand motor performance when a long-term intervention protocol from 2 hours was used [4,46]. However, in our opinion, a shorter stimulation period is much more attractive for rehabilitation purposes. There is convincing evidence that short stimulation periods can induce structural changes accompanied with significant improvement in motor performance. Fraser et al. (2002) described that a short-term period (10 min) of sensory electrical stimulation (5Hz, 0,2 ms, mean intensity 16 ± 2 mA) of the pharynx in dysphagic stroke patients was not only associated with enhanced excitability of the human swallowing motor cortex, but also with improvements in swallowing function. However, the short-term intervention protocol used in the present study (20 min) did not reveal any significant effects on sensorimotor function in the dominant hand of elderly.

The result cannot be explained by fatigue during the experiment or by pain as a result of the intervention. More likely, it appears that the stimulation period in this study was too short or that stimulation parameters such as pulse duration and intensity were not optimal. On the other hand, it is possible that the elderly participating in this study had no further potential for sensorimotor improvement and that the results can be explained by a ceiling effect. Hummel et al. (2009) reported mean baseline values of JTT ranging from 28,53s to 29,89s in a healthy study population with a mean age of 69 years. Mean baseline values of JTT in the present study are remarkably lower with scores ranging from 21,38s to 22,73s despite the fact that the mean age of the study population was 73 years.

However, imaging studies report that (some) elderly are able to reach motor performance levels comparable to younger subjects (unpublished pilot data) when overactivating sensorimotor brain regions or by activating additional brain regions e.g. frontal area [40,48]. Another aspect that has to be taken in account in the explanation of our results is that there are great differences in physical activity in elderly. Most European elderly are categorized in two extreme groups: no activity or 3.5 or more hours per week activity. Of these European countries, Belgium is known as a country with a high proportion (56%) of sedentary elderly [41].

Probably the sample of healthy elderly who participated in this study was not representative for the Belgian population. All participants reported to be physically active at least one hour a week and most of them even more.

Training task

Clinical studies indicate that peripheral nerve stimulation should not be used as a single therapy, but rather in combination with active training. In this way, the effectiveness of training can be enhanced [7]. Previously TENS was successfully combined with a motor training task [4]. Compared to motor training alone, it was reported that the combination of TENS with a motor training task (JTT) leads to significantly better performance of this task after the end of intervention [4]. In the present study a combination with a tactile sensitivity task was applied. Nonetheless, touch thresholds did not change.

In contrast to our findings, a recent study by Kalisch et al. (2008) did found significant improvements in sensitivity using a comparable technique. However, there are several differences to take in account. First of all, Kalisch et al. (2008) applied a paradigm based on Hebbian synaptic plasticity, i.e. tactile coactivation (CA) instead of TENS. In this paradigm neural activity was evoked by a 2h-3h passive sensory stimulation of skin portions of the finger. Secondly, in the present study we used the SWMT, whereas Kalisch et al. (2008) included the 2-point discrimination test to measure sensitivity. Both tests measure different properties of sensitivity [49]. While the 2-point discrimination test measures spatial discrimination, the SWMT measures detection threshold. Whereas the SMW is known for its validity, reliability, reproducibility and responsiveness; the validity and the responsiveness of the 2-point discrimination test has been questioned [49]. Nonetheless, Kalisch et al. (2008) used a specifically designed apparatus that allows a standardized and objective form of testing 2-point discrimination. However, its validity, reliability and responsiveness for assessing spatial discrimination in the fingers has not been studied so far.

2. Interaction of the BDNF Val66Met polymorphism and TENS

The second aim of this study was to investigate differences between BDNF Val and Met carriers in sensorimotor performance after peripheral nerve stimulation. BDNF has been identified as a key regulator in synaptic plasticity. It is expressed by neurons in response to neuronal activity and controls basic steps in the process of synaptic plasticity [14]. It is known that the BDNF Val66Met polymorphism has anatomical [30] and behavioural [28] consequences in healthy people.

In contrast to Val carriers, Met carriers of the BDNF gene are associated with poorer capability to induce functional and/or structural neuroplasticity after motor task learning [32] or median nerve paired associative stimulation [50]. This reduced ability to induce neuroplasticity in Met carriers could lead to a detrimental rehabilitation outcome. If this is true, it will be useful to take BDNF in account in the prognosis of neurorehabilitation.

A previous study [32] reported differences in corticospinal excitability between Val and Met carriers after conducting a motor task. We expected that difference in task performance would be more pronounced because the active training was accompanied with TENS. However, our findings show that there are no differences in sensitivity or JTT performance between the two different genotypes (Val/Val or Val/Met) after short-term TENS therapy. Corresponding with our results, Kleim et al. (2006) did not found differences between the BDNF genotypes on simple motor tasks such as peg-board test, pinch-grip test or tapping rate. Probably a more complex motor task with new movements might be required to detect differences between the BDNF genotypes (Kleim et al. 2006). However changes in hand motor performance might be found according to differences in cognition. Interestingly, a recent study (Nagel et al. 2008) showed a correlation of age, cognitive performance and the BDNF genotype. Nagel et al. 2008 has proven that genetic factors, such as the BDNF Val66Met polymorphism contribute to an increasing heterogeneity of cognitive performance in elderly. As a consequence of this, we can hypothesize that sensorimotor tasks including a cognitive component are easier to perform by Val carriers as compared to Met carriers, with increasing age.

Conclusion

Different protocols based on prolonged peripheral nerve stimulation (1-2 hours) can induce plastic changes in SM1. Hence, peripheral nerve stimulation in the form of TENS has the potential to be a therapeutic tool in neurodegenerative diseases including ageing. However, in our opinion a shorter stimulation period for rehabilitation purposes is much more attractive. The present results indicate that a 20 min low frequency TENS elicits no improvement in sensitivity or hand motor function in the dominant hand of healthy elderly. Furthermore, functional performance was not influenced by the BDNF Val66Met polymorphism. To obtain an optimal behavioural outcome after 20 min peripheral nerve stimulation, more experiments are necessary to discover the appropriate stimulation parameters i.e. frequency, pulse duration, intensity, waveform (monophasic/biphasic). In order to achieve more pronounced effects a more complex task could be used in future experiments. In addition, the study population must be chosen with caution i.e with potential to improve on sensorimotor performance. Because the possible significance of BDNF in prognosis of neurorehabilitation it would be interesting to investigate a larger study population for the influence of the BDNF Val66Met polymorphism, once an affective protocol is acquired. A limitation of the study is that no transcranial magnetic stimulation (TMS) measurements were performed. Despite the fact that subjects show no significant improvement in behavioural performance, reorganization of SM1 was expected. However additional TMS measurements are necessary to confirm this judgement.

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Supplement 1: DNA extraction

a. Genomic DNA extraction out of blood samples

Day 1

Red blood cell lysis

- Transfer blood sample (10ml) into a 50 ml tube
- Add 30ml hemolysisbuffer (1x)
- Place sample 20 min on ice
- Centrifuge at 2000rpm for 15 min in a cooled centrifuge
- Drain supernatant
- Wash pellet with 10ml hemolysisbuffer (1x)
- Vortex
- Centrifuge at 2000rpm for 10 min in a cooled centrifuge
- Drain supernatant
- Wash pellet with 10ml hemolysisbuffer (1x)
- Vortex
- Centrifuge at 2000rpm for 10 min in a cooled centrifuge
- Drain supernatant

White blood cell lysis

- Add 3ml lysisbuffer
- Vortex
- Weigh 2 mg proteinase K powder and solve in 1 ml proteinase K-solution: add 0,5 ml to sample
- Add 100 µl 20% SDS
- Incubate overnight at 37°C

Day 2

- Transfer sample into a 15ml tube
- Add 1ml saturated NaCl-solution
- Shake
- Add 4ml saturated chloroform
- 20 min on rotex separator
- Centrifuge at 2000rpm for 10 min at room temperature
- Transfer upper phase into a 50ml tube
- Add 2 volumes 100% ethanol
- Mix softly until a DNA-prop becomes visible
- Transfer DNA-prop into a eppendorftube
- Wash with 200µl 70% ethanol
- Transfer entire ethanol with a pipette
- Evaporate DNA
- Dissolve DNA in 500µl milliQ water
- Store at -20 °

b. Solutions

Hemolysisbuffer (10x)	*Lysisbuffer*	*Proteinase K-solution*
82,9g NH_4Cl 10g $KHCO_3$ 3,7g EDTA Dilute with 1L milliQ water Setting pH at 7,4 Autoclave solution Store at 4°C	0,12g Tris 2,33g NaCl 0,07g EDTA Setting pH at 8,2 Dilute with 100ml milliQ water Autoclave solution Store at 4°C	1g SDS 0,07g EDTA Dilute with 100 milliQ water Autoclave solution Store at 21°C
Saturated NaCl solution	*SDS 20% solution*	*Saturated chloroform*
35g NaCl Dilute with 100ml milliQ water Autoclave solution Store at 4 °C	10g SDS Dilute with 50ml milliQ water Autoclave solution Store at 21°C	60ml chloroform 20ml autoclaved milliQ water Shake until 2 phases appear Wrap with aluminum foil Store at 21°C

Supplement 2: PCR-RFLP

a. PCR-RFLP

Primer design

Primers that were previously used (green letters) in PCR-RFLP experiments (Cheeran et al. 2008, Kleim et al., 2006, Sen et al., 2003) were confirmed using the NCBI primer designing tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/).

The following FASTA sequence was entered as PCR template. The red letters indicate the polymorphism of interest (rs6265). A 'G' to 'A' nucleotide substitution produces a valine to methionine amino acid substitution.

>gnl|dbSNP|rs6265|allelePos=318|totalLen=1458|taxid=9606|snpclass=1|alleles='A/G'|mol=Genomic|build=130

CTGCAGAAAGGCCTGGAATTACAATCAGATGGGCCACATGGCATCCCGGTGAAAGAAAGCCCTAACCAGTTTTCT
GTCTTGTTTCTGCTTTCTCCCTACAGTTCCACCAGGTGAGAAGAGTGATGACCATCCTTTTCCTTACTATGGTTA
TTTCATACTTTGGTTGCATGAAGGCTGCCCCCATGAAAGAAGCAAACATCCGAGGACAAGGTGGCTTGGCCTACC
CAGGTGTGCGGACCCATGGGACTCTGGAGAGCGTGAATGGGCCCAAGGCAGGTTCAAGAGGCTTGACATCATTGG
CTGACACTTTCGAACAC[A/G]TGATAGAAGAGCTGTTGGATGAGGACCAGAAAGTTCGGCCCAATGAAGAAAACA
ATAAGGACGCAGACTTGTACACGTCCAGGGTGATGCTCAGTAGTCAAGTGCCTTTGGAGCCTCCTCTTCTCTTTC
TGCTGGAGGAATACAAAAATTACCTAGATGCTGCAAACATGTCCATGAGGGTCCGGCGCCACTCTGACCCTGCCC
GCCGAGGGGAGCTGAGCGTGTGTGACAGTATTAGTGAGTGGGTAACGGCGGCAGACAAAAAGACTGCAGTGGACA
TGTCGGGCGGGACGGTCACAGTCCTTGAAAAGGTCCCTGTATCAAAAGGCCAACTGAAGCAATACTTCTACGAGA
CCAAGTGCAATCCCATGGGTTACACAAAAGAAGGCTGCAGGGGCATAGACAAAAGGCATTGGAACTCCCAGTGCC
GAACTACCCAGTCGTACGTGCGGGCCCTTACCATGGATAGCAAAAAGAGAATTGGCTGGCGATTCATAAGGATAG
ACACTTCTTGTGTATGTACATTGACCATTAAAAGGGGAAGATAGTGGATTTATGTTGTATAGATTAGATTATATT
GAGACAAAAATTATCTATTTGTATATATACATAACAGGGTAAATTATTCAGTTAAGAAAAAAATAATTTTATGAA
CTGCATGTATAAATGAAGTTTATACAGTACAGTGGTTCTACAATCTATTTATTGGACATGTCCATGACCAGAAGG
GAAACAGTCATTTGCGCACAACTTAAAAAGTCTGCATTACATTCCTTGATAATGTTGTGGTTTGTTGCCGTTGCC
AAGAACTGAAAACATAAAAAGTTAAAAAAAATAATAAATTGCATGCTGCTTTAATTGTGAATTGATAATAAACTG
TCCTCTTTCAGAAAACAGAAAAAAACACACACACACACAACAAAAATTTGAACCAAAACATTCCGTTTACATTTT
AGACAGTAAGTATCTTCGTTCTTGTTAGTACTATATCTGTTTTACTGCTTTTAACTTCTGATAGCGTTGGAATTA
AAACAATGTCAAGGTGCTGTTGTCATTGCACCCCCAAGGGGAACTAACCGCCTCCCACACACTATATTCCTGCCA
CCCCCGCCCCACCCTACACCGGCCCCGCACCGCCCC

Next a forward primer (5'-AAAGAAGCAAACATCCGAGGACAAG-3') and a reverse primer (5'-ATTCCTCCAGCAGAAAGAGAAGAGG-3') were entered in primer parameters. The result for these query's are shown in the next figure.

Primer pair 1

	Sequence (5'->3')	Strand on template	Length	Start	Stop	Tm	GC%
Forward primer	AAAGAAGCAAACATCCGAGGACAAG	Plus	25	732	756	56.06	44.00%
Reverse primer	ATTCCTCCAGCAGAAAGAGAAGAGG	Minus	25	1005	981	56.04	48.00%
Product length	274						

Products on intended target

>NM_170731.4 Homo sapiens brain-derived neurotrophic factor (BDNF), transcript variant 3, mRNA >gi|152926251|gb|EF689021.1| Homo sapiens brain derived neurotrophic factor variant (BDNF) mRNA, complete cds

```
product length = 274
Forward primer  1    AAAGAAGCAAACATCCGAGGACAAG  25
Template        732  .........................  756

Reverse primer  1     ATTCCTCCAGCAGAAAGAGAAGAGG  25
Template        1005  .........................  981
```

Restriction mapping

The electronic restriction mapper (http://www.restrictionmapper.org/) was used to determine the length of the DNA fragments. The *Hsp*92II enzyme, which recognize the 5'-CATG-3' sequence, was selected and the following sequence was entered. This sequence is the resulted 274bp PCR product. Green letters represent forward and reverse primer. The red letters represent the polymorphism of interest.

AAAGAAGCAAACATCCGAGGACAAGGTGGCTTGGCCTACCCAGGTGTGCGGACCCATGGGACTCTGGAGAGCGTG
AATGGGCCCAAGGCAGGTTCAAGAGGCTTGACATCATTGGCTGACACTTTCGAACAC[A/G]TGATAGAAGAGCTG
TTGGATGAGGACCAGAAAGTTCGGCCCAATGAAGAAAACAATAAGGACGCAGACTTGTACACGTCCAGGGTGATG
CTCAGTAGTCAAGTGCCTTTGGAGCCTCCTCTTCTCTTTCTGCTGGAGGAAT

Virtual digest for 'G' carriers or Val carriers resulted in 2 products, 217 and 57 bp

Enzymes: Hsp92II

Length	5' Enzyme	5' Base	3' Enzyme	3' Base	Sequence
217	Hsp92II	58	none	274	GGACTCTGGA GAGCGTGAAT GGGCCCAAGG CAGGTTCAAG AGGCTTGACA TCATTGGCTG ACACTTTCGA ACACGTGATA GAAGAGCTGT TGGATGAGGA CCAGAAAGTT CGGCCCAATG AAGAAAACAA TAAGGACGCA GACTTGTACA CGTCCAGGGT GATGCTCAGT AGTCAAGTGC CTTTGGAGCC TCCTCTTCTC TTTCTGCTGG AGGAATA
57	none	1	Hsp92II	57	AAGAAGCAAA CATCCGAGGA CAAGGTGGCT TGGCCTACCC AGGTGTGCGG ACCCATG

Virtual digest for 'A' carriers or Met carriers resulted in 3 products, 140, 77 and 57 bp

Enzymes: Hsp92II

Length	5' Enzyme	5' Base	3' Enzyme	3' Base	Sequence
140	Hsp92II	135	none	274	ATAGAAGAGC TGTTGGATGA GGACCAGAAA GTTCGGCCCA ATGAAGAAAA CAATAAGGAC GCAGACTTGT ACACGTCCAG GGTGATGCTC AGTAGTCAAG TGCCTTTGGA GCCTCCTCTT CTCTTTCTGC TGGAGGAATA
77	Hsp92II	58	Hsp92II	134	GGACTCTGGA GAGCGTGAAT GGGCCCAAGG CAGGTTCAAG AGGCTTGACA TCATTGGCTG ACACTTTCGA ACACATG
57	none	1	Hsp92II	57	AAGAAGCAAA CATCCGAGGA CAAGGTGGCT TGGCCTACCC AGGTGTGCGG ACCCATG

Auteursrechtelijke overeenkomst

Ik/wij verlenen het wereldwijde auteursrecht voor de ingediende eindverhandeling:
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Richting: **master in de biomedische wetenschappen-milieu en gezondheid**
Jaar: **2010**

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Datum: **14/06/2010**