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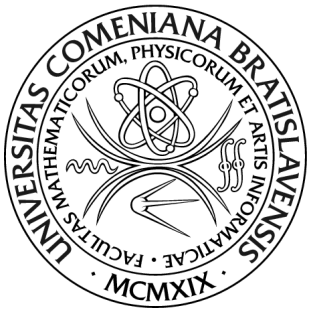
EEG MU RHYTHM DESYNCHRONISATION: AN ELECTROPHYSIOLOGICAL EVIDENCE FOR MIRROR NEURONS ACTIVITY

Diplomová práca

Bratislava 2012 **Bc. Ján Šilar**

COMENIUS UNIVERSITY IN BRATISLAVA
FACULTY OF MATHEMATICS, PHYSICS AND INFORMATICS



EEG MU RHYTHM DESYNCHRONISATION: AN ELECTROPHYSIOLOGICAL EVIDENCE FOR MIRROR NEURONS ACTIVITY

Diploma thesis

Study program:	Cognitive science
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Supervisor:	Kristína Rebrová, RNDr.
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Bratislava 2012 **Ján Šilar, Bc.**

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THESIS ASSIGNMENT

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Title: EEG mu rhythm desynchronisation: An electrophysiological evidence for mirror neurons activity

Aim:
1. Explore the literature about mu rhythm desynchronisation and mirror neurons.
2. Using an EEG experiment investigate mu rhythm desynchronisation in subjects observing simple motor movements.
3. Explore the effect of various experimental conditions (different hand movements) on desynchronization and evaluate the results.

Supervisor: RNDr. Kristína Rebrová
Department: FMFI.KAI - Department of Applied Informatics

Assigned: 19.11.2010

Approved: 19.10.2011 prof. RNDr. Pavol Zlatoš, PhD.
Guarantor of Study Programme

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ZADANIE ZÁVEREČNEJ PRÁCE

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Názov: Desynchronizácia EEG mí rytmu: elektrofyziologický dôkaz aktivity zrkadliacich neurónov

Cieľ:
1. Preskúmajte literatúru o desynchronizácii mí rytmu a zrkadliacich neurónoch.
2. Experimentálne preskúmajte desynchronizáciu mí rytmu pri pozorovaní jednoduchých pohybov v rámci EEG experimentu.
3. Preskúmajte možnosti rôznych experimentálnych podmienok resp. rôznych pohybov ruky a vyhodnoťte výsledky.

Vedúci: RNDr. Kristína Rebrová
Katedra: FMFI.KAI - Katedra aplikovanej informatiky

Dátum zadania: 19.11.2010

Dátum schválenia: 19.10.2011

prof. RNDr. Pavol Zlatoš, PhD.
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študent

.....
vedúci práce

Declaration

Herewith I declare, that I wrote this diploma thesis by myself, with use of the referenced literature, and under the careful supervision of my thesis supervisor.

Prehlásenie

Týmto čestne prehlasujem, že som túto diplomovú prácu vypracoval samostatne s použitím uvedenej literatúry a pod dohľadom mojej diplomovej vedúcej.

Bratislava, 25th of May 2012 Ján Šilar

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I would like to thank my supervisor, RNDr. Kristína Rebrová, for her spent time and energy for leading me not only through the process of writing this thesis, but also during the whole master studies.

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Abstract

Mirror neurons are multimodal association neurons with motor properties in premotor and posterior parietal cortex that increase their activity and fire not only during action execution, but also while observing or hearing another individual performing the same or a similar action. Mirror neurons play important role in action recognition, imitation, empathy, and also in the theory of mind. Electrophysiological index of mirror neuron system activity is an EEG oscillation called mu rhythm. We created an EEG experiment with focus on mu rhythm as the indicator of mirror neuron system activity. The core research question was to find the highest mu power in relax condition and similar mu suppressions in self motor movement, and the same observed movement condition. Within the second research question we expected to find contralateral correlations in mu rhythm strength according to index finger side in nonrest conditions and hemispheric differences in mu power also in nonrest conditions. The results showed statistically significant suppression from baseline in mu oscillations over both hemispheres during nonrest conditions, supporting our expectations of the first research question. Results for our second question were statistically insignificant, what contradicts our expectation. However, from this finding we can assume that the mirror neuron system activity is not hemispherically differentiated for distinguishing left and right side movement, but rather approximate to distinguish and understand any kind of movement.

Keywords

mirror neurons, motor movement, motor resonance, mu rhythm, EEG

Abstrakt

Zrkadliace neuróny sú multimodálne asociačné neuróny s motorickými vlastnosťami, nachádzajúce sa v premotorickej a posteriórnej parietálnej kôre, ktoré zvyšujú svoju aktivitu a pália, nielen pri vykonávaní akcií, ale aj pri pozorovaní alebo počúvaní iného jedinca vykonávať rovnakú alebo podobnú akciu. Zrkadliace neuróny hrajú dôležitú úlohu pri rozpoznávaní akcií, imitácii, empatii, a tiež v teórii mysle. Elektrofyziologický ukazovateľ aktivity systému zrkadliacich neurónov je EEG oscilácia nazývaná mí rytmus. Vytvorili sme EEG experiment zameraný na detekciu mí rytmu ako indikátora aktivity systému zrkadliacich neurónov. Základným očakávaním bolo nájdenie najvyššej sily signálu mí rytmu v stave motorickej relaxácie a podobnú supresiu tohto rytmu v podmienkach vlastného pohybu a rovnakého pozorovaného pohybu. Naviac, sme očakávali nájdenie kontralaterálnych korelácií sily mí rytmu v závislosti na aktívnej ruke v podmienkach pohybu a hemisférické rozdiely v sile mí rytmu počas podmienok v pohybe. Výsledky ukázali štatisticky signifikantné zníženie sily mí oscilácií oproti základnej podmienke v oboch hemisférach počas pohybových podmienok, čo potvrdzuje naše prvé očakávanie. Výsledky nášho druhého pokusu boli štatisticky nesignifikantné, čo protirečilo nášmu očakávaniu. Z tohto zistenia, ale môžeme usúdiť, že činnosť systému zrkadliacich neurónov nie je hemisféricky rozdelená pre rozoznávanie ľavej a pravej strany pohybu, ale skôr zovšeobecňujúca pre rozpoznanie a pochopenie akéhokoľvek druhu pohybu.

Kľúčové slová

zrkadliaci neurón, motorický pohyb, motorická rezonancia, mí rytmus, EEG

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1 Introduction

The discovery of mirror neurons properties, first in macaque monkeys and later on in humans, has evoked a number of new research studies and thoughts of the possible, and most probable roles the mirror neurons play in variety of cognitive functions, that the brain possess.

We use our brains every day, 24 hours a day completely automatically without even knowing about them. So do we use mirror neurons, which are inseparable parts of our brains. Mirror neurons are multimodal association neurons with motor properties in premotor and posterior parietal cortex that increase their activity and fire not only during action execution, but also while observing (1) or hearing (2) another individual performing the same or a similar action.

Mirror neurons facilitate us with the understanding of motor actions of other human beings and even some animal species capabilities, which is one of the most crucial cognitive skill that our brains possess.

When we observe another individual's actions, we are able to make predictions about the mental state of the observed individual, leading to "theory of mind" capabilities (3), which plays important role in social cognition and social interactions. The understanding of actions is often explained by activity of mirror neuron system and motor resonance in our brains, which can be experimentally proved through measurements and observations of particular EEG oscillation called mu rhythm (3).

In our experiment we have focused on the mu rhythm as the indicator of mirror neuron system activity. Through observation of this rhythm using EEG device the cognitive processing and the electrophysiological consequences of action processing and recognition can be studied.

2 Mirror neurons

In this chapter we introduce theoretical background of concept and functionality of brain cells called “mirror neurons”. We will discuss basic anatomy, special properties and electro-physiological index of mirror neurons activity, which is called the mu rhythm.

2.1 Mirror neurons

Mirror neurons are multimodal association neurons with motor properties in premotor and posterior parietal cortex that increase their activity and fire not only during action execution, but also while observing (1) or hearing (2) another individual performing the same or a similar action. Their main goal is to transform specific sensory information into a motor format. Because of these properties, which suggest that the individual is observing its own actions reflected by a mirror, these cells were called mirror neurons (4).

2.1.1 Mirror neurons in monkeys

Mirror neurons have been originally discovered in the ventral premotor cortex, area F5, of the macaque monkey (1). This discovery was carried out using single-cell recording technique. Single-cell recording is invasive neurophysiological method that records electric activity of single neuron using implanted microelectrode to the particular cell in the brain tissue. The premotor cortex of the macaque brain, is a cortical region important for coding the planning, preparation, and selection of movements and coordinated actions, where its ventral sector called area F5, has physiological properties relevant to the neural control of mouth and hand movements, especially grasping (4). Subsequently, they have been also found in macaques inferior parietal lobule (5) (Figure 2.1). This suggests that the visual information travels to F5 through the parietal area PF/PFG, which is heavily interconnected with F5 (6). The common defining characteristic of both ventral premotor mirror neurons and inferior parietal mirror neurons is the close relationship they show between the motor acts they code and the visual motor acts they respond to. In other words

they become active both when the monkey makes a particular action (for example, when grasping an object or holding it), and when it observes another individual (monkey or human) making a similar action (Figure 2.2). Typically,mirror neurons do not respond to the sight of a hand mimicking an action in the absence of the target. Similarly, they do not respond to the observation of an object alone, even when it is of interest to the monkey (7).

Anterior part of the macaque ventral premotor cortex (area F5) is histoarchitectually heterogeneous and it is subdivided into three sections (Figure 2.1 – F5 closeup). A section located on the anterior part of the arcuate sulcus (F5a), a section located on the posterior part of the arcuate sulcus (F5p), and a section lying on the cortical area called "convexity" (F5c) extending on most of the postarcuate convexity cortex (8). There have been found and described two functionally distinct categories of visuomotor neurons in area F5. “Canonical neurons”, which become active when monkey observes graspable 3D object (5). These neurons are located in the posterior part of the inferior arcuate sulcus, particularly in area F5p (Figure 2.1 – F5 closeup) (8). The second group are “mirror neurons”, which discharge when monkey performs a particular motor action (for example, grasping), and when it observes another individual making the same or similar action (Pic. 2.2) (7). Mirror neurons are located almost exclusively in the cortical convexity, area F5c (Pic. 1 – F5 closeup) (8).

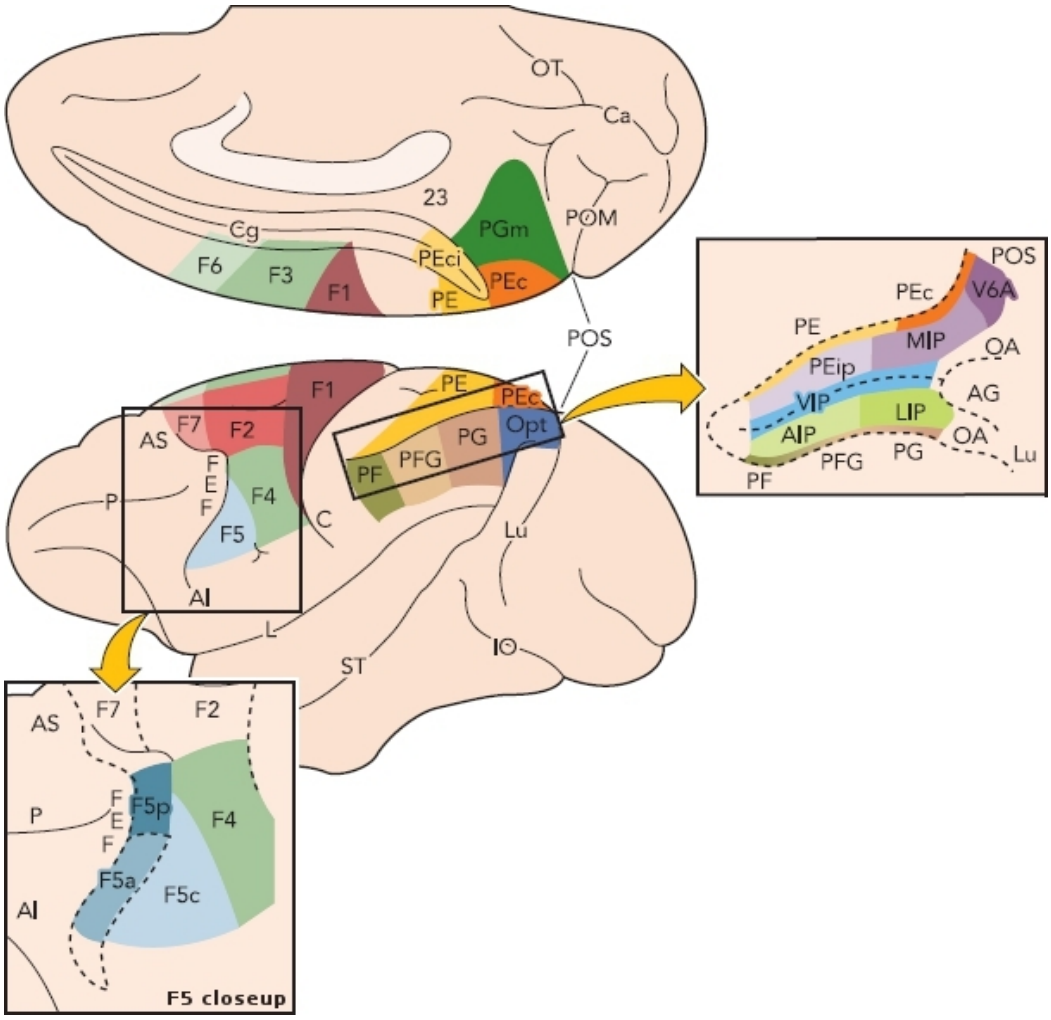


Figure 2.1: ***Medial and lateral views of the macaque brain****. The central part of the figure shows the cytoarchitectonic parcellation of the frontal motor cortex (areas indicated with F and Arabic numbers) and of the parietal lobe (areas indicated with P and progressive letters). The enlargement of the frontal region (rectangle on the left) shows the parcellation of F5. The rectangle on the right shows the areas buried within the intraparietal sulcus. AIP, anterior intraparietal area; IP, intraparietal sulcus; LIP, lateral intraparietal area; MIP, medial intraparietal area; POs, parieto- occipital sulcus; As, superior arcuate sulcus; AI inferior arcuate sulcus; C, central sulcus; Ca, calcarine fissure; CG, cingulate cortex; FEF, frontal eye field; L, lateral sulcus; Lu, lunate sulcus; P, principal sulcus; STS, superior temporal sulcus.(5)*

When we examine the congruency criterion between execution and observing of motor actions in mirror neurons activity, there are two significantly distinguishable groups of

mirror neurons from this perspective in macaque monkey brain. The first group are strictly congruent mirror neurons, which discharge when the observed and executed effective motor acts are identical both in terms of goal (for example, grasping) and in terms of the way in which that goal is achieved (for example, precision grip)(5). Strictly congruent mirror neurons form approximately 30% of all mirror neurons in F5 area, whereas the rest of them is included in the second group (9). Mirror neurons in the second group are called broadly congruent. Broadly congruent mirror neurons are less precise in the mapping of the observed and executed motor acts. They are triggered by achieving a similar goal (for example, grasping an object) but can often involve another effector (for example, grasping with the mouth or with the hand) (10). These neurons seem to generalize the goal of the observed action across many instances of it (7). Finally, Gallese and colleagues defined third group of those neurons in which there was no clear relationship between the effective observed actions and the effective motor action of the monkey as non-congruent (9).

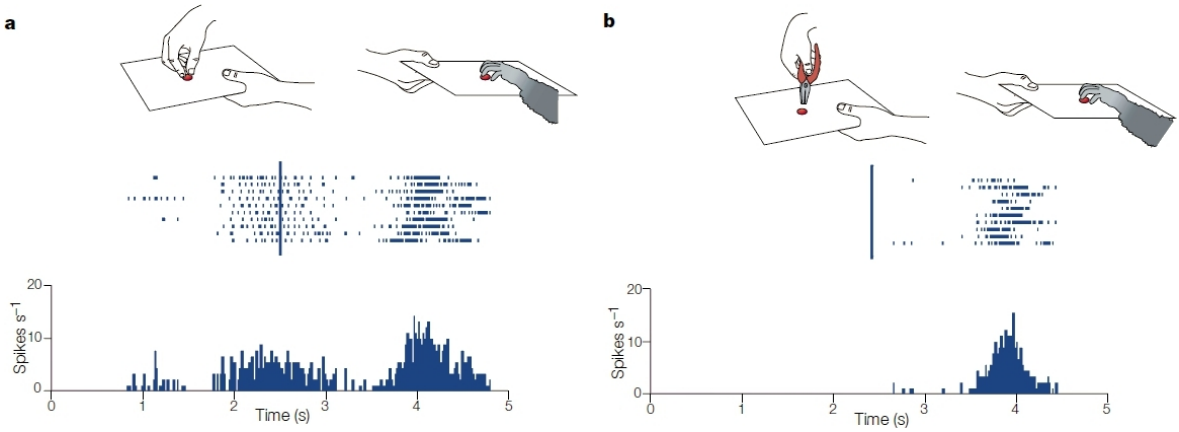


Figure 2.2: ***Visual and motor responses of a mirror neuron in area F5****.* ***a)*** *A piece of food is placed on a tray and presented to the monkey. The experimenter grasps the food, then moves the tray with the food towards the monkey. Strong activation is present in F5 during observation of the experimenter's graspingmovements, and while the same action is performed by the monkey. Note that the neural discharge (lower panel) is absent when the food is presented andmoved towards the monkey.* ***b)*** *A similar experimental condition, except that the experimenter grasps the food with pliers. Note the absence of a neural response when the observed action is performed with a tool. Rasters and histograms show activity before and after the point at which the experimenter touched the food(vertical bar). (7) © 1996 Elsevier Science.*

2.1.1.1 Mirror neuron circuit

Regions of the posterior lateral temporaloccipital cortex, particularly within and nearby the posterior superior temporal sulcus (STS) also respond to the perception of biological motion (8). Pelphrey and colleagues have demonstrated in other studies that the posterior lateral temporal-occipital region is sensitive to violations of the apparent goal of the observed motion, and have suggested that it participates in social perception via the analysis of the intentions conveyed by others' actions (8). Neurons in this region respond to particular set of movements, that consists of walking, turning the head, bending the torso, and moving the arms. A small set of STS neurons discharge also during the observation of goal-directed hand movements (Perrett et al. 1990).

When we compare the functional properties of STS and F5 neurons, there are two apparent differences in these two regions. First, STS appears to respond to a much larger number of movements than F5. This may be ascribed, however, to the fact that STS output reaches the whole ventral premotor region and not only F5. Second, STS neurons do not appear to be endowed with motor properties (1) because they do not discharge in association with motor activity (5).

Recent experimental research using functional magnetic resonance and other anatomically constrained imagining techniques showed that there are two main anatomical and functional streams that, via inferior parietal lobule (IPL), connect area F5 with the higher order visual areas of the STS. The first stream originates from a sector of the upper bank of the STS ("STPm"), reaches parietal area prefrontal gyrus (PFG), and terminates in area F5c. The second stream arises in the lower bank of the STS, reaches parietal area AIP, and then area F5a (11, 5).

Another cortical area where there are neurons that respond to the observation of actions done by other individuals is the parietal lobe, specifically area PF (5). This area (in Figure 1) forms the rostral part of the inferior parietal lobule. It PF receives input from STS and sends an output to the ventral premotor cortex including area F5. PF neurons are functionally heterogeneous. Most of them (about 90%) respond to sensory stimuli, but about 50% of them have also motor properties, discharging when the monkey performs specific movements or actions (12). PF neurons responding to sensory stimuli have been

subdivided into “somatosensory neurons” (33%), “visual neurons” (11%), and “bimodal (somatosensory and visual) neurons” (56%). About 40% of the visually responsive neurons respond specifically to action observation and about two thirds of them have mirror properties (12, 1).

In conclusion, the cortical mirror neuron circuit is formed by two main regions: the anterior part of the inferior parietal lobule and the ventral premotor cortex. STS is strictly related to it but, lacking motor properties, cannot be considered a true part of it (1), however it is neccesery for its functioning. There are two main anatomical and functional streams that, via inferior parietal lobule, connect area F5 with the higher order visual areas of the superior temporal sulcus (STS) (5). The first stream originates from a sector of the upper bank of the STS (“STPm”), reaches parietal area PFG, and terminates in area F5c. The second stream arises in the lower bank of the STS, reaches parietal area AIP, and then area F5a (5, 13).

2.1.1.2 Functional properties of macaque mirror neurons

Before we describe which functional roles might the mirror neurons play, it is important to define the basic terms of motor organization: movement, motor act, and action. Movement indicates a mere displacement of a body part. It does not include the idea of goal. Motor act defines a series of movements performed to reach a goal (e.g., grasping an object). Finally, motor action is a series of motor acts (e.g., reaching, grasping, bringing to the mouth) that allows individual to fulfill its intention (5).

The most widely accepted hypothesis on the functional role of the parieto-frontal mirror circuit is that it plays a role in understanding the goal of motor acts, which is what an individual is doing in a given moment (e.g., grasping, holding, tearing) (7). In other words we anticipate that these neurons are part of a system that recognizes actions performed by others. This recognition is achieved by matching the observed neuronal action motorically coding the same action. By the means of such a neural matching system, the observer during action observation in the same “internal” situation is placed as when actively executing the same action (14).

After selecting mirror neurons that responded exclusively during the observation of the

late phase of grasping and/or during object holding, these neurons were tested in two conditions (14). In one, the monkey saw the hand of the experimenter grasping and holding an object ("full vision" condition); in the other, the monkey saw only the experimenter's hand moving toward a screen but not the final critical part of the motor act, e.g., the hand object interaction ("hidden" condition) (14). The results showed that more than a half of the F5 mirror neurons discharged in the hidden condition. This shows that when the monkey has sufficient clues to create a mental representation of the observed motor act, mirror neurons describe it even if the crucial part of the motor act is not visible (14).

This fact is demonstrated in Figure 2.3, where the lower part of each panel illustrates the experimenter's hand performing an action of grasping an object (panel a and b) and pretending doing the same action (panels c and d) as presented to examined monkey. The behavioral paradigm was based on two basic conditions: full vision condition (a) and the hidden condition (b), and two control conditions: mimicking of grasping non-existing object in full vision (c) and the same mimicking in the hidden condition (d). In panels (b) and (d), the grey square represents an opaque sliding screen that prevented the monkey from seeing the experimenter's action performed behind it. The vertical line in rasters and histograms, shows the point in which the experimenter's hand began to disappear from the monkey's vision. The upper part of each panel shows rasters and histograms of 10 consecutive trials recorded during the corresponding experimenter's hand movement. Kinematic recordings (gray traces) of the experimenter's hand are shown above each raster. The illustrated neuron responded to the observation of grasping and holding in full vision (a) and in the hidden condition (b), in which the interaction between the experimenter's hand and the object occurred behind the opaque panel. The neuronal response was virtually absent in the two conditions in which the observed action was mimicked and there was no graspable object presented in the scene (c and d) (7).

Actions may be recognized also when presented acoustically, from their typical sound. It was found that many mirror neurons that responded to visual observation of motor acts accompanied by sounds also responded to the sound alone. These neurons were named "audio-visual" mirror neurons (15). This means that motor action done by other individual can be recognised via audio-visual mirror neurons just by hearing the sound of it.

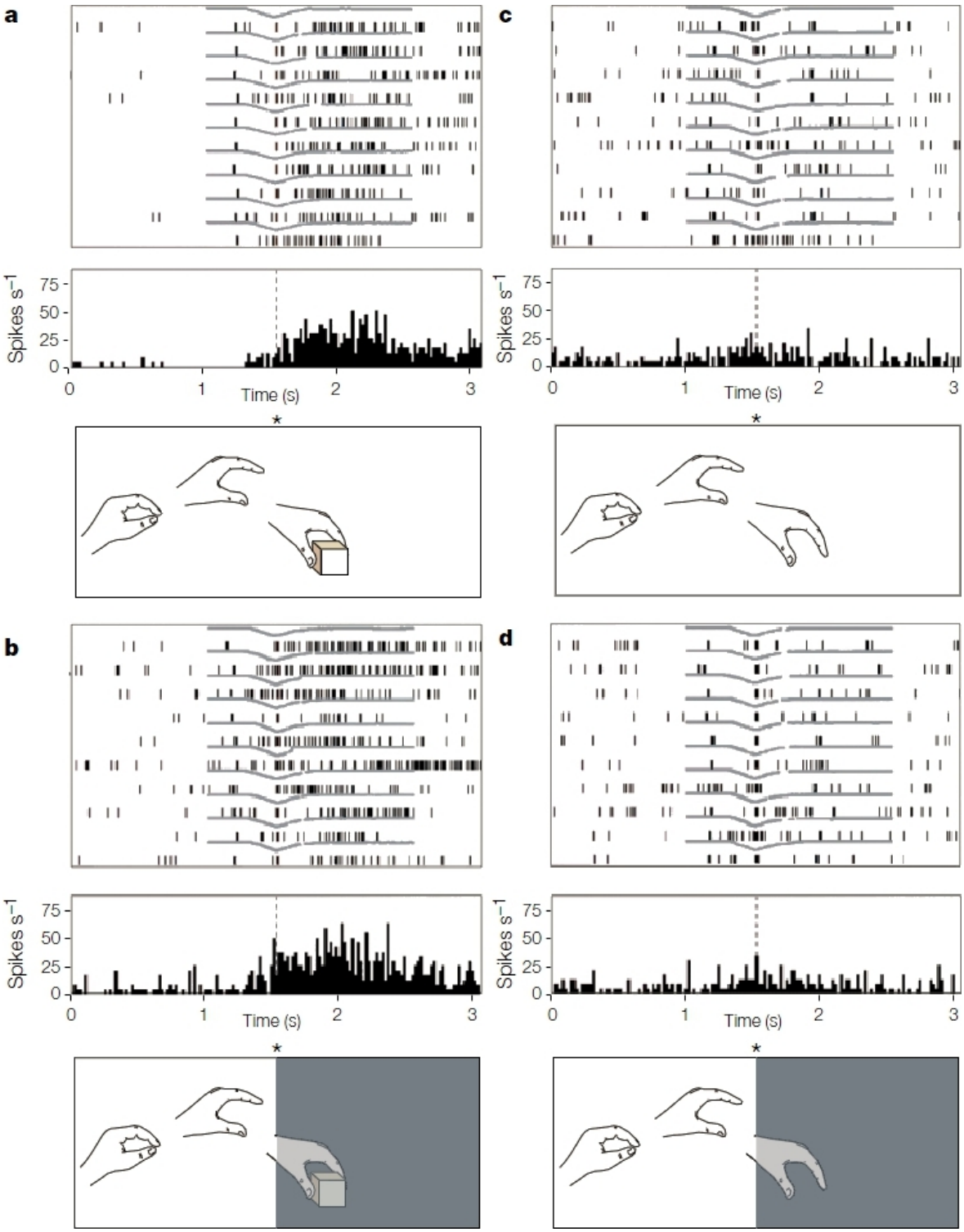


Figure 2.3: ***Activity of a mirror neuron in F5 in response to action observation in full vision and inhidden conditions.*** *Adapted from: (7)*

2.1.2 Mirror neurons in humans

In the human brain, there is not direct evidence for mirror neurons, because there is no single-neuron recording study performed in human cortex areas believed to be associated with mirror neuron system activity. However we have indirect proves that show the existence of mirror neurons in humans.

Neurophysiological and brain imagining experiments using hemodynamic methods such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) revealed activation in the possible homologuous human brain Brodman area (BA) 44 and the adjacent ventral BA 6 to monkey area F5 during action observation (1).

Furthermore, electrophysiological methods like magnetoencephalography (MEG) and electroencephlaography (EEG) have shown activation of motor cortex during observation of finger movements (16, 17). The EEG method and its specific reflection of mirror neuron system activity is in focus of our study. Therefore we will describe this method in detail in following chapters of this thesis.

Transcranial magnetic stimulation (TMS) provided more direct evidence that the human motor system has mirror properties. TMS is a noninvasive technique used for stimulation of the cortex. TMS creates strong localized transient magnetic field that induces current flow in underlying neural tissue, causing a temporary disruption of activity in small regions of the brain. When TMS is applied to the motor cortex, at appropriate stimulation intensity, as a response motor-evoked potentials (MEPs) can be recorded from contralateral muscles. The amplitude of these potentials is modulated by the behavioral context. The modulation of MEPs' amplitude can be used to assess the central effects of various experimental conditions (1). TMS revealed that observation of goal-directed and also meaningless hand actions determined an increase of the recorded MEPs. The increase concerned selectively those muscles that the participants use for producing the observed movements (18).

2.1.2.1 Cortical structures involved in human mirror neuron system

Action observation done by other individuals activate in humans a complex network formed by occipital, temporal, and parietal visual areas, and cortical regions with predominantly motor functionality. These motor-asociated regions are the anterior part of the inferior parietal lobule and the inferior part of the precentral gyrus plus the posterior part of the inferior frontal gyrus (IFG) (1) and posterior part of superior temporal sulcus (STS) .These regions are considered as former structures of the core of the human mirror-neuron system (19).

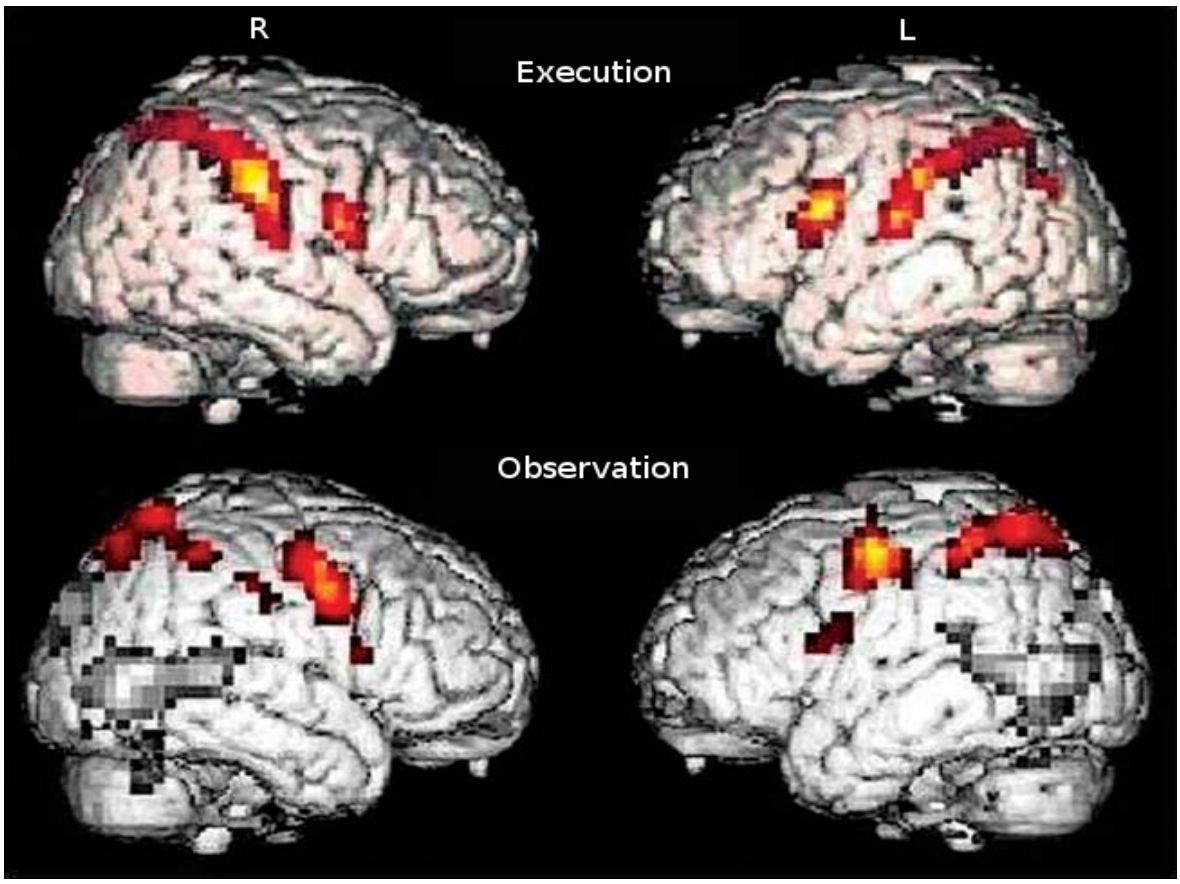


Figure 2.4: ***The core of the human mirror system****. Cortical activation pattern overlaid on lateral views of the human cortex, measured using fMRI while participants manipulated an object executing self motor actions (top) and observed another individual grasping an object (bottom). There is considerable overlap in the frontal and parietal activations in the two conditions. There is also significant activation in the visual cortex which is shown in gray to differentiate it from the parietal and frontal activity common to both conditions. Figure adapted from (6) © 2007 Elsevier Ltd. All rights reserved.*

Figure 2.4 shows an image obtained by fMRI imagining technique of the frontal cortex in which, the opercular part of the inferior frontal gyrus constitutes the ventral premotor cortex and is bilaterally activated both by the execution and the observation of hand actions such as object grasping and manipulation. The anterior part of the interparietal sulcus of the parietal cortex shows the same activations in the same time. These two brain areas are anatomically connected (not only via STS) and therefore thought to create an integrated fronto-parietal mirror neuron system in human brain (6).

2.1.2.2 Human mirror neuron system properties

Similarly as in macaque monkeys it is believed that mirror neuron system plays role in action recognition of other individuals also in humans. Moreover human mirror neuron system is involved also in imitation and empathy (20, 4).

2.1.2.2.1 Action understanding

By action understanding, we mean the capacity to achieve the internal description of an action and to use it to organize appropriate future behaviour (7). There are two essential hypotheses that might explain how the understanding actions of others uprises.

The first one, which is refered to as the “visual hypothesis”, claims that action understanding is based on a visual analysis of the different elements in the observed scene that form the action. This hypotheses states that for action recognition no motor involvement is required. For example, when we observe a hand grasping a graspable object, the analysed elements would be the hand, the object and the movement of the hand towards the object. According to this theory the associations between the elements, and inferences about their interaction, are sufficient to allow the individual to understand the observed action. From this point of view, the understanding of action has to essentially be mediated by the activity of the extrastriate visual areas (adjacent to primary visual cortex) in occipital cortex, the inferotemporal lobe and the superior temporal sulcus (STS). The activity of these cortical structures in monkeys and also humans, responds selectively to objects, body parts and biological motion. Neurons in the posterior part of the STS, respond to interactions between hands and objects (7). According to (21) higher-order

visual neurons in STS combine the output of neurons that are specifically responsive to the observation of limb reaching with the output of neurons that respond specifically to the direction of attention, provided by the direction of sight. These properties of the STS neurons indicate, that the visual analysis of observed action is surprisingly complex. But the existence of neurons that interconnect different types of visual features of an observed action, is not sufficient condition for action understanding just by itself. The biggest drawback of the visual hypothesis is the explanation of the "validation" process of the meaning of the observed action. It is by no means obvious how the complex properties of STSa neurons could have emerged (7).

Giacomo Rizzolatti and colleagues proposed the second, alternative theory, which is called "the direct matching hypothesis". This hypothesis claims that action understanding is the outcome of the visual representation mapping process of the observed action onto individuals own motor representation of the same action. In this theory, an action is understood when its observation causes "resonation" in the motor system of the observer. So, when we observe a hand grasping an graspable object, the same population of neurons that control the execution of grasping movements becomes active in the observer's motor areas just by observing the particular motor action. By this approach, the "motor knowledge" of the observer is used to understand the observed action. In other words, we understand an action because the motor representation of that action is activated in our brain. The idea that we understand others through an "internal act", that recaptures the sense of their acting, was defended by philosophers mainly from phenomenology stream (for example M. Heidegger). Of course, the hypothesis that action understanding is based on a direct-matching mechanism does not exclude the possibility that also other, cognitive processes based on object and movement descriptions, could participate in this function. It stresses out, however, the primacy of a direct matching between the observation and execution of action (7). This hypothesis has found a strong neurophysiological support in the discovery of the mirror neuron system properties.

2.1.2.2.2 Imitation

The concept of "imitation" can be explained by variety of different definitions. In everyday life, it simply means "to do after the manner of" or "to copy". It is clear that this

broad definition includes a large variety of phenomena.

Imitation has very broad application during development as a form of learning, providing the acquisition of many skills within a time-saving process contrary to the trial-and-error learning. Imitation plays also crucial role in the development of fundamental social skills such as reading facial and other body gestures and for understanding the goals, intentions and desires of other people (22).

In major cases imitation concerns motor behaviours that are determined by the observation of similar motor behaviours made by an individual of the same species. Imitation might be accompanied by an understanding of the action meaning. It can be an approximate or a precise copy of the observed action, and it might concern a series of motor acts that were never performed by the observer before(7).

Imitation is not a unitary phenomenon, and because of that, it is possible that different imitative behaviors, covered under this name, are involving different mechanisms. However, the elementar ability to copy basic actions should be based on simple neural mechanisms. The neurophysiological core of this type of imitating might involve a "resonance" mechanism that directly maps a pictorial or kinematic description of the observed action onto an internal motor representation of the same action (7). Therefore, a comparable direct matching mechanism between the observed and executed action is a reasonable candidate for human imitation. The direct matching hypothesis predicts that the brain areas where the matching uprises must contain neurons that discharge during action execution regardless of how action is initiated and that at least a subset of them should receive input representing the action they actually encode. Cortical areas endowed with a matching mechanism should, therefore, have motor properties, and, more importantly, they should become more active when the action to be executed is evoked by the observation of that action (20).

The fMRI study done by Iacoboni and colleagues revealed that cortical regions involved in mirror-neuron mechanism sufficiently support the above mentioned direct matching hypothesis assumptions. Their paradigm involved observation conditions and observation-execution conditions. In the observation-execution conditions, imitative and nonimitative behavior of simple finger movements was compared. In an imitative condition task was to execute the observed finger movement. In the nonimitative

conditions, participants' task was to execute the same movement triggered by spatial or symbolic cues. Within the imitation task they recorded larger signal intensity in contrast to the observation-execution tasks. This effect was found in three areas: the left frontal operculum, the right anterior parietal region, and the right parietal operculum. In the first two areas, activation was also present during observation tasks. These findings indicate, that the left frontal operculum (BA 44) and the right anterior parietal cortex (PE/PC) are endowed with an imitation mechanism as postulated by the direct matching hypothesis (20).

According to fMRI study by Lisa Koski and colleagues (2002) inferior frontal mirror neuron areas show greater activations when participants imitate the motor action as it was their mirror reflection, compared to imitating in an anatomically correct way, for example when actor raises the left index finger, the imitator also raises the left index finger. This suggests that the relative facility with which humans imitate in the specular configuration may be a function of greater dependence on a simple mechanism for matching observed and executed actions (23). Imaging studies have shown that action goals during imitation are also coded by inferior frontal mirror neurons (20, 24). Inferior parietal mirror neuron area in humans seems to be more involved in processing of the motor aspects of the action to be imitated (24).

In conclusion, the imaging data in humans indicate that the human MNS forms, with higher-order visual areas situated in area of the STS, a core circuit for imitation (25). The schema of the MNS circuit for imitation is displayed in the Figure 2.5. In this circuit, the STS serves as a higher-order visual descriptor of the observed action to be imitated, the parietal component of the MNS processes the motor aspects of the imitated action and the frontal component of the MNS is concerned with the goal of the imitated action (22).

It is important to stress that this neuronal core circuit for imitation is not sufficient for covering all forms of imitative behaviour. However, there are extant data suggesting that large-scale interactions between the core circuit for imitation and other neural networks are necessary for the implementation of two main forms of imitative behaviour — imitative learning and social mirroring (22).

In developmental psychology, the work by Meltzoff and Moore in the 1970s about imitation of facial and manual gestures by human neonates showed that newborns posses

very basic forms of imitative behaviour in their early development. The study suggested that imitation is a key form of learning in early life of humans. Another important theme emerging from the developmental psychology findings is the functional interconnection between imitation and social cognition, from which we can infer the ability to develop a theory of mind (22).

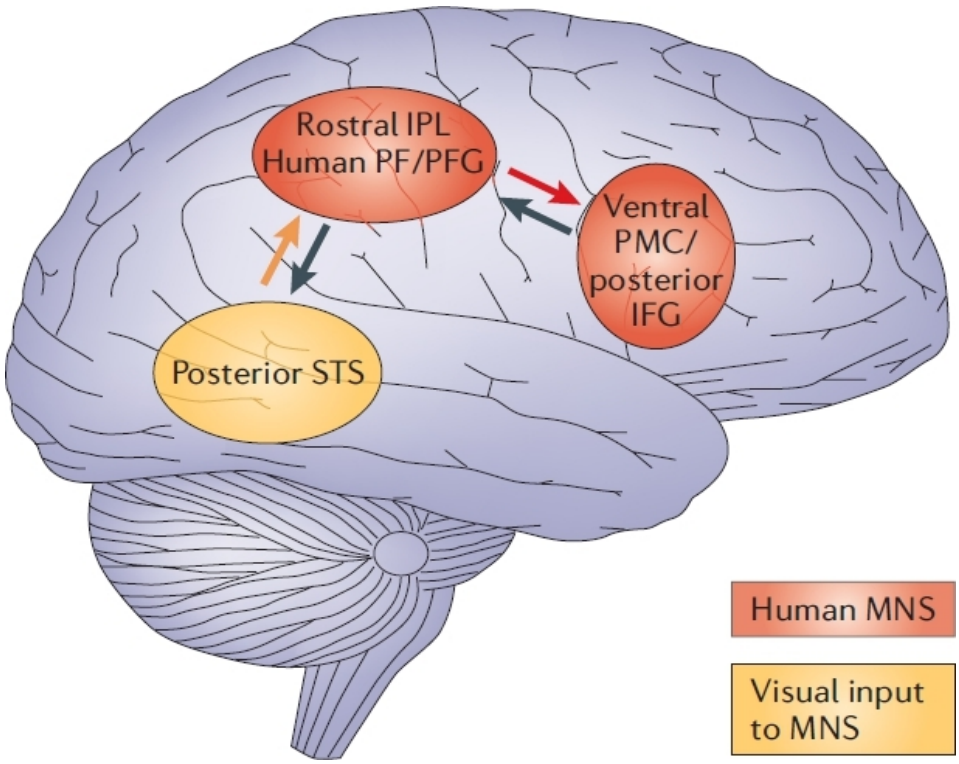


Figure 2.5: ***Basic neural circuit for human imitation.*** *The Figure shows schematic overview of the fronto-parietal mirror neuron system (MNS) (red) and its main visual input area involving superior temporal sulcus (STS) (yellow) on the lateral wall of the right cerebral hemisphere of the human brain. An anterior area with mirror neuron properties is located in the inferior frontal cortex, encompassing the posterior inferior frontal gyrus (IFG) and adjacent ventral premotor cortex (PMC) (20). A posterior area endowed with mirror neuron properties is located in the anterior part of the inferior parietal lobule (IPL). This area of the human brain is considerably homologous to the area PF/PFG in the macaque. The main visual input to the MNS originates from the posterior sector of the STS. These three areas form a "core circuit" for imitation (25). The visual input from the STS to the MNS is represented by an orange arrow. The red arrow represents the information flow from the parietal MNS, which is mostly processing the motoric description of the action, to the frontal MNS, which is more concerned with the goal of the action. The black arrows represent efference copies of motor imitative commands that are sent back to the STS to allow matching between the sensory predictions of imitative motor plans and the visual description of the observed action (22). Anatomical image adapted from (22).*

3 Electroencephalography

Electroencephalography (EEG) is non-invasive electorphysiological imagining method used for recording brain's electric activity over the scalp. Richard Caton (1842–1926), a professor of physiology, was the first who documented the existence of electrical potentials emanating from the brains of rabbits and monkeys in the British Medical Journal in 1875. The pioneer researcher in field of human EEG recordings and analysis of measured signal was German psychiatrist and physiologist Hans Berger (1873–1941). In 1924 he recorded the first non-invasive scalp EEG recordings from human participants. Berger wrote numerous landmark publications for the new field of research, in which he for example: established term “electroencephalogram” or investigated and described the 10 Hz activity characterising the awake relaxed EEG oscillation of adult humans which he named “alpha waves”(26).

3.1 Physiological source of the EEG signal

The electroencephalogram is the visualised form of the spontaneous brain electrical activity generated by cerebral neurons. From the physiological point of view, neurons, like other body cells, have high concentrations of potassium (K+) and chloride (Cl−) ions inside the cell bodies, and high concentrations of sodium (Na+) and calcium (Ca2+) ions are kept behind the cell membrane. This configuration of positively and negatively charged particles leads to a voltage difference of about −60 to −70 mV with respect to the outer side of the cell membrane (27). This voltage difference is not fixed, but fluctuating with changing concentrations of cations and anions in the inner- and outer-space of the cell body. The fluctuation depends on the opening and closing of ion channels, that represent “gates” for the charged particles, which are induced by electrical or chemical stimuli. A decrease of charge separation across the membrane, due to an influx of positive charged ions into the cell, results in a less negative membrane potential. This effect is called depolarisation. The opposite effect, which is an increase in charge separation creating a more negative membrane potential is called hyperpolarisation (27).

When a sufficient amount of Na+ ions diffuses into the cell and the membrane potential reaches a threshold level, the opening of additional Na+ channels is facilitated leading to a sudden marked increase of depolarisation. This fast depolarising event corresponds to the rising phase of the action potential, which is the point of the highest electric activation increase, while the falling phase is related to the outflux of K+ ions which, combined with a decrease of Na+ influx, induces a repolarisation of the cell. After an action potential, there is a transitory inactivation of Na+ channels that causes a refractory period during which another action potential cannot be generated (27). The time course of the described membrane potential changes is visualised in Figure 3.1.

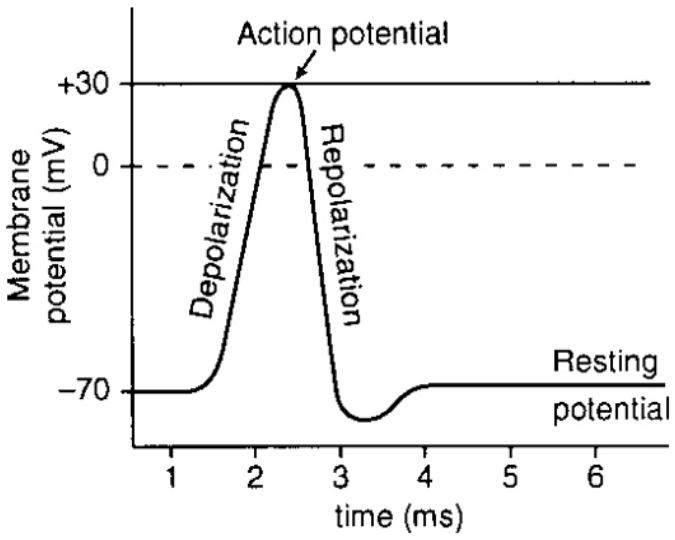


Figure 3.1: ***The time course of membrane potential changes.*** *The graph is showing the time course of depolarisation phase of the cell membrane reaching the action potential peak and repolarisation phase reaching resting potential values.*

The action potentials last too short a time to contribute to scalp-recorded EEG, however they can traverse long axonal distances, without loss of amplitude reaching the adjacent neuron in the circuit. An action potential induces an excitatory postsynaptic potential (EPSP) in the excitatory presynaptic fibre and an inhibitory postsynaptic potential (IPSP), in the postsynaptic neuron. An EPSP produces a flow of positive charges into the cell (current sink), while an IPSP acts in the opposite way by inducing a flow of positive charges out of the cell (current source) (27). Even though action potentials have higher amplitudes, EPSP and IPSP represent the most significant source of scalp-recorded EEG

signals. This fact is caused by the length of their duration. In comparison with action potentials (milliseconds) (see Figure 3.1), synaptic potentials have a longer duration period (tens of milliseconds), which increases their probability to occur with a temporal overlap, and involve a larger membrane surface. The electrical activity summation of extracellular temporal overlaps and spatial membrane surfaces of the neuron populations are the main sources of the EEG signal (27).

3.1.1 Polarity and other characteristics of the EEG signal

The surface electric potentials have different polarity, which is determined by the location of the synaptic activity within the cortex. Scalp EEG electrodes detect the extracellular electrical fields generated closer to the cortical surface. An EPSP in a dendrite produces electrical negativity in the closest surrounding area. As the EPSP traverses along the neuronal fiber, the electrical field becomes positive with increasing distance from the source. The opposite effect occurs with an IPSP, which generates an electrically positive charge in the nearest surrounding and a negative field in the distance. In the end, a deep IPSP and a superficial EPSP are both generating a scalp negativity and vice versa (27).

Postsynaptic potentials, create a dipole with separation of charge that is orientated vertically in the cortex. Therefore, the cortical areas situated parallel to the scalp surface induce radial electric fields which are more easily detectable with the scalp electrodes. Their maximal field is directly above the source and opposite polarity field on the opposite side of the head. Source areas situated in the sulci of the cortex, which are orthogonally oriented to the scalp surface, induce tangential dipoles. These dipoles are harder detectable by the scalp EEG electrodes, because both positive and negative voltage maxima are displaced to either side. Moreover, there is substantial impedance to electrical conduction from skin, skull, dura and brain tissue between the source of electrical potentials and the detecting electrode on the scalp, which are decreasing detection of weak electrical signals. For these reasons spatial and temporal summation of cortical activity is necessary to produce a voltage field recordable from the scalp. The amount of tissue needed to produce an EEG spike has been calculated to be rectangle 2 cm $\times$ 3 cm (27).

In the end, EEG waves properties like size, shape and duration are directly influenced

by the distance of the recording electrode from the current generator, the anatomical orientation of the layer of cells generating the signal, and the duration and number of synchronously activated postsynaptic potentials.

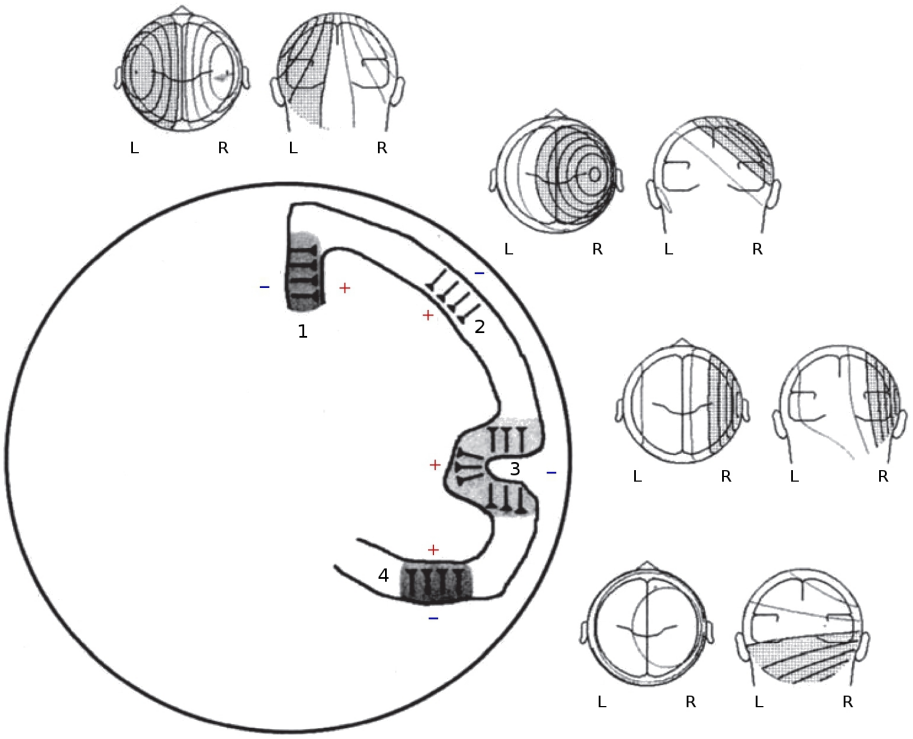


Figure 3.2: ***Schematic illustration of four representative cortical EEG signal generators.*** *Sources 2 and 3 produce radial fields which have a maximum field directly above the source and another one with opposite polarity on the opposite side of the head; sources 1 and 4 produce tangential fields which are not well seen by scalp EEG since both positive and negative voltage maxima are displaced to either side. Adapted Figure from (26).*

3.2 Technique and technical details

Electroencephalography stands for detection and recording of brain electric activity generated by the cortex. The detection is obtained by electrodes attached to a specific EEG cap, that are applied to the scalp using specialised conductive gel. Each electrode is connected to a highly sensitive technical device – the EEG amplifier. The amplitudes of brain activity and rhythms are very weak electric signals floating within the range of microvolts. Therefore amplification and filtering are needed to detect and distinguish brain

activity from other biological signals or technical artefacts. EEG machines and software are needed for amplification, recording, filtering, presentation and storage of brain electric activity.

The brain signals detection takes place on the scalp surface via electrodes mounted at specific locations covering the skull. There are several international standardised Electrode Placement Systems, that differ in amount of electrodes and density of skull surface coverage. The most widely used are international 10-20 electrode placement system, 10-10 electrode placement system, and 10-5 electrode placement system. The detection of the very low amplitude EEG signal requires a stable electric conductance between scalp surface and amplifiers, provided by electrodes and electrically shielded wires. The conductivity between skin and electrodes can be enhanced using electrode paste, conductive gel, alcohol or other fluids (28).

3.2.1 Requirements

The basic requirement for performing electroencephalography is to have an device, which is called electroencephalograph, capable of detecting and recording weak electric currents propagating through the scalp. Typical electroencephalographs consist of a electronic board containing a set of separate electrode channels, which are connecting the electrodes with the electroencephalograph. The number of channels is essential for the spatial reliability of the EEG signals. It can vary from eight electrode channels to 128 and more channels.

External disturbance of for instance power supplies (50 or 60 Hz oscillations) or other electromagnetic factors are almost always present while recording EEG signal. This external noise is influencing electrodes and signal recorded by the amplifier. Therefore, a differential amplifying technique is required so that only differences of amplitudes between two input signals per channel will be amplified, and any unwanted signal, similar at both inputs, will be rejected or suppressed (Figure 3.3) (28). This means that similar input signals are decreased by a certain ratio, depending on the quality of the amplifier.

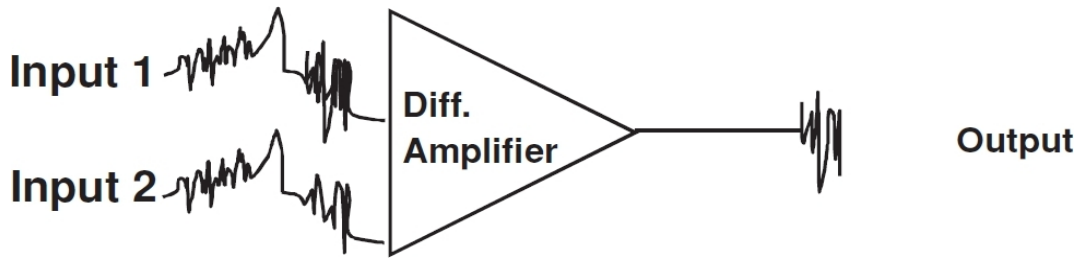


Figure 3.3: ***Differential amplification.*** *Figure adapted from (28).*

When using differential amplification method, the connection and combination of the electrodes are crucial factors for acquiring the output signal. The output can be determined by two different kinds of montages, the referential and the bipolar montage. For EEG recording and analysis, both types of montage should be used, because they are complementary and have different advantages and disadvantages (28).

Under the referential montage setting there is chosen one electrode, which is used as common reference (indifferent or reference electrode) for all other electrodes (different or active). The reference electrode is always connected to one of the inputs for each amplification channel. In order to amplify the clearest brain signal, the reference electrode should be placed at a position, through which is the least likely to propagate the brain electric activity. Therefore, earlobe positions and mastoid positions for single reference are often used. Other frequently used examples of referential montages are the Cz reference and the common average reference, which is a average signal obtained by all scalp electrodes (28). Reference montages are very good at detecting general alterations of signal, while excluding the reference electrode. However, these montages are less reliable in detecting and describing focal abnormalities, especially if the reference electrode is within the focus.

The bipolar amplification montage is based on determination of the differences between active scalp electrodes. Within this montage the EEG channels receive input signals from two different active, usually neighbouring, electrodes that are sequentially interconnected. The differential amplification is being processed on neighbouring electrodes that are usually arranged in ‘chains’, reaching from frontal to occipital regions, parasagittal and temporal, longitudinally or transversally (28). Because of the electrode chains are spread over the skull, this montage provides a good anatomical overview of the

distributed brain activity and is more suited for the detection of focal disturbances than bilateral or generalised changes (28).

In order to decrease unwanted low and high frequency signals (artefacts), which are certainly not produced by the brain activity of measured participant, EEG devices are additionally equipped with high- and low-pass filters that can be adjusted separately. Common settings are high-pass filters of 0.5 Hz and low-pass filters of 70 Hz to suppress or eliminate activity below 0.5 Hz or above 70 Hz (28). So called “notch” filtering is used for elimination of the 50 or 60 Hz artefacts usually produced by power supplies, poorly shaded electric lines in walls or other electric devices. The modern EEG amplifiers support digital signal processing, which allows flexible filtering (filter settings such as frequencies, characteristics, etc.), offline modification of signals, like re-referencing or artefact elimination.

Another necessary equipment for EEG measurements are electrodes, which usually consist of flat metal discs made of silver, gold or tin, connected to the amplifier through a shielded wire. Electrodes are attached to the scalp according to one of standardised systems (international 10-20, 10-10 or 10-5 system). Fluid substances like electrode conductive paste or gel, or alcohol are used to enhance contact with the skin, which provide better conductivity, and keep the impedance between skin and electrode below 5 kΩ. The brain electric activity is recorded via these discs or cups with the EEG machine.

3.2.2 Electrode placement systems

The first widespread standardised electrode placement system in electroencephalography was international 10-20 system. This system describes head surface locations via relative distances between cranial landmarks over the head surface (29). The name of this system consists of two numbers (10-20), where the second number 20 stands for percentage of distance between neighboring electrodes (Figure 3.4 A, B). The first number 10 is the percentage of distance between the closest electrode and one of four distinct primary reference points on the scalp anatomy: nasion (Nz), a dent at the upper root of the nose bridge; inion (Iz), an external occipital protuberance; left preauricular point (LPA), an anterior root of the center of the peak region of the tragus; and right preauricular point (RPA) determined as for the left (29) (Figure 3.4 A). Each electrode has

an identifying name with a letter indicating the general brain area (F – frontal, C – central, P – parietal, T – temporal, O – occipital, A – earlobes) and a figure indicating the location relative to the midline, with higher numbers for more lateral placements (28). Electrodes over the left hemisphere have odd numbering and those over the right hemisphere are numbered with even numbers.

The primary purpose of the 10-20 system was to provide a reproducible method for placing a relatively small number (typically 21) of EEG electrodes over different studies, and there was only a little need for high spatial resolution and accurate electrode placement. Since the topographic signal source localization methods have began to advance, enhanced need for denser electrode coverage of the skull arose. Therefore, an extended electrode placement systems stemmed from the international 10-20 system. Nowadays, there are broadly used the 10-10 system with channel density up to 81 electrodes and also the 10-5 system, which is the further extension of 10-10 system, with the maximum of 320 explicitly described electrode positions (29). In our study we used 10-20 and 10-10 international system (see Figure 3.5).

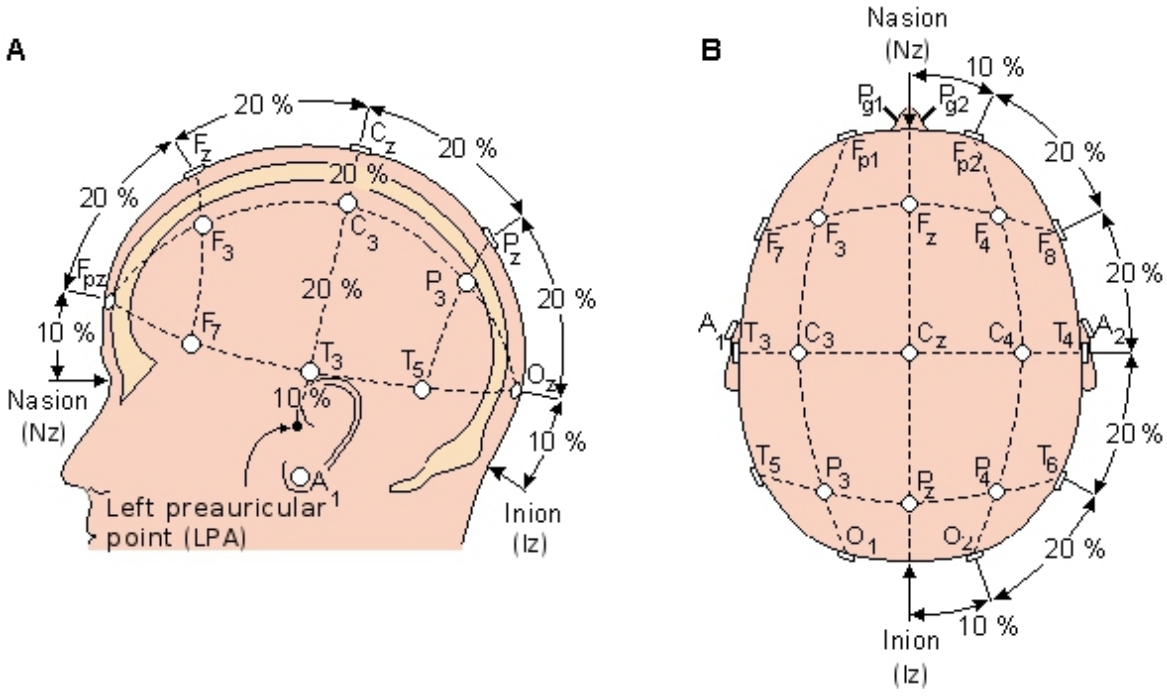


Figure 3.4: ***International 10-20 electrode placement system scheme.*** *Figure adapted from http://www.bci2000.org/wiki/index.php/User_Tutorial:EEG_Measurement_Setup.*

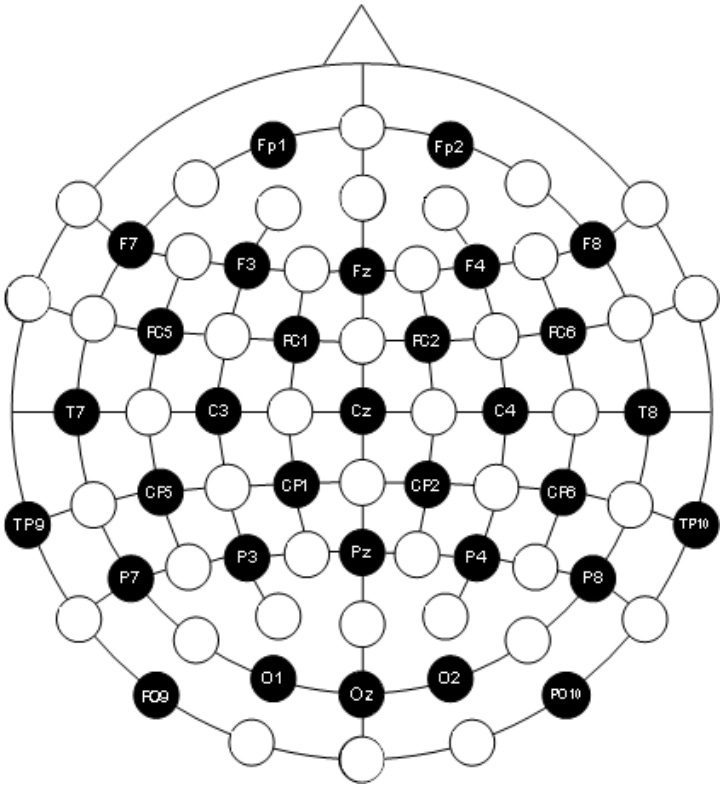


Figure 3.5: ***Inernational 10-10 electrode palcement system.*** *The same palcement as shown in this Figure was used in our experiment.*

3.2.3 EEG recording process

EEG recordings should take place in a quiet, sound attenuated and electrically shielded environment. Participants should be seated in relaxed position, without any unnecesary muscle tension, in a comfortable chair with eyes closed while being in a wakeful resting state. A typical recording should, include reactivity tests (eye opening), activation observation during at least 4 minutes of deep hyperventilation and of course the actual task conditions, which are in focus of particular measurement. Participants should be observed and monitored during the whole time of recording and every clinical change (alertness, vigilance, attention, etc.) has to be documented (29). After the recording phase, artefacts should be identified and eliminated from the recorded signal wherever possible.

3.3 EEG brain signal analysis

The analysis of various brain EEG patterns and also interpretation of artefacts, which are detected and recorded oscillations, that were not produced by brain gray matter are two crucial electrophysiological factors that need to be considered when evaluating results after measurements. Participant's individual characteristics, like health and age also contribute to output signal, hence these properties also have to be reviewed in EEG brain signal analysis.

3.3.1 Interpretation of artefacts

The most important part in the analysis of the EEG is the clear detection and discrimination of alterations and disturbances that occur in the recording, but do not arise from the brain. According to this explanation an EEG artefacts are defined as an electrical signals from various sources not related to brain electric activity. These artefacts can be divided into two categories: biological (bioelectrical signals from the participant himself/herself) and technical artefacts (electrical signals detected from the environment).

Biological artefacts include electric signals of: muscle activity (electromyogram (EMG) pulses), electrocardiogram (ECG) pulses, heart beat, eye and eyelid movements, wet skin (sweating), body movements, breathing, tongue movements.

Technical artefacts include electric signals of: defective electrodes, wires, ground, loose electrodes, electrostatic disturbances, electromagnetic interference, 50 Hz and 60 Hz power sources.

Several examples of these artefacts are visualised in Figure 3.5 – Figure 3.18. Each epoch shows 10 seconds long record of EEG signal.

3.3.1.1 EMG artefacts

Muscle activity artefacts are the most common artefacts that are present in almost each EEG signal. EMG artefacts are caused by any kind of movement, however motor activity like yawning, smiling, chewing, swallowing, increased tension in facial and neck muscles, has considerable influence on especially temporal and frontal electrodes. Activity from

muscles under the scalp (e.g.M. Frontalis, M. temporalis, M. occipitalis) or even from remote areas (face, neck, throat) can remarkably interfere with EEG signal (29).

EMG artefacts are sometimes short and focal with single deflections of spike-like appearance. Swallowing leads to a characteristic generalised “muscle activity pattern” of approximately 1 second duration.

The tongue can be characterised as an electric dipole with positivity at the base and negativity at the tip of the tongue. Rhythmic tongue movements are reflected as a slowing waves of the EEG signal. The artefact produced by the tongue has a broad potential field that drops from frontal to occipital areas.

Muscle activity can also be long lasting and widespread, diffusely affecting all channels and, especially in tense, anxious or stressed subjects, these artefacts can lead to severe disturbances affecting the entire brain activity.

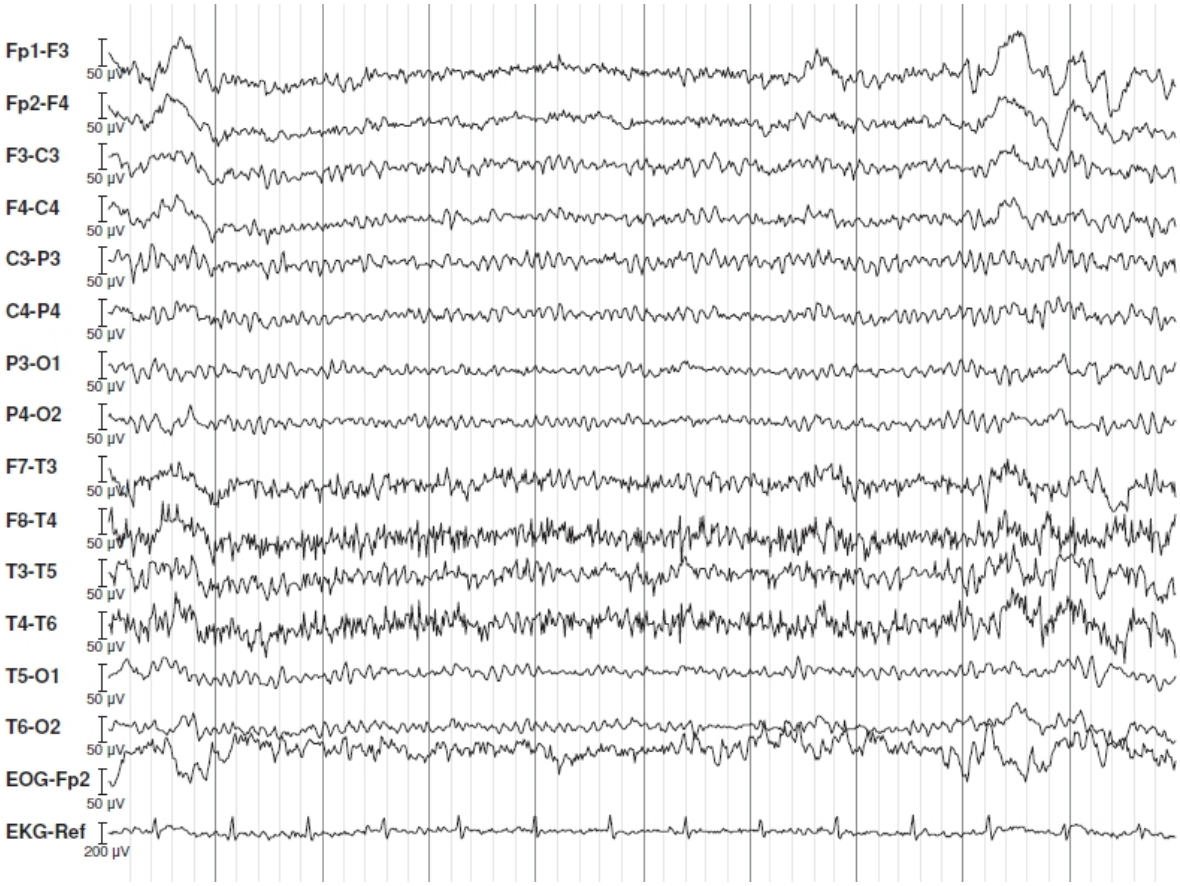


Figure 3.6: ***Muscle activity artefact in temporal electrodes.*** *(29)*

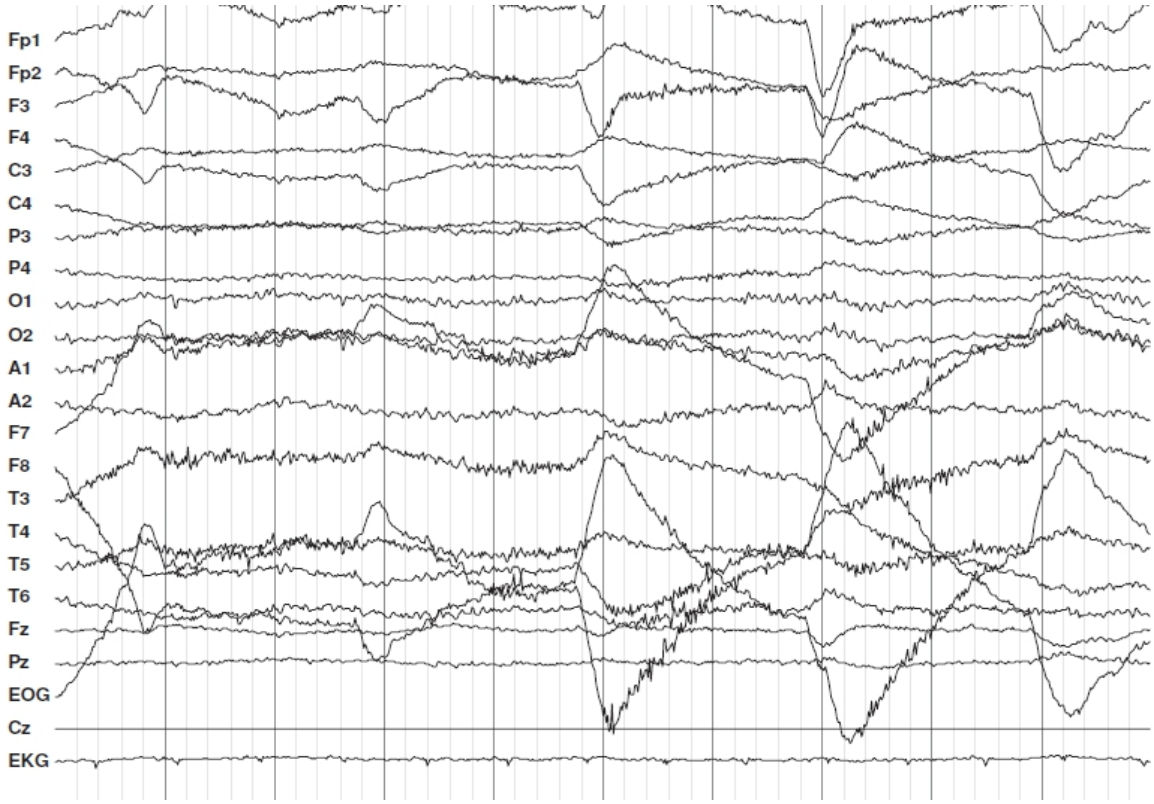


Figure 3.7: ***Muscle artefact due to head movements.*** *(29)*

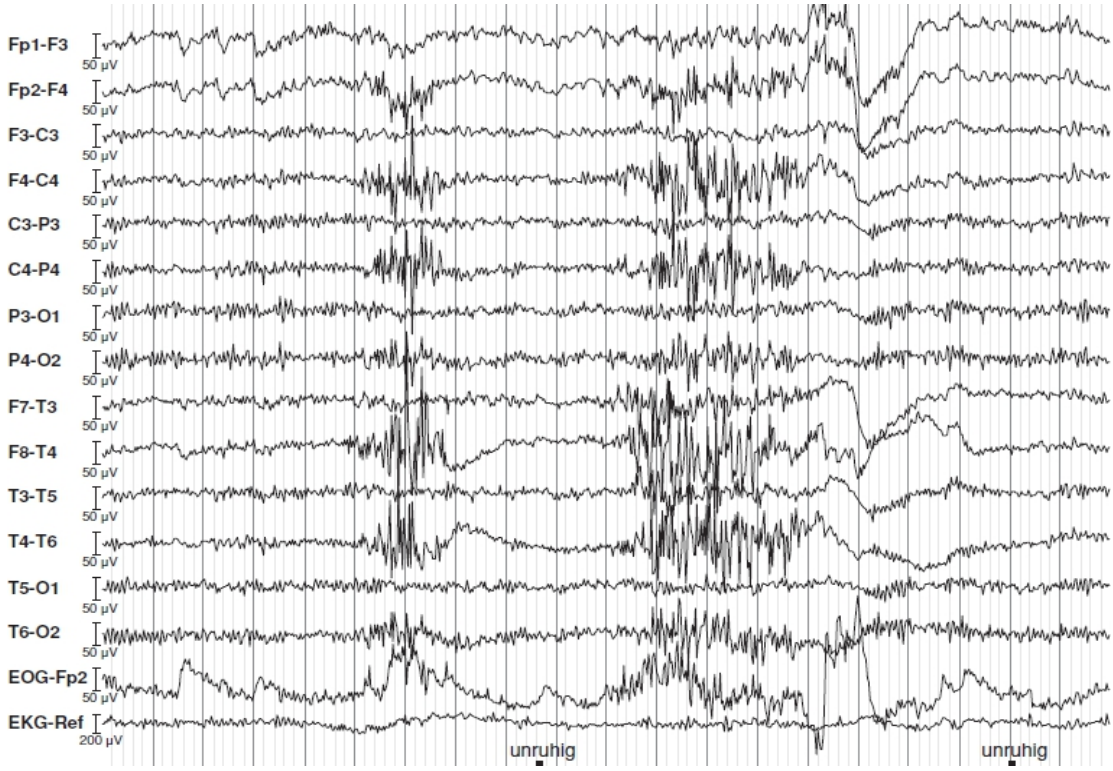


Figure 3.8: ***Muscle artefact due to swallowing.*** *(29)*

3.3.1.2 ECG and vascular artefacts

The activity of heart and blood vessels can produce a different kind of artefact. The electrocardiogram, which reflects the electric activity of the heart, can be recorded all over the surface of the body. Generally, people with short and wide necks have the largest ECG artifacts visible in their EEG. Especially earlobe electrodes, in referential montage, can be mostly influenced by the ECG projecting to the head surface. ECG artifact can be easily recognized because of its rhythmical appearance and coincidental onset with the ECG tracing signal. This artefact can easily be recognised if ECG and EEG are recorded simultaneously (29).

Another type of artefact is the vascular artefact. It appears when an electrode is placed above or near a pulsating vessel. The slight movements within each beat can produce changes in resistance leading to pulse synchronised (slow) deflections and rhythmic slowing with a temporal correlation to the electrocardiogram (29).

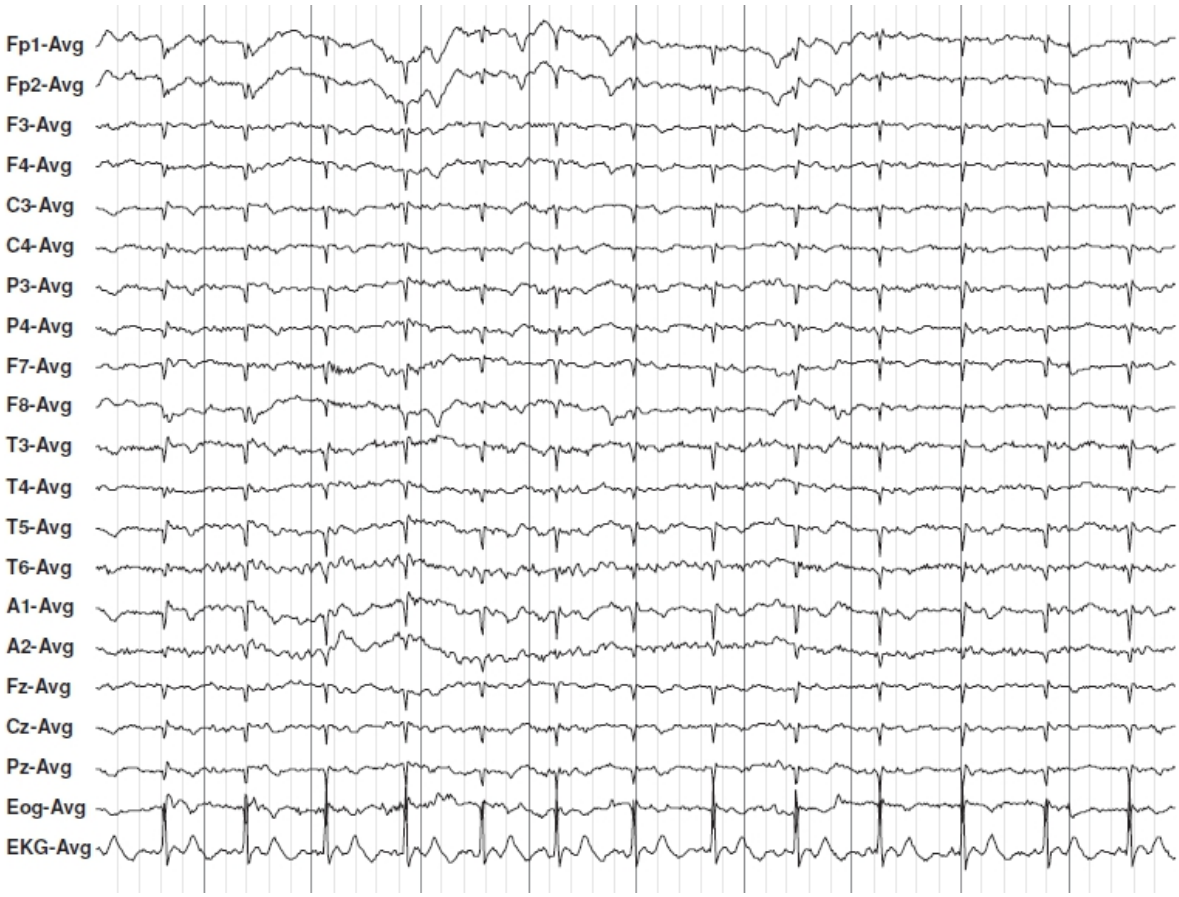


Figure 3.9: ***ECG artefact.*** *(29)*

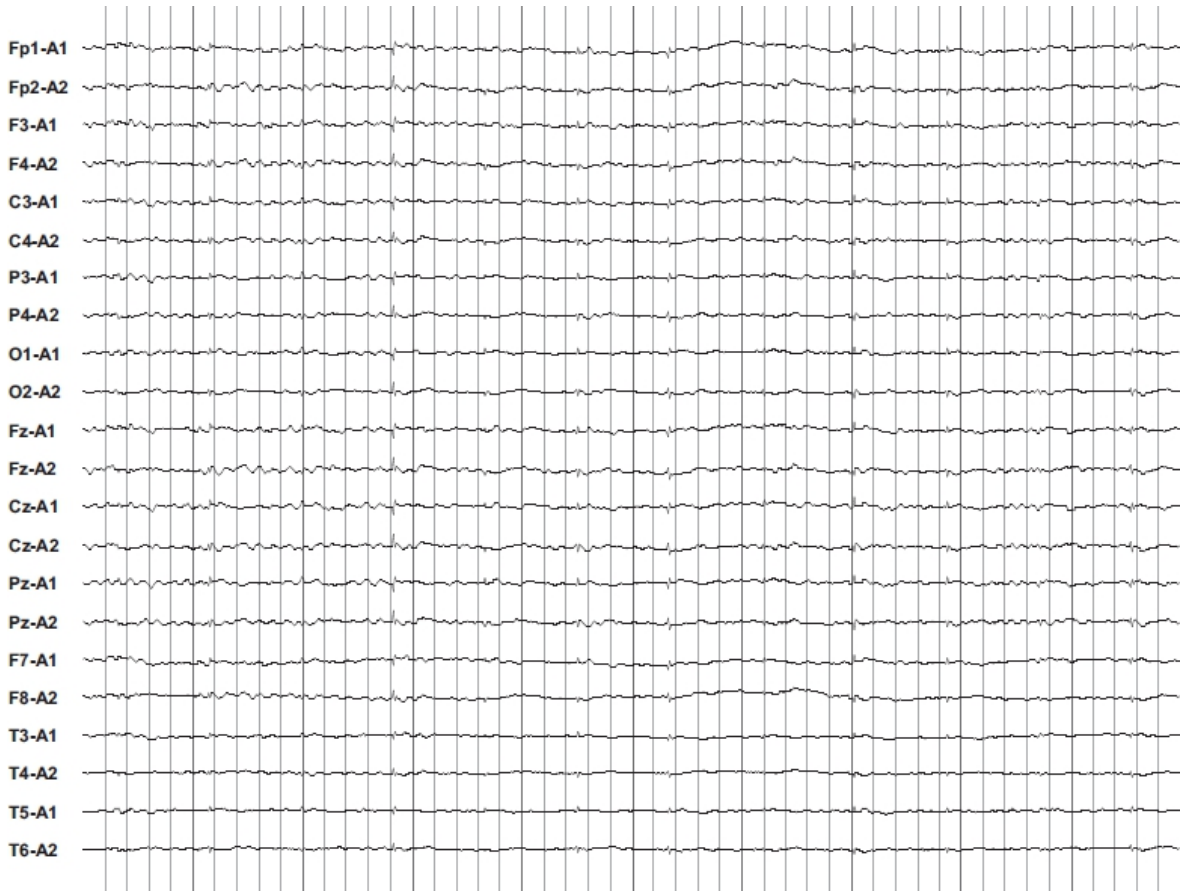


Figure 3.10: ***Rhythmical artefact due to heart pace stimulator.*** *(29)*

3.3.1.3 Eye movements artefacts

From the electric activity perspective, the eye can be described as a strong electric dipole with a positivity over corneal surface and a negative charge over retinal surface. Compared to brain induced EEG activations, eye dipole shows larger amplitudes in EEG signal. Therefore, all eye movements lead to apparent disturbances of the electric fields on the scalp surface, especially in frontal region electrodes.

According to Bell's phenomenon, every blink (when the eyelids close) leads to an upward movement of the eyes with a sudden increase of positivity (positive charge of the cornea) in frontal electrode positions, which means that Fp1 and Fp2 electrodes become more positive than the surrounding electrodes, leading to marked deflections of the EEG signals (29). When the eyes go down again, a relative negativity causes a reverse deflection. In case of vertical eye movements the disturbances of the EEG signals are in phase on both sides (Fp1 and Fp2) (29). Lateral (side to side) eye movements lead to

deflections, especially of the lateral frontal electrodes F7 and F8. Movements to the right (the positive corneal pole goes to the right side, the negative retinal pole is more on the left side) lead to a positivity around electrode F8 but to a relative negativity around F7, these deflections are separate and out of phase (29).

The eye movement artefacts are the most widely present disturbances in EEG recordings. Basically there are two main methods, correction and rejection, used for signal clearing. Rejection method is based on detection of ocular artefact in the particular EEG measurement and consequent rejection (deletion) of the artefact part in it. The second, ocular artefact correction, method is more sophisticated in the perspective, that it does not reject any data. The base of ocular correction consists in precise eye movement detection, which is obtained via electrooculogram (EOG). EOG signal is obtained simultaneously within the particular measurement, usually using additional electrodes aimed exclusively at eye movement detection. Afterwards, in the offline signal processing period, this EOG signal is used, by specialised software for EEG data handling, for the actual eye movement correction at all electrodes excluding those used for EOG recording.

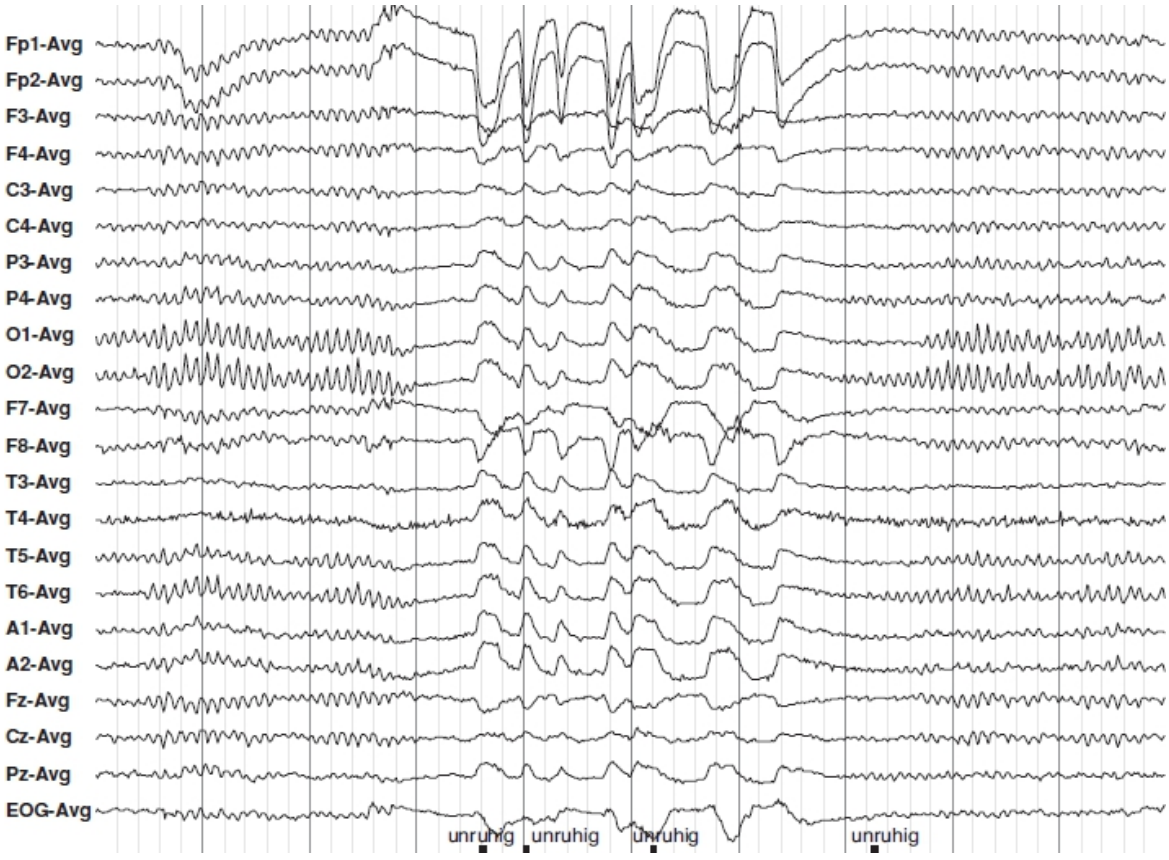


Figure 3.11: ***Eye blink artefact.*** *(29)*

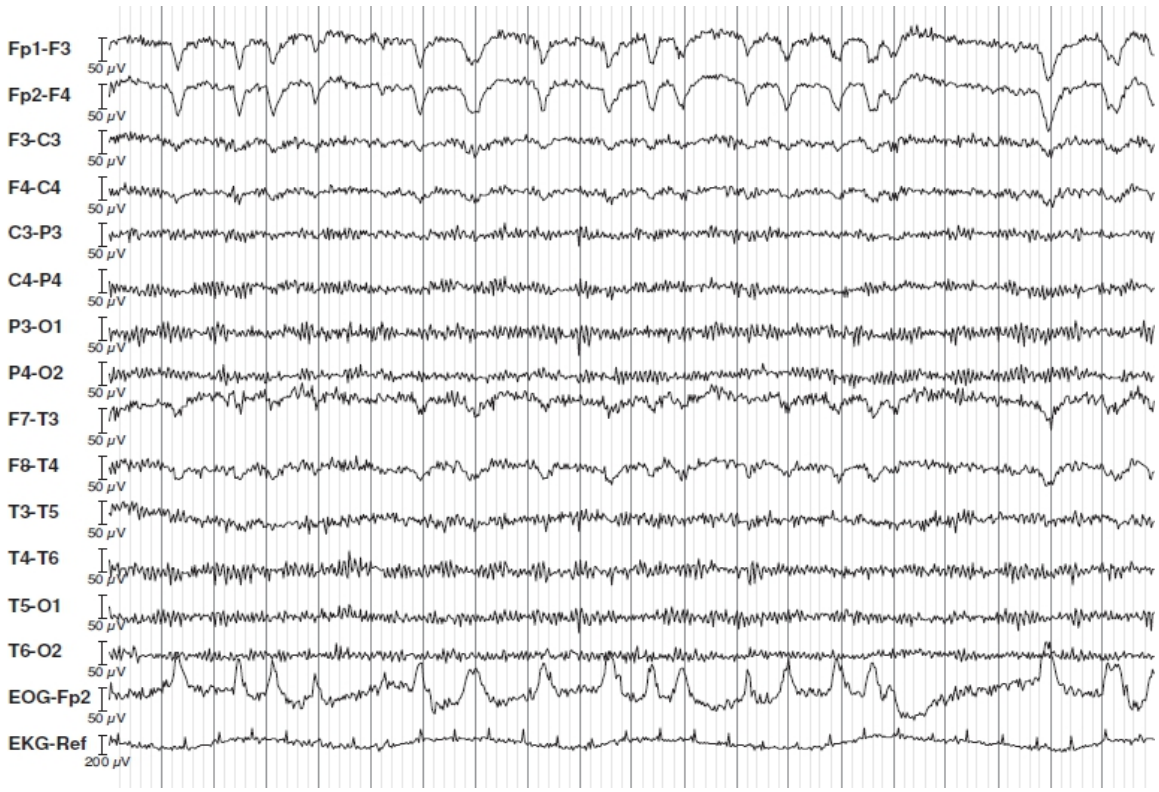


Figure 3.12: ***Eye movement artefact.*** *(29)*

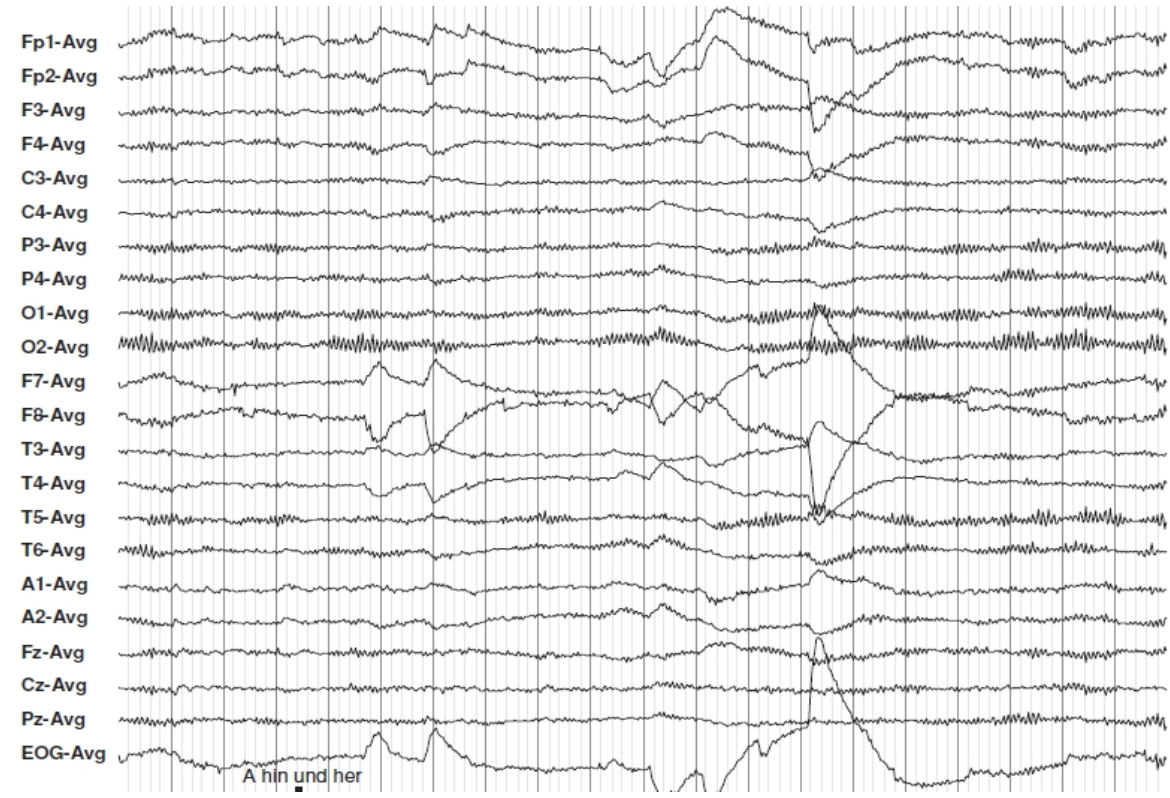


Figure 3.13: ***Lateral eye movement artefact.*** *(29)*

3.3.1.4 Sweating artefact

High skin humidity under the electrodes decreases conductance and increases resistance of the electrodes. This effect leads to an increase in slow frequencies of various amplitudes. Sometimes, sweating can lessen the skin contact with the electrodes. As a result, very slow artefacts of the involved channels (slow irregular deflections) can occur, masking the brain activity (29).

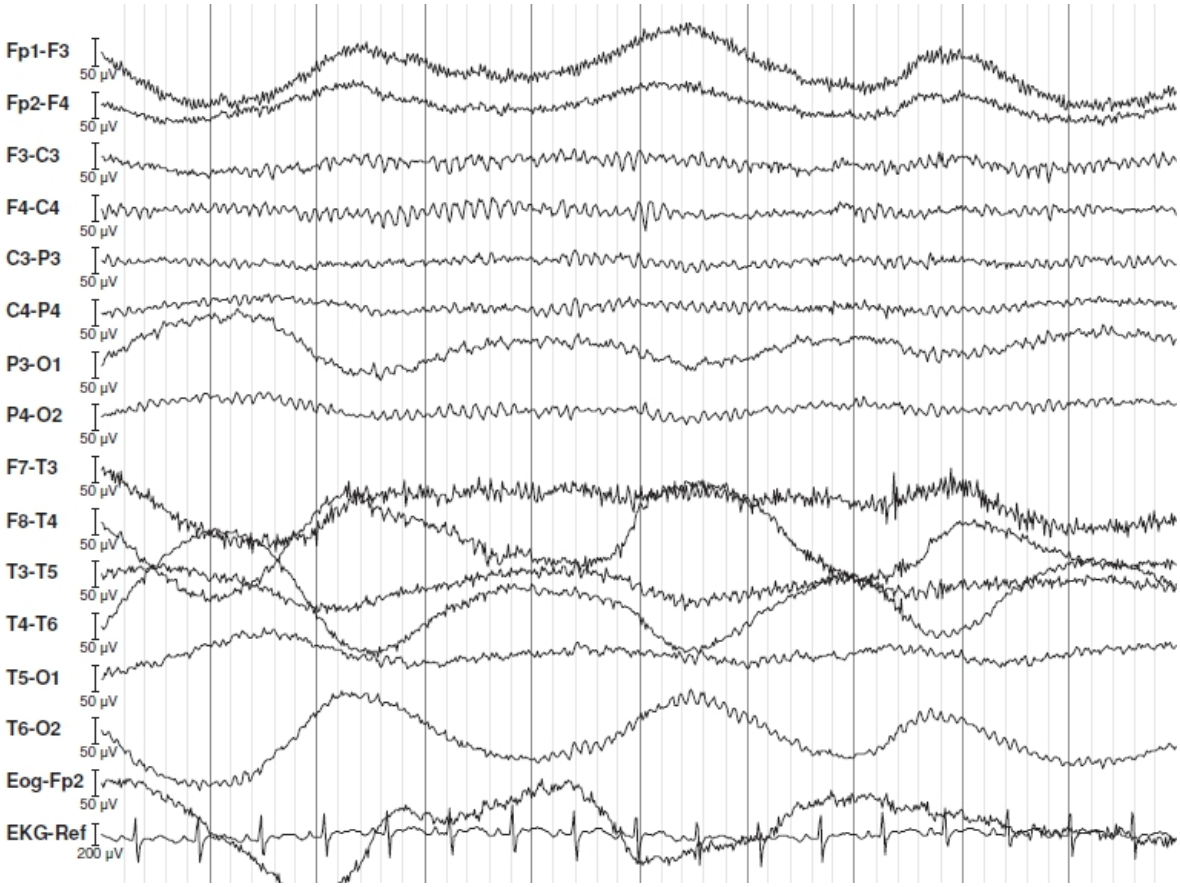


Figure 3.14: ***Artefact due to increased scalp skin humidity.*** *(29)*

3.3.1.5 Power sources artefacts

Another frequent type of artefacts are power source artefacts, which originate in the alternating current power sources. These artefacts are present when problems with the grounding of the electrical devices or the participants occur, and also when the electrodes show a high resistance. These artefacts show alternating 50 or 60 Hz deflections of uniform appearance (29). Notch filters are often used for minimising of these artefacts. However, it

is recommended to avoid them by thorough preparation before recording is started or by using special electricity shielding like the Faraday cage.

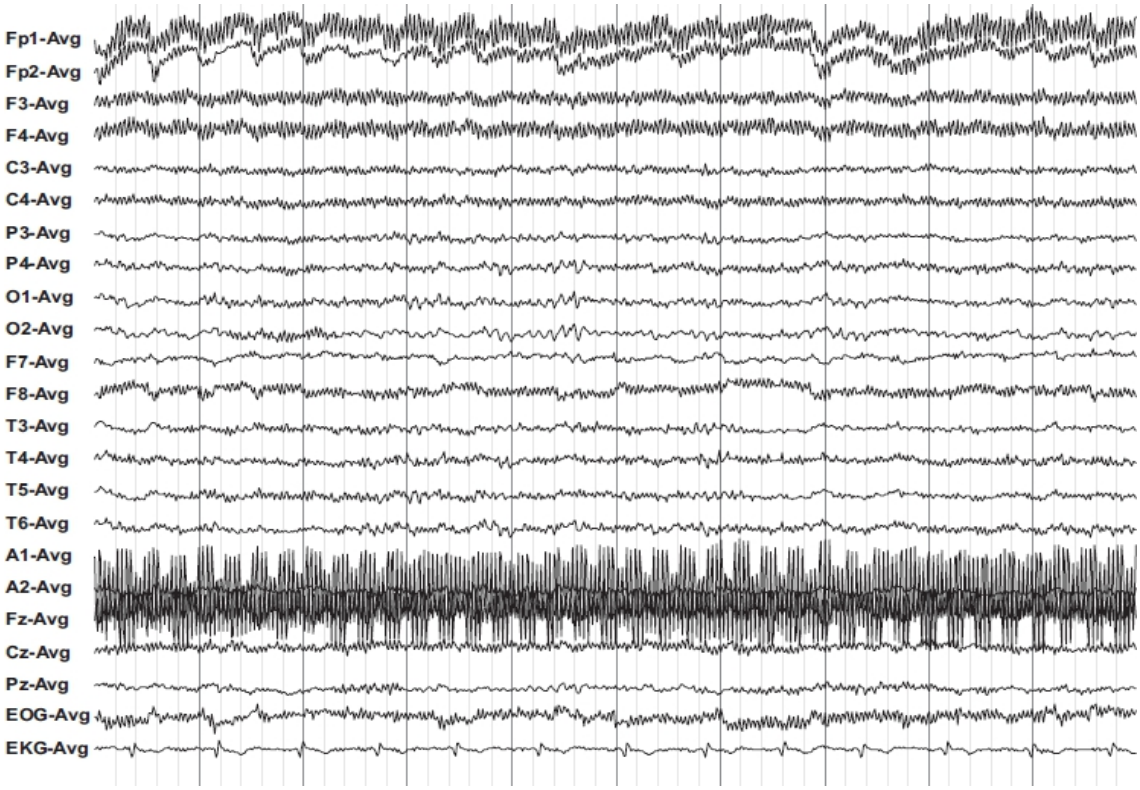


Figure 3.15: ***Power source artefacts.*** *(29)*

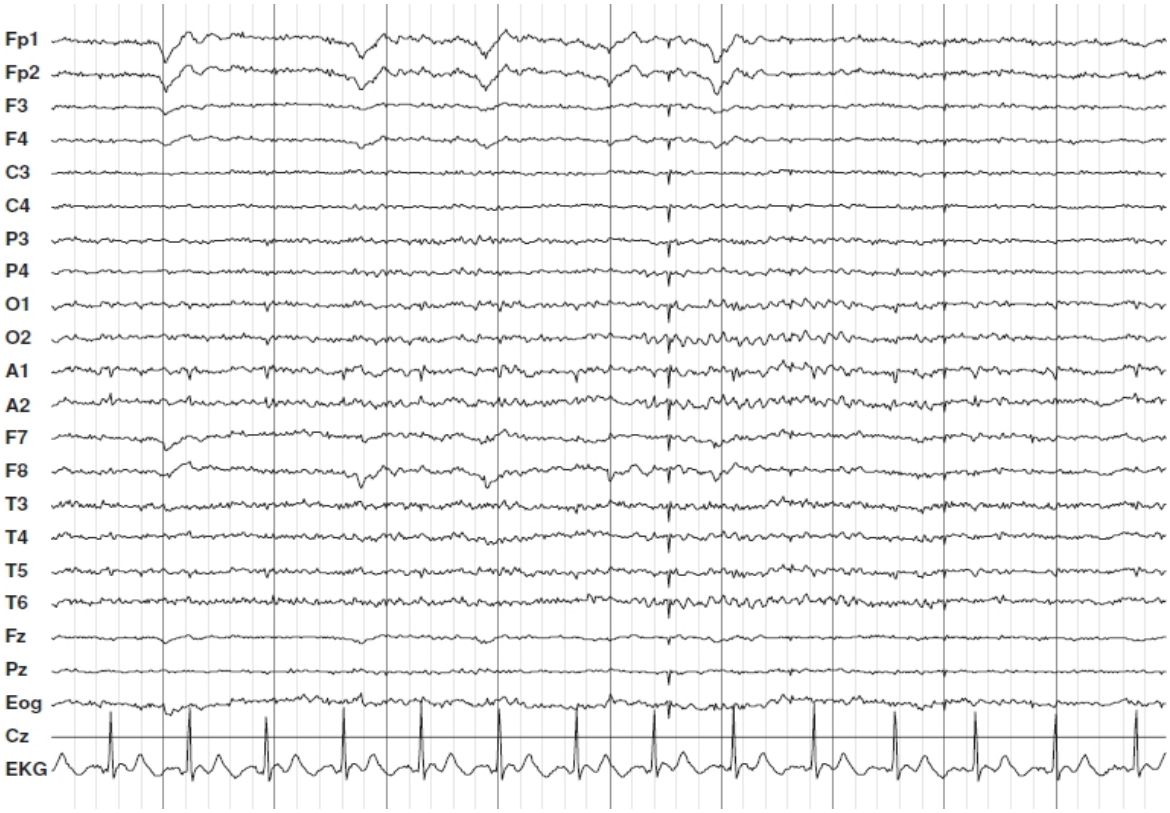


Figure 3.16: ***Light switching artefact.*** *(29)*

3.3.1.6 Electrode artefacts

The most common electrode artefact is the electrode popping. This effects appear as single or multiple sharp waveforms due to abrupt impedance change. It is identified easily by its characteristic appearance (e.g. abrupt vertical transient that does not modify the background activity) and its usual distribution, which is limited to a single electrode. In general, sharp transients that occur at a single electrode should be considered artifacts unless proven otherwise.

At other times, the impedance change is not so abrupt, and the artifact may mimic a low-voltage arrhythmic delta wave.

If electrodes are defective, sudden mostly positive deflections can occur in all channels the respective electrodes are linked with. Especially, if the defective electrode is reference, the signal in all other electrodes is affected. The defective electrodes should be immediately removed and replaced.

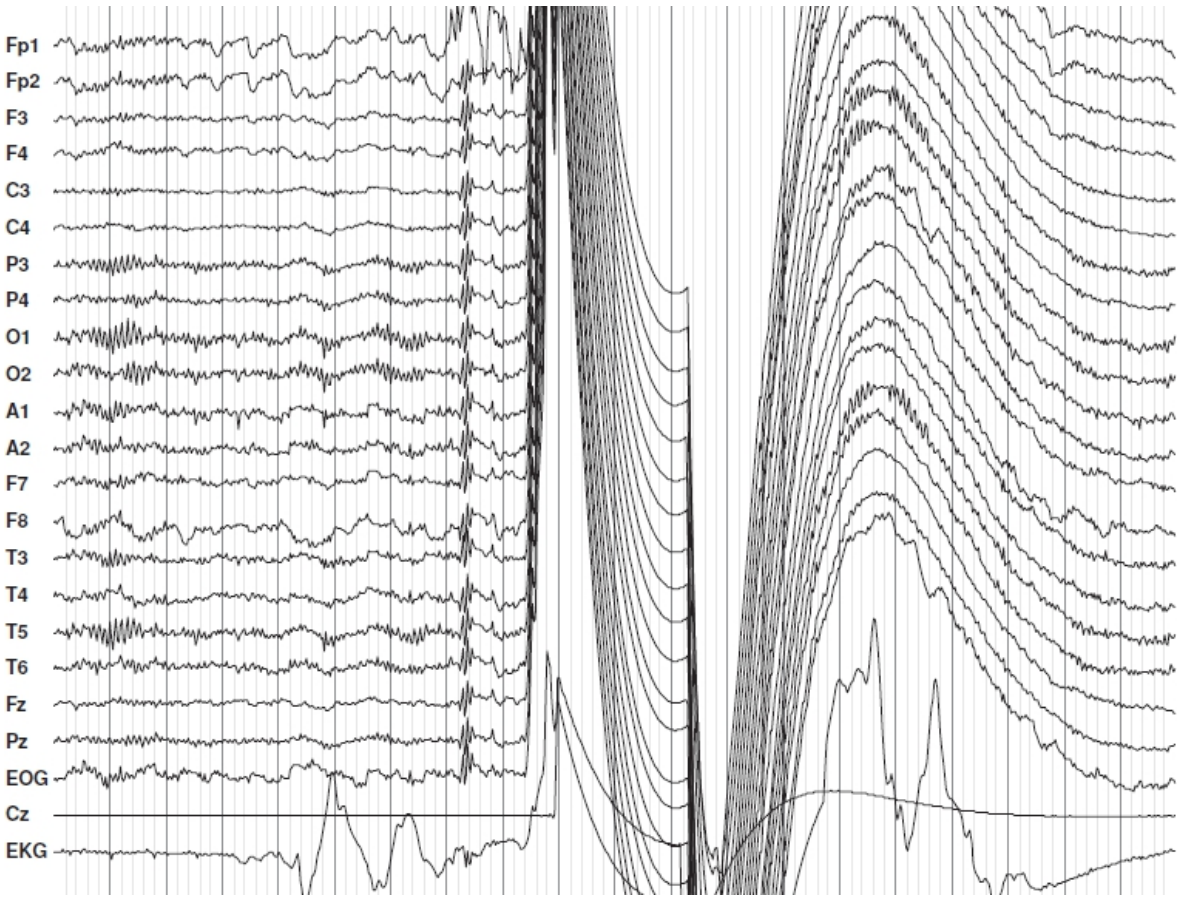


Figure 3.17: ***Defective reference (Cz) electrode artefact.*** *(29)*

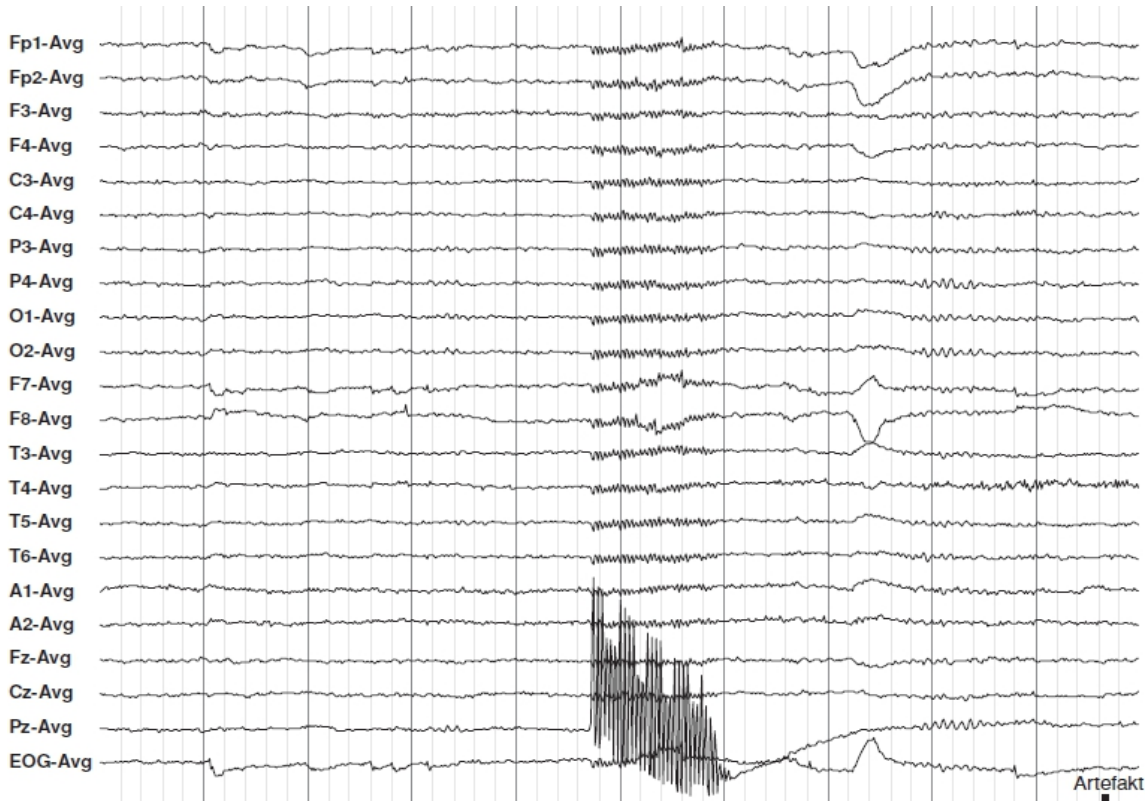


Figure 3.18: ***Single (Pz) electrode technical artefact.*** *(29)*

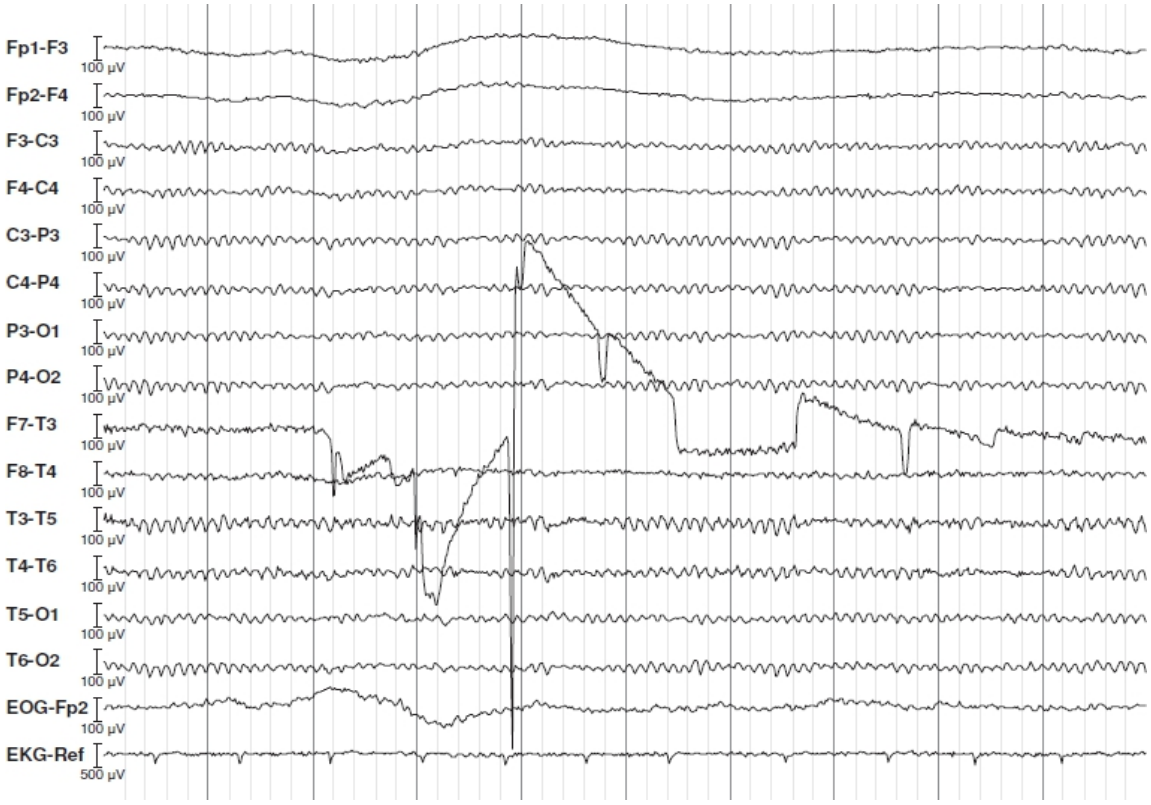


Figure 3.19: ***Single electrode (F7) artefact.*** *(29)*

3.3.2 Typical EEG oscillations

There are several types of oscillations in the EEG signal, which reflect variety of ongoing cognitive processes and apparently differ from each other. These typical oscillations are referred to as EEG rhythms. The differences of EEG rhythms are described by several classification criteria, from which frequency range (the most significant factor), amplitude, shape, topographic distribution and reactivity are taken into consideration. To give significance to the observed waveforms, these parameters must be integrated with information on factors influencing the EEG rhythms, such as age of the subject, health, state of alertness or sleep, use of psychotropic drugs (as an example, a rhythm of 7 Hz or less is considered abnormal in awake adults, while it is normal in children or in adults who are asleep) (29). Moreover, EEG activities can be affected by activation procedures such as hyperventilation and intermittent photic stimulation. Table 1 shows brief outlook at the typical characteristics of the common EEG rhythms in healthy humans.

3.3.2.1 Alpha (α) rhythm

In the EEG signal of healthy participant, there is a posterior dominant rhythm represented bilaterally over the posterior head regions and lies within the 8 to 13 Hz bandwidth, which represents the alpha frequency. When this rhythm is attenuated with eye opening, it is referred to as the alpha rhythm (30). During normal development, alpha appears after 3 years of age in 8 Hz frequency and remains stable during normal aging to the late years of life in 8 to 12 Hz bandpass. In approximately one fourth of normal adults, the alpha rhythm is poorly visualized, and in less than 10 %, voltages of smaller than 15 μV may be seen (30). The strength of the alpha rhythm is maximal in the occipital regions, and shifts anteriorly during drowsiness. Voltage asymmetries of more than 50 % should be considered as abnormal, moreover when the alpha on the left hemisphere is greater than the right alpha. It is best observed during relaxed wakefulness, and has a side to side difference of less than 1 Hz (30). The alpha frequencies usually temporally increase their activity immediately after eye closure, what is called alpha squeak. Alpha variants include forms that are one-half (slow alpha) or two times (fast alpha) the frequency with similar distribution and reactivity (30). Paradoxical alpha occurs when alertness results in the

presence of alpha, and drowsiness does not (30).

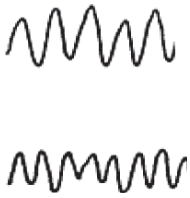
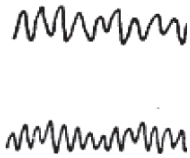
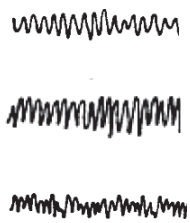
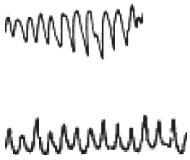
Name	Appearance (1 sec.)	Frequency range (Hz)	Amplitude (μV)	Main scalp area	Participant condition
Delta (δ)		0,1 – 3,5	50 – 350	Variable	Drowsiness and deep sleep; Hyperventilation; Infancy and childhood.
Theta (θ)		4 – 7,5	10 – 150	Variable	Drowsiness and deep sleep; Hyperventilation; Infancy and childhood. Small amount in awake adults.
Alpha (α)		8 – 13	20 – 100	Posterior (occipital-parietal)	Relaxed wakefulness with eyes closed.
Beta (β)		13 – x, x > 13;	10 – 30	Frontal or diffuse	Increase during cognitive efforts as well as during drowsiness and light sleep.
Mu (μ)		8 – 13 15 – 25	10 – 50	Central	Relaxed wakefulness with both eyes open and closed. Blocked by movements of the contralateral body parts.
Lambda (λ)		0,2 – 0,3	< 50	Occipital	Visual exploration.

Table 1: ***Typical EEG oscilations and their properties.*** *(29)*

3.3.2.2 Beta (β) rhythm

Beta rhythms are frequencies that are faster than 13 Hz. These rhythms are common, and normally observed within the 18 to 25 Hz bandwidth with a voltage lower than 20 μV, while voltages beyond 25 μV in amplitude are considered to be abnormal. Benzodiazepines, barbiturates, and chloral hydrate are potent generalized beta activators of "fast activity" higher than 50 μV for more than 50% of the waking tracing within the 14 to 16 Hz bandwidth (30). Beta activity normally increases during drowsiness, light sleep, and with mental activation. Persistently reduced voltages of more than 50% suggest a cortical gray matter abnormality within the hemisphere having the lower amplitude. However, lesser asymmetries may simply reflect normal skull asymmetries, which are normal unless associated with spikes or focal slowing (30).

3.3.2.3 Tetha (θ) rhythm

Theta rhythms are typical 4 to 7 Hz oscillations with varying morphologies and amplitudes. Approximately one-third of normal awake, young adults show intermittent 6 to 7 Hz theta rhythms with amplitude lower than 15 μV that is maximal in the frontal or frontocentral head regions (30). The appearance of frontal theta can be facilitated by emotions, focused concentration, and during mental tasks (30). Hyperventilation, drowsiness, or sleep are also enhancing factors of theta activity. Discrete 4 to 5 Hz activity bitemporally, or even with a lateralized predominance (usually left > right), may occur in about one-third of the asymptomatic elderly and is not abnormal (30).

3.3.2.4 Lambda (λ) rhythm

Lambda waves have been initially described as surface positive sharply contoured theta waves appearing bilaterally in the occipital region (30). These potentials have a duration of 160 to 250 msec, and may at times be quite sharply contoured, asymmetrical, with higher amplitudes than the resting posterior dominant rhythm (30). When they occur asymmetrically, they may be confused with interictal epileptiform discharges, and potentially lead to the misinterpretation of the EEG. They are very well visible in young

adults, although they are more typical for children. Lambda waves are best elicited when the participant visually scans a textured or complex Figure with fast saccadic eye movements (30).

3.3.2.5 Delta (δ) rhythm

Delta rhythm activity includes frequencies slower than 4 Hz that comprises less than 10 % of the normal waking EEG by age of 10 years. In the waking states, delta activity can be considered as normal waveform in the young children and in the elderly. The normal elderly may have rare irregular delta complexes in the temporal regions (30). This delta is normal in people older than 60 years, at the onset of drowsiness, in response to hyperventilation, and during slow-wave sleep (30). Excessive generalized delta is abnormal and indicates an encephalopathy that is etiology nonspecific (30). Focal arrhythmic delta usually indicates a structural lesion involving the white matter of the ipsilateral hemisphere, especially when it is continuous and unreactive (30).

3.3.2.6 Mu (μ) rhythm

Mu (also known as the central, Rolandic, sensorimotor, wicket, or arceau) rhythms are typically identified as 8 to 13 Hz and ~ 20 Hz bands oscillations (31). These oscillations are limited to brief periods of 0.5 to 2 s duration, maximal over sensorimotor cortex when the individual is in resting state (3). Mu rhythm is attenuated by voluntary movement or somatosensory stimulation, but is minimally affected by visual stimulation (3). While mu resembles the alpha rhythm, it does not block with eye opening, but instead with body movement (30). Mu rhythms may be visible only on one side, and may be quite asymmetrical and asynchronous, despite the notable absence of any underlying structural lesion (30). The mu rhythm waves may slow with advancing age, and is usually of lower amplitude than the existent alpha rhythm. When persistent, unreactive, and associated with focal slowing, mu-like frequencies are abnormal (30).

Because mu rhythm frequencies overlap those of the occipital or classical alpha rhythm (~ 10 Hz), it is sometimes difficult to separate them and often these two have been confused and misidentified. However, mu rhythms show a more anterior focus compared to

the more posterior one for the classical alpha oscillations (3). In general, mu rhythms reflect sensorimotor processing in frontoparietal networks, while classical alpha reflects primarily visual processing in occipital networks (3). When an individual performs a motor action, which means that he/she changes from resting into an active state, the power of the EEG mu is rapidly decreased or suppressed and mu oscillations become desynchronised, because the underlying sensorimotor tissue fires asynchronously supporting the movement of different body parts. This phenomenon is often refered to as event related desynchronisation (ERD), which is an electrophysiological correlate of activated or excited cortical neurons that tend to work independently and this is displayed in low amplitudes of the EEG signal (32). The opposite effect is called event related synchronisation (ERS), which has been assumed to reflect deactivation, inhibition, or at least the natural "idling" state of the underlying cortical network (32).

Because the sensorimotor areas are believed to be generators of the mu rhythm (3), and mirror neurons are located mostly in premotor cortex (1), it has been hypothesized that the mu rhythm may be a specific index of downstream modulation in primary sensorimotor areas by activity of the mirror neurons (33). In this perspective, when the transformation from viso- and/or auditory-inputs into internal representation of "doing" occurs, or, in other words, when action observation/comprehension occurs, then mu rhythms reflect primarily modulation of mirror neurons activity (3). There are several facts that support these claims. Firstly, mu power recorded from central electrodes at scalp locations C3 and C4 is attenuated by self-initiated movement and observed movement (ERD) (34). The mu rhythm perturbations show another important similarity to mirror neuron activity, in null response to nonbiological directional motion such as bouncing balls (35). Moreover, the frontal mirror neuron system seems to be the only network in the region of sensorimotor cortex that has been identified as responding to observed hand actions (36). Taken together, these results suggest that mu wave suppression to observed actions, in terms of ERD, can be used as a selective measure of activity of mirror neuron system (36).

4 Preliminary experiments

The main role of the preliminary experiments was to test several different nonresting conditions and consider the most suitable motor stimulus for the leading study, that we wanted to perform. The power spectral density of the event related desynchronisation (ERD) effect in the mu typical band was considered to be the critical criterion for the competing conditions.

4.1 Participants

EEG data from four healthy participants, from which three were men and one woman, were collected. All of them were university students, their age varied from 22 to 27 years.

4.2 Data acquisition

We focused on mu ERD and expected to find the highest mu power in relax conditions and similarities of mu rhythm suppression in self motor movement and the observed movement conditions. We recorded event related EEG signal for each stimulus using 8 active electrodes within international 10-20 placement situated in F3, F4 Fz, C3, C4, Cz, T7 and T8 positions. While recording, online lowpass and highpass filters along with 50 Hz notch filter were applyed to all 8 electrodes. Individual filtering was applyed to few more noisy electrodes. Each experiment consisted of several conditions, that were presented for exact amount of time, which was 30 sec. per condition. During each condition participants were asked to perform the given task. Their performance was observed and controlled by the experimenter. The behavioral data showed absolute accuracy in performance of all four participants.

4.3 Experiments

We created four preliminary EEG experiments examining ERD, that differed in movement stimuli conditions also in type of execution.

The first preliminary experiment consisted of six conditions, which were:

- Motor relax
- Performing a PC mouse scrolling movement
- Motor relax
- Observing a PC mouse scrolling movement performed by another individual
- Motor relax
- Observing a PC mouse scrolling movement performed by an artificial rubber hand

The second preliminary experiment consisted of four conditions, which were:

- Motor relax
- Performing a grasp of a cup
- Motor relax
- Observing a grasp of a cup performed by another individual

The third preliminary experiment consisted of six conditions, which were:

- Motor relax
- Performing a painting movement
- Motor relax
- Observing a painting movement performed by another individual
- Motor relax
- Observing a painting movement performed by an artificial rubber hand

The fourth preliminary experiment consisted of six conditions, which were:

- Motor relax
- Performing a finger walk movement
- Motor relax

- Observing a finger walk movement performed by another individual
- Motor relax
- Observing a finger walk movement performed by an artificial rubber hand

4.4 Data analysis

After the recording phase frequency-domain analysis was used for data evaluation. We created a Matlab application using Fast Fourier Transformations function to obtain power spectral density (PSD) of the EEG signal in 3 to 25 Hz band, which includes as we showed in chapters above, mu typical (8 to 13 Hz, 15 to 15 Hz) bands.

4.5 Results

The results were computed for each electrode including all conditions per experiment and plotted into graphs. After evaluation, graphs were compared in mu power amplitudes, to find the most significant stimulus.

The comparison revealed results, which are presented in following table:

Condition	Electrode	Frequency band *(Hz)*	PSD of particular task (uV^2/Hz)			PSD difference of relax and nonrest tasks (uV^2/Hz)	
			Relax	Self movement	Observed movement	Relax - Self movement	Relax - Observed movement
Scrolling	**C3**	**~ 10**	**6,5**	**0**	**0**	**6**	**6**
	C4	**~ 16**	**8**	**0,1**	**0,1**	**7,9**	**7,9**
	T7	~ 13	2,8	0,1	0,6	2,7	2,2
Grasping	C3	~ 11	2,5	0,1	0,8	2,4	1,7
		~ 11,5	5,5	1,1	0,7	4,4	4,8
Painting	C3	~ 9	4,2	0,5	0,3	3,7	3,9
	C4	~ 10	2,5	0,2	0,3	2,3	2,2
Fingerwalk	C3	~ 9,5	5	0,8	0,2	4,2	4,8
		~ 12	2,6	0,8	0,4	1,8	2,2
	C4	~ 11	2,2	1	0,7	1,2	1,5

The results showed that the highest PSD suppression between relax and nonrest (self movement and observed movement) conditions is evoked by scrolling. Importantly, this suppression was found at scalp positions C3 and C4, which are central electrodes collecting the EEG brain signal mostly from sensorimotor cortex, which are the brain areas including the mirror neurons. Another remarkable finding is that the suppression was found in the frequency bands (~ 10, 16 Hz bands), that are typical for mu rhythm oscillations. From this finding we can conclude that ERD of mu rhythm is mostly present while moving and observing movement of fingers of hand. We can assume that finger movement is the most suitable trigger for motor resonance, hence four our leading study.

5 Experiment

With focus on previous knowledge of mirror neuron system properties and EEG mu rhythm characteristics, we created and designed a psychological experiment with an aim to examine previous findings and will to show some other, maybe not so broad accepted, properties of mirror neurons and mu perturbations. In our experiment we chose electroencephalography as the essential imaging technique for mirror neurons activity observation, with focus on mu rhythm as the indicator of the mirror neuron system (MNS) activity. We conducted two main scientific questions for our experiment. Firstly, we wanted to prove the previous findings about ERD of mu rhythm, which is linked with MNS activity. Our direct expectation was to see the highest mu power in relax condition and similarities of mu rhythm suppression in self motor movement and the same observed movement condition. Secondly we expected to find contralateral correlations in mu rhythm strength according to left/right hand in nonrest conditions and hemispheric differences in the mu power also in nonrest conditions.

We aimed our focus on mu rhythm as the indicator of mirror neuron system activity and designed EEG experiment, whose results should have proved the theoretical knowledge and our expectations about the mu ERD and hemispheric differences in mu power associated with contralaterality of brain motor movements control.

Before the main experiment establishment several performed preliminary EEG measurements were executed. We designed simple experiments with variation of different stimuli, from which after the evaluation arose the most significant one, that pointed out the better outlook for future experimentation. These measurements served as indicators for selection of the most suitable nonrest stimulus in terms of mu suppression.

5.1 Stimuli

The stimuli in the experiment were presented in visual form only. Two different kinds of visual stimulation were used – video and pictogam stimuli. The most critical problem

was to find suitable motor stimulus for triggering the high response of MNS activity.

5.1.1 Motor stimulus type selection

In the beginning we had to carefully choose appropriate motor stimulus which will be the most probable trigger of the significant response in mu rhythm signal power differences. For this purpose we used our preliminary experiments results. These showed the best ERD response in terms of PSD differences of rest and nonrest conditions for mouse scrolling movement. In line with this finding we considered similar types of movement applicable for our experiment, which required movement of both hands, while we wanted to examine also hemispherical differences of mu power according to contralateral movement control theory. Avoidance of motor activity noise in EEG signal was another important requirement that we had to take into consideration. Because of these constraints we decided for horizontal movement of the index finger (tapping, see Figure 4.1, Figure 4.2) of both hands as the accurate stimulus, that should lead to expected effects and prevent unwanted artefacts.

5.1.2 Video stimuli

The motor movement was recorded and presented in short videos, which we recorded in advance using Pentax Optio WG-1 digital camera. With focus on preventing distractors, like facial expression or environment outlook, in the stimuli the close-shot of hands lying on the desk and body up to the chest of the actor was filmed. The videos were recorded separately for each left and right index finger movement. The perspective of opposite observer was used, which means that if right index finger movement stimulus is presented the observer sees it at his/her left side. This simulates the most common perception of other individuals in everyday life. After the filming phase videos were cut to appropriate duration. The sound was removed, what ensured stimulation of "visual" mirror neurons.

The two short video stimuli were used:

- right index finger movement video (snapshots in Figure 5.1)
- left index finger movement video (snapshots in Figure 5.2)

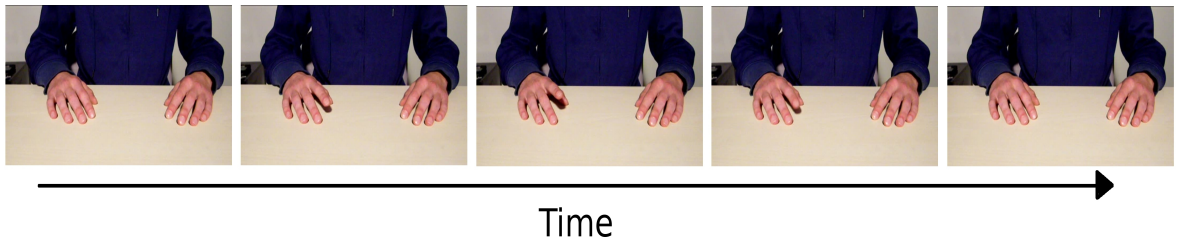


Figure 5.1: ***Right index finger movement.***

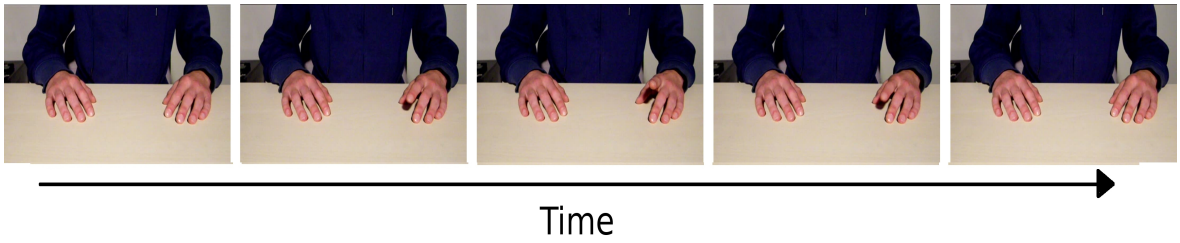


Figure 5.2: ***Left index finger movement.***

5.1.3 Pictogram stimuli

All the nonvideo stimuli were chosen according to KISS (keep it simple stupid) principle, which provides the best solution for stimulus run and understanding of the task presented via the stimulus.

Pictograms were designed with focus on the clarity of their presentation, and therefore high contrasting colours were chosen (shiny lime green for the stimulus over black background color). Three simple pictogram stimuli were used for triggering intended tasks:

- left arrow composed of three text elements "<" and two "-" (see Figure 5.3). This stimulus was used for triggering self motor movement of the left index finger.
- right arrow composed of three text elements two "-" and ">" (see Figure 5.4). This stimulus was used for triggering self motor movement of the right index finger.
- the cross sign "+" was used as gaze fixation point for motor rest conditions (see Figure 5.5).

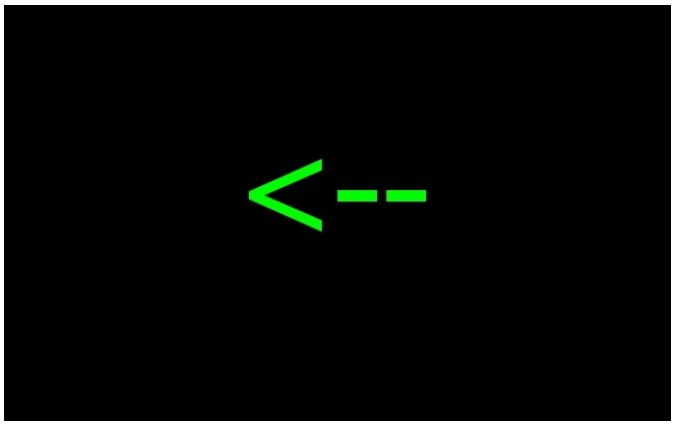


Figure 5.3: ***Left index finger movement stimulus.***

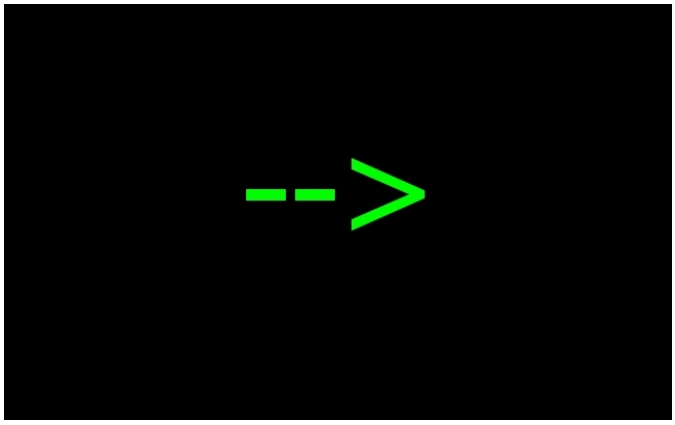


Figure 5.4: ***Right index finger movement stimulus.***

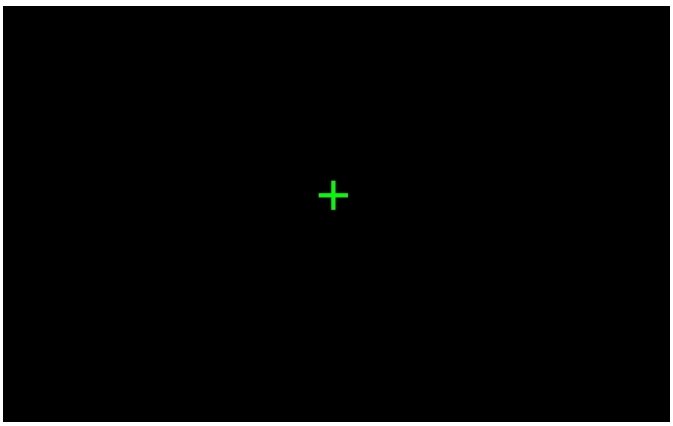


Figure 5.5: ***Motor rest stimulus.***

5.2 Experimental conditions design

After solving the appropriate motor stimulus problem, we stepped to the actual experiment design. Firstly, we programed an E-Prime application that was handling and projecting our stimuli across different types of conditions. E-Prime is a suite of applications offering audited millisecond-timing precision, enabling to develop a wide variety of paradigms that can be implemented with randomized or fixed presentation of text, Figures and sounds. Our experiment consisted of three different types of conditions:

- The first type condition was observing simple vertical motor movement (finger tapping on the desk) of either left or right index finger (see snapshots in Figure 5.2 and Figure 5.1).
- The second type condition was self performing simple vertical motor movement (finger tapping on the desk) of left or right index finger identical with the first type condition.
- The third type condition was motor relax state.

The task for the first type condition was only observing presented video stimuli. Important part of it was avoidance of any self motor movement, because it would cause bias with second self movement condition, and thereby mislead results of the whole experiment. Therefore participants were monitored and controlled by the experimenter.

The second type condition task was to perform simple horizontal movement of either left or right index finger, which was the same movement as presented in the first type conditions. This movement execution had to last the same amount of time as the previous video stimulation, to prevent possible incongruity with first type condition stimuli, whereas these two were later on compared in data analysis.

The last condition type was motor relaxing. It was crucial to stay absolutely still in this condition, since any even the smallest intentional motor activity would evoke desynchronistion of the mu rhythm, which would bias the data that we wanted to acquire.

5.3 Experiment design

Experiment consisted of three main parts, which were:

- instructing,
- measurement preparation,
- measurement and signal recording.

Each part will be described and explained in more details within the following chapters. The design of the stimuli presented across the conditions, during the measurement phase is presented in chapter 5.4 Testing process.

5.3.1 Instructing

To ensure clear explanation of the experiment process, actual experimental tasks, providing exactly the same conditions for each participant, and to prevent misunderstanding, participants were instructed by a set of instructions read before each experiment start. The instructions were read every time with the same speed in the exact order, without skipping any line.

"*This measurement consists of two parts. The first part is preparation and attaching EEG electrodes to your head. This involves putting on a cap with electrodes and decreasing impedance between electrodes and skin with use of conductive gel. This process will last approximately 15-25 mins.*

The second part is the experiment itself. It lasts approximately 7-8 minutes. The experiments consists of four following tasks which we ask you to fulfill. The first task is just observation of short videos presenting small movement. Your second task is to relax, with opened eyes staring at the cross presented in the middle of the screen. The third task is performing the same small movement as you have seen in the first task, either with your left or right index finger according to the direction of arrow presented on screen. Please, keep on performing the movement till the arrow disappears. Your last task is again relaxation with opened eyes while looking at the cross presented in the middle of the screen.

It is very important that you do not move during the experiment, except the task you are instructed to move. Please seat yourself in a comfortable position and try to avoid every unnecessary muscle movement, including talking and all facial expressions.

All the instructions are presented step by step on the screen with proceeding of the experiment. If you have any question, please, ask them now."

After answering all questions, we stepped to the measurement preparation.

5.3.2 Measurement preparation

This process was necessary for setting up the EEG device, proper mounting of all electrodes including electrooculogram (EOG) ones and initialisation of applications responsible for stimuli handling and data recording.

In the first step the computers were turned on. On the stimuli presenting one, our E-Prime application was initialised. On the other one, used for data recording, BrainVision Recorder application was run.

The second step was enabling the EEG amplifier and mounting the electrodes over participant's encephalon. Before the actual electrode mounting process, the size from inon to nasion for each participant was measured. This was necessary for accurate individual electrodes cap placement. Hence, we used international 10-10 electrode placement system, the first electrode had to be placed in the 10 % distance from the beginning of either inion and nasion bones. The cap was fixed with chest bandage and elastoplast on forehead.

After proper cap placement the pair of vertical (V) and horizontal (H) EOG electrodes was mounted. The HEOG electrodes were placed over the left and right sphenoid bones, one VEOG electrode over the frontal bone near the right eye and second over the maxillary bone near the right eye.

The next step was the impedance between electrodes and head reduction, with help of an electric conductive gel. We used 32 active electrodes with led impedance indication. The impedance change was signalised with three colors, red (> 60 kΩ), orange (25 - 60 kΩ) and green (< 25 kΩ). After applying the conductive gel with a syringe to all of the electrodes and making them light all green, visual signal check of the online signal

displayed on data saving computer was performed. When everything was well the procedure continued to the actual experiment run and signal recording.

5.3.3 Measurement and signal recording

After EEG setup and successful low signal impedance stabilisation, the experiment was started. Right after start, identification data of the participant, like participant number, session id, were filled into experiments form. More specific details about the participant like name, gender, age, dominant hand, and e-mail address were recorded into a paper form.

Then the experiment program itself started running from the appropriate computer. EEG signal with encompassed triggers, signalising the onset of particular stimulus was saved on the hard disk of data saving and projecting computer. In the end, the application announced the end of the session. Each participant was offered a clean towel and shampoo to wash the conductive gel out of their hair.

The experiment run details are described in the next chapter 5.4 Testing process.

5.4 Testing process

The experiment was designed in four logical parts of three groups of conditions. The actual alternation of the conditions was following:

1. video stimuli of random hand movement observation
2. motor relaxed state
3. self motor movement according to the direction of arrow projected on the monitor screen
4. motor relaxed state

After the initialisation of experiment, brief instructions about on-coming task were presented and the actual testing process begun when participant decided to start. The instructions text was:

"In the first part short videos of movement will be presented. Your task is just to observe them!
When you'll be ready press ENTER to start the experiment, please!"

The first part, which was observing of simple motor movement, consisted of 15 trials per each hand (see Figure 5.1, Figure 5.2). The stimuli in trials were presented randomly, with the overall count of 30. Each trial of this part lasted 5 seconds. The trial was divided to the video stimulus presentation, that lasted for 4 seconds and 1 second of white blank page, which was the length of break before next stimulus onset.

Before the second part, which was motor relaxation state, there were presented short instructions for 2,5 seconds, saying:

"Get prepared for the next task!
Look at the cross in the middle of the screen when presented and relax."

After the instructions, the second part of the experiment, lasting 15 seconds, ran. During this time, fixation point (see Figure 5.5) in the middle of the screen was projected and motor relaxation EEG signal was recorded.

Another instruction set, present for 5 seconds, preceded the third part of our experiment. The instructions were:

"Get prepared for the next task!

Your next task is self motor movement according to the direction of an arrow that will be presented on the screen.

When you'll see left arrow (<--) tap your left index finger, as you've seen in previous videos, please.

When you'll see right arrow (-->) tap your right index finger, as you've seen in previous videos, please.

When you'll be ready press ENTER to continue, please!"

The third part consisted of 15 trials per each hand. The stimuli in trials were presented randomly, with the overall count of 30. Each trial of this part was 5 seconds long. The trial was divided to the pictogram (arrow in the middle of the screen) stimulus presentation, that lasted for 4 seconds and 1 second of black blank page, which was the length of break

before next stimulus onset. In this part, the EEG signal of self motor movement was recorded.

Last set of instructions was presented before the fourth part of the experiment, lasting for 2,5 seconds. The instructions said:

"Get prepared for the next task!
Look at the cross in the middle of the screen when presented and relax."

The motor relaxation task lasted again for 15 seconds, during which motor relaxation data were collected.

In the end of the experiment the goodbye screen with following text was presented:

"YOU ARE DONE!
Thank you very much for participating in our experiment!!!"

5.5 Participants

We recorded data from group of 28 healthy adult participants, in age from 20 to 32 years, from which 16 were females and 12 males. However, for final analysis data from 25 of them, 14 females and 11 males, were used. The two participants were excluded due to slight changes in experimental design and one because of unbearable noise in the recorded data.

Most of the participants were exchange Erasmus mobility students, from different European countries, at that time studying at different faculties and departments of University of Zagreb. The rest of our participants were Croatian students and PhD colleagues from University of Zagreb. Only two participants from the whole group had previous experience with electroencephalography and EEG measurements.

Each one of the participants did not have any previous knowledge about the experimental tasks nor the purpose of the experiment. It is arguable if the previous knowledge could influence the results, because the mirror neuron activity should be spontaneous, however we wanted to be sure that no influence will propagate into our data.

All of them participated voluntarily, and as a reward received photos taken during their

measurement, which were sent via e-mail.

5.6 Methods and technical background

This experiment was elaborated in the Laboratory for Psycholinguistic Research (POLIN), Faculty of Education and Rehabilitation Sciences of University of Zagreb, Croatia, under the great supervision of assistant professor Dr. Marijan Palmović.

The laboratory is equipped with an EEG "QuickAmp amplifier" device developed by Brain Products company, encompassed with 256 channels and 4 integrated bipolar as well as 4 auxiliary channels for sensor input, the maximal sampling frequency od this device is 2 kHz. The EEG equipment of this lab involves also several "actiCAP" electrode sets with 32 active electrodes based on Ag/AgCl sensors with integrated noise subtraction circuits delivering low noise levels and ActiveShielding support, made by the Brain Products company. All of these are highly sensitive devices that provide excellent conditions for precise EEG data acquisition. For data recording BrainVision Recorder software and for EEG/ERP data analysis BrainVision Analyzer 1 and BrainVision Analyzer 2 were used.

The programing of our experiment was done on an IBM desktop computer with adequate hardware and computational power. For the programing E-Prime software package for experimental psychology with E-Studio programming interface was used. The stimuli were presented in 21,5" LCD monitor with a screen resolution of 1280x1024 pixels and 60 Hz refresh rate.

For the run of the experiment and data recording a framework of two computers interconnected with the EEG amplifier was used. The first computer used for data recording was directly interconnected with the EEG device, whole the second computer used for the experiment run and stimuli presentation was interconnected with the first data saving one. The connection of the two computers was essential, in terms of the appropriate stimulus onset integration into the EEG data, which was crucial for the signal analysis. Each stimulus had specific identification marker, with which it could been recognised. These markers were sent and propagated into the recorded data, serving as specific time point identifier for the brain response on the presented stimulus associated with the particular marker.

We were recording event related EEG signal for each stimulus using 32 actiCAP active electrodes in international 10-10 electrode placement system, interconnected with QuickAmp amplifier. Two Ag/AgCl bipolar electrodes for VEOG and HEOG data recording were used. Conductive gel was applied at each position between electrode and the skin surface to reduce the impedance of the electrode-skin contact. The impedances on all electrodes were measured and held lower than 2,5 kΩ during the whole testing process. The sampling frequency was set to 1 kHz. For the nonrest conditions we recorded 4 seconds continuous chunk of data from the stimulus onset and for the rest condition it were 15 seconds long data chunks with the beginning in the stimulus onset. The recording was performed in well electrically shielded room.

5.7 Data analysis

The raw data analysis was done using BrainVison Analyzer software. During the recording phase the raw signal for each participant was saved. In this phase only 50 and 60 Hz notch filters were applied. The main part of the signal processing was done in offline analysis. During the offline analysis high-pass 0,05 Hz filter and low-pass 40 Hz filtering was done. We used automatic noise rejection function to cut out mostly muscle artefacts, but also other unwanted signals that propagated into our data. The ocular artefacts correction according to VEOG and HEOG signal obtained by bipolar electrodes was performed. All of these filters and filtering techniques were applied to all 32 electrodes. After the filtering and correction the signal of each participant was visually checked. Individual filtering was applied to few more noisy electrodes, if needed.

The EEG data measured from 25 healthy participants were analysed. The core of the whole analysis consisted from of parts. The first part was the frequency-domain analysis used for acquiring power spectral data. These data were used in statistical analysis, which was the second part of the experiment analysis.

5.7.1 Frequency-domain analysis

The next step after data filtering was the frequency-domain analysis. This type of analysis was used to obtain power spectral density (PSD) data from the measured and filtered signal.

Firstly, with use of BrainVison Analyzer, we did the averages of the filtered signal according to particular condition for each participant from the whole run of the experiment. This averaging was done for all of the 32 electrodes involved in the measurement one by one. We computed very precised average data per participant per electrode, consisting from the average of:

- left index finger movement observation
- right index finger movement observation
- left index finger movement execution
- right index finger movement execution
- motor relaxation

The precision of the average was faciliated by the stimuli markers sent, by the stimuli handling and experiment running computer, and saved to the exact time point in the recorded signal by the data handling computer. The averages were done with the segmented data taken out of the signal according to an appropriate stimulus with precision on thousandth of second. The particular segment length of the nonrest condition was 4 seconds, the rest data segments were 15 seconds long. In the end of this process we had averaged five different conditions for each participant.

In the second step, we used BrainVison Analyzer's Fast Fourier Transformations (FFT) function to obtain PSD data in 8 to13 Hz and 15 to 25 Hz bands, which are the mu rhythm typical bands, as we showed in chapter 3.3.2.6 Mu (μ) rhythm. FFT ran on above described averaged data for each participant for each electrode for particular condition. The visal analysis of the PSDs showed remarkable changes in power of the signal when compared rest to nonrest conditions, preferably on electrodes placed over sensorimotor cortex and temporal cortex regions, in most of the reviewed participants. This was a positive sign of

possibly statistically significant differences of the PSD suppression in these bands.

All the averaged PSD data of both examined frequency bands were exported into the text format for following statistical analysis.

5.7.2 Statistical analysis

With use of the statistical methods we wanted to prove the effects, which we were concerned about in the experimental questions.

Firstly, we wanted to prove the previous findings about ERD of mu rhythm, which is directly linked with MNS activity. Our expectation was to see the highest mu power in the relax condition and similarities of mu rhythm suppression in self motor movement and with the same observed movement condition.

Secondly, we wanted to find out if the MNS is hemispherically differentiated for distinguishing left and right body side movement. Our expectation was to find contralateral correlations in mu rhythm strength according to left/right index finger in both nonrest conditions, and hemispheric differences in the mu power also in nonrest conditions. In other words we anticipated higher mu power in left hemisphere when the participant was observing or preforming the motor task of the right hand and vice versa.

According to the knowledge of brain areas involving mirror neurons, we focused on brain signal obtained by electrodes, which were located directly above these positions or very near them. Therefore, from the 32 electrode system we have chosen C3, C4, T7 and T8 electrode to be most plausible for our research. The T7 and T8 electrodes were taken into consideration especially in respect to their suitable position for recording the signal produced by primary motor cortex area, according to motor homunculus, thought to be responsible also for volitional index finger movement control. Electrodes C3 and C4 were chosen because they have the widest coverage of scalp locations corresponding to left and right sensorimotor cortex.

The computations and also graphs plotting were created in statistical software STATISTICA 8.

5.7.2.1 Mu rhythm ERD

Power in the mu frequency bands (8–13 Hz, 15-25 Hz) at above mentioned scalp locations during the observation and self movement conditions was compared to mu power during the relaxed state (baseline) condition. This was done by computing the natural logarithmic ratio of the power in the mu frequency bands during the observed movement and self motor movement conditions, over the power in the rest (baseline) condition. A ratio was used to control for variability in absolute mu power as a result of individual differences in brain activity. Since ratio data are not normal, as a result of lower bounding, a natural logarithmic transformation was used for analysis. We designed the equation formula as follows:

$$\mu_c' = \ln(\frac{n_c}{b})$$

In this equation where *n* is PSD average of left and right side of particular nonrest condition *c*. The *b* represents PSD of the baseline (motor rest) condition. Then the μ' represents a mean logarithmic ratio of suppression or enhancement of mu rhythm activity at particular electrode in particular condition *c*. The μ_c' values were computed for both observing and self movement conditions for all of the 25 participants and all selected electrodes. For all values, a mean logarithmic ratio greater than zero indicates enhancement of the mu power and a mean logarithmic ratio smaller than zero indicates suppression of the power in the mu band in particular condition. For the actual statistics t-test comparing the μ_c' values of different conditions to the new baseline 0 was. The new baseline arose from the logarithmic calculation of our equation. The mu difference was considered to be significant if $p < 0.001$.

5.7.2.2 Hemispherical differences in mu PSD

To prove the hemispherical differences in mu PSD, we similarly computed the mean natural logarithm ratio of PSD in the mu frequency (8–13 Hz and 15–25 Hz) during the observation of index finger movement and the same self movement conditions, over the power in the same movement conditions in contra- and ipsi-lateral scalp locations to obtain ipsilateral and contralateral mu power suppression or enhancement values. Again a ratio was used to control for variability in absolute mu power as a result of individual differences in brain activity. Natural logarithmic transformation was used for normalising the data. We designed the equation formulae as follows:

$$(a)\ \mu_{Li}' = \ln\left(\frac{l_n}{r_n}\right) \qquad (b)\ \mu_{Lc}' = \ln\left(\frac{r_n}{l_n}\right) \qquad (c)\ \mu_{Ri}' = \ln\left(\frac{r_n}{l_n}\right) \qquad (d)\ \mu_{Rc}' = \ln\left(\frac{l_n}{r_n}\right)$$

These equations are pretty similar in the logic with the mu rhythm ERD one. Index *n* in all of the equations represents one of the nonrest conditions. *l* and *r* are indexes for PSD of the electrodes over the left (*l*) and right (*r*) hemispheres. μ_{Li}' stands for ipsilateral and μ_{Lc}' for contralateral changes in mu power according to left (*L*) hand at particular nonrest condition. μ_{Ri}' stands for ipsilateral and μ_{Rc}' for contralateral changes in mu power according to right (*R*) hand at particular nonrest condition.

$\mu_{Li}' > 0$ means that the activity of the left hemisphere was enhanced over the right hemisphere, which means that mu rhythm in the left hemisphere was more suppressed than in the right hemisphere. When $\mu_{Li}' < 0$ the activity of the right hemisphere was enhanced over the left hemisphere, indicating greater mu suppression of the right hemisphere. If $\mu_{Lc}' < 0$ the mu rhythm activity in the right hemisphere was more suppressed than in the left hemisphere, which indicates increased activity of the right hemisphere over the left hemisphere. $\mu_{Lc}' > 0$ stands for activity increase of the left hemisphere over the right hemisphere, what means higher suppression of mu rhythm in the left hemisphere than in the right one.

When $\mu_{Ri}' > 0$ the activity of the right hemisphere was enhanced over the left hemisphere, indicating greater mu suppression of the right hemisphere. When $\mu_{Ri}' < 0$ the activity of the left hemisphere was enhanced over the right hemisphere, which means that mu rhythm in the left hemisphere was more suppressed than in the right hemisphere. $\mu_{Rc}' < 0$ stands for activity increase of the left hemisphere over the right hemisphere, which means higher suppression of mu rhythm in the left hemisphere than in the right one. If $\mu_{Rc}' > 0$ the mu rhythm activity in the right hemisphere was more suppressed than in the left hemisphere, which indicates increased activity of the right hemisphere over the left hemisphere.

The values for each equation (a), (b), (c), (d) were computed for both observing and self movement conditions using the data from all of the 25 participants and the selected electrodes. For the actual statistics consisted of t-tests comparing the μ_{Li}', μ_{Lc}', μ_{Ri}', μ_{Rc}' values to the new baseline 0. The new baseline arose from the logarithmic

calculation of our equation. The mu difference was considered to be significant if $p < 0.001$.

6 Results and discussion

The statistical analysis was the most important part of the experiment data evaluation, because it provided exact answers to our experimental questions, which we wanted to scientifically prove or confute.

6.1 Mu ERD significance

ERD of mu rhythm, evoked by observation of a simple motor movement and the same self motor movement, was expected to occur. We expected to find the highest mu power in the rest condition and similarities of mu rhythm suppression in nonrest conditions. The results for our first question are plotted in the following graphs.

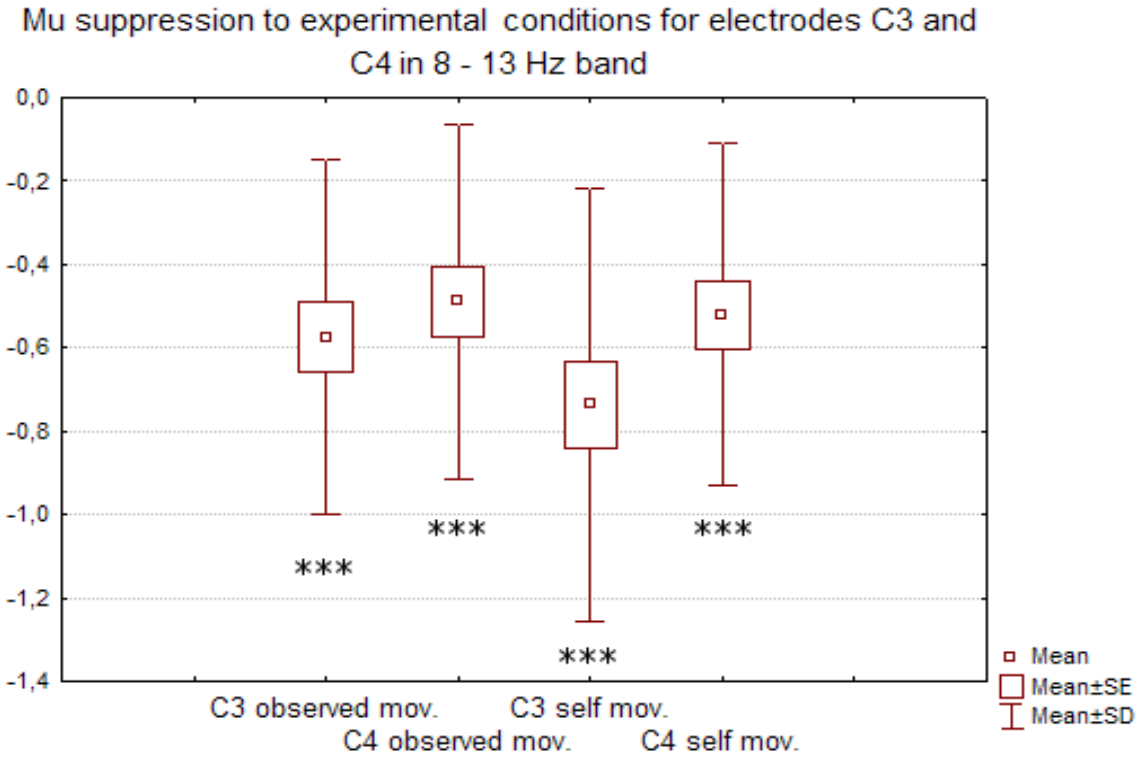


Figure 6.1: ***Mu suppression in different conditions on C3 and C4 electrodes.***

T-tests comparing mu suppression during each of the experimental conditions to zero showed significant suppression from baseline in mu oscillations over C3 and C4 electrodes in 8-13 Hz band during both the observed (Figure 6.1: C3 $t(24) = -6.75$, $p < 0.000001$; C4 $t(24) = -5.78$, $p < 0.000006$) and executed (Figure 6.1: C3 $t(24) = -7.10$, $p < 0.000000$; C4 $t(24) = -6.33$, $p < 0.000002$) finger movement conditions.

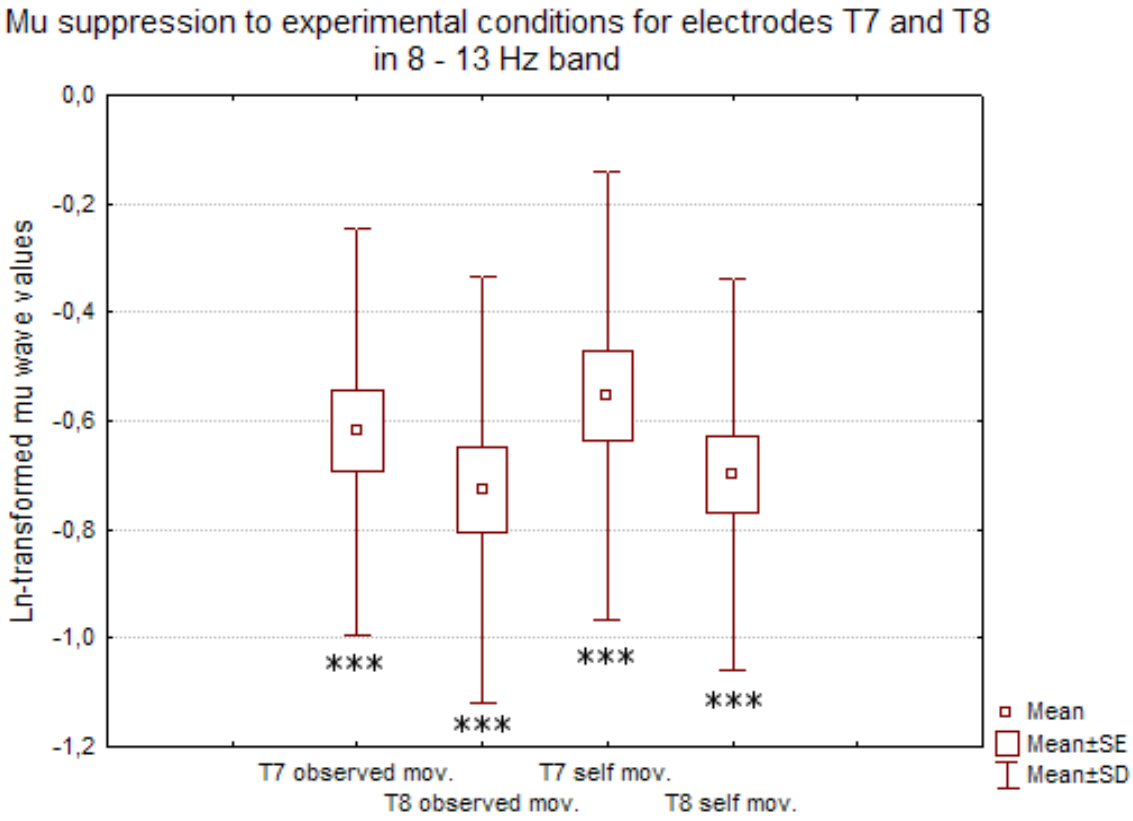


Figure 6.2: ***Mu suppression in different conditions on T7 and T8 electrodes.***

T-tests comparing mu suppression during each of the experimental conditions to zero showed significant suppression from baseline in mu oscillations over T7 and T8 electrodes in 8-13 Hz band during both the observed (Figure 6.2: T7 $t(24) = -8.27$, $p < 0.00000$; T8 $t(24) = -9.23$, $p < 0.00000$) and executed (Figure 6.2: T7 $t(24) = -6.70$, $p < 0.000001$; T8 $t(24) = -9.69$, $p < 0.000000$) finger movement conditions.

T-tests comparing mu suppression during each of the experimental conditions to zero showed significant suppression from baseline in mu oscillations over C3 and C4 electrodes in 15-25 Hz band during both the observed (Figure 6.3: C3 $t(24) = -6.75$, $p < 0.000001$; C4 $t(24) = -5.78$, $p < 0.000006$) and executed (Figure 6.3: C3 $t(24) = -7.10$, $p < 0.000000$; C4

t(24) = -6.33, p < 0.000002) finger movement conditions.

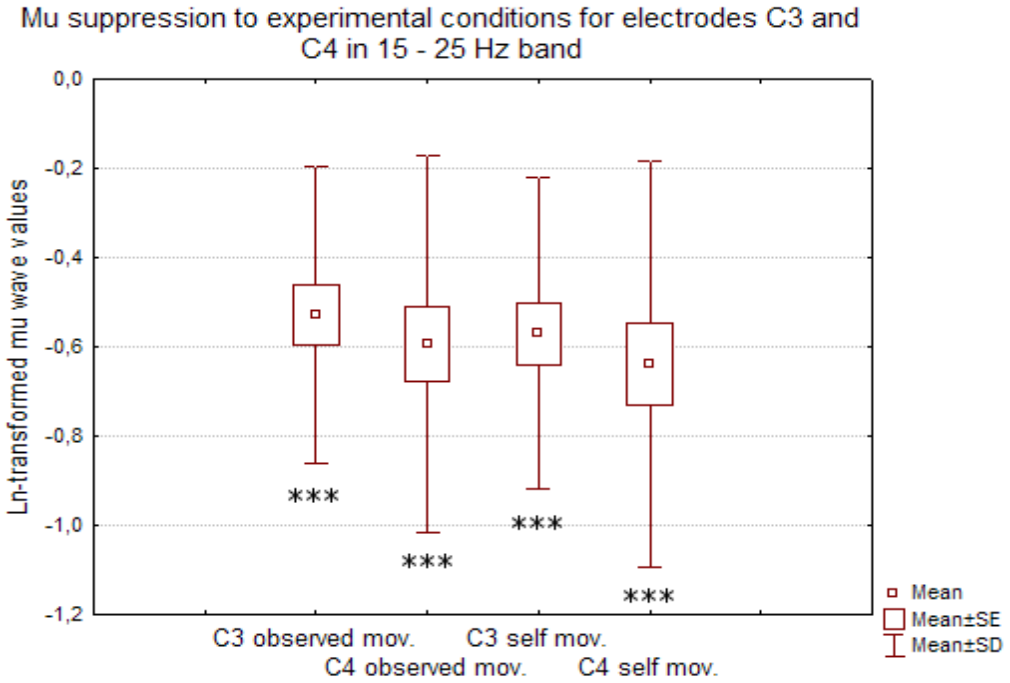


Figure 6.3: ***Mu suppression in different conditions on C3 and C4 electrodes.***

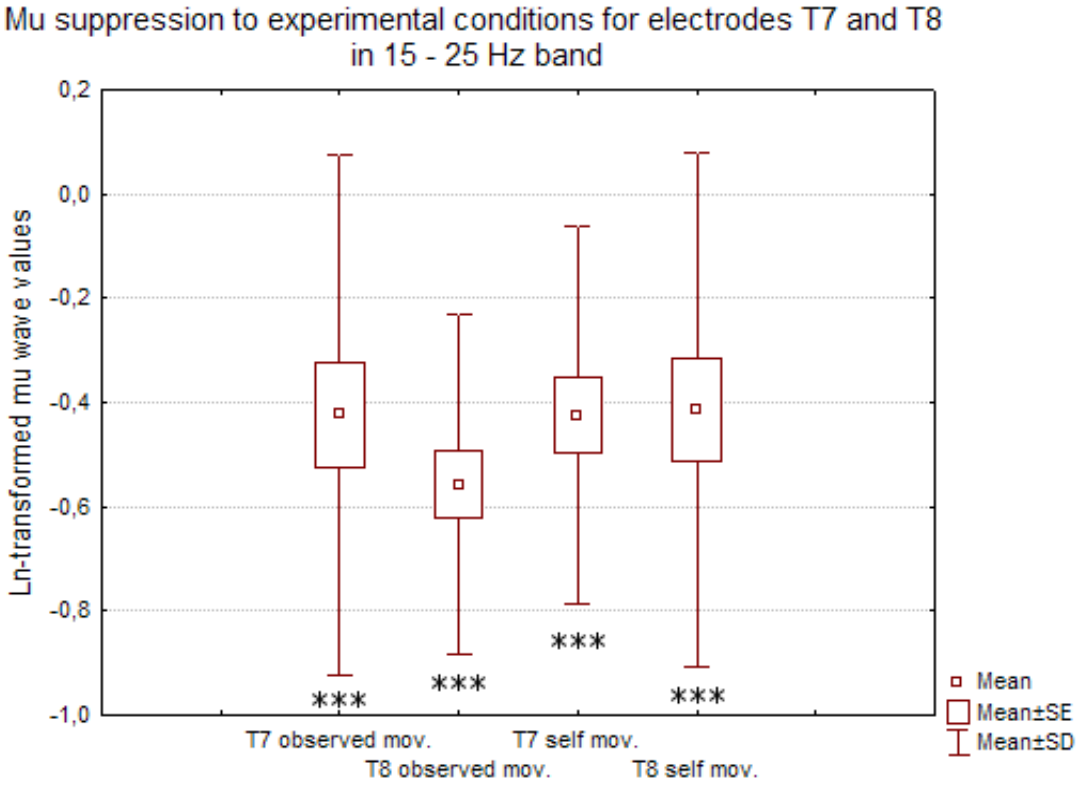


Figure 6.4: ***Mu suppression in different conditions on T7 and T8 electrodes.***

T-tests comparing mu suppression during each of the experimental conditions to zero showed significant suppression from baseline in mu oscillations over C3 and C4 electrodes in 15-25 Hz band during both the observed (Figure 6.4: C3 $t(24) = -4.26$, $p < 0.000276$; C4 $t(24) = -8.56$, $p < 0.000000$) and executed (Figure 6.4: C3 $t(24) = -5.85$, $p < 0.000005$; C4 $t(24) = -4.19$, $p < 0.000319$) finger movement conditions.

The overall t-tests comparing mu log ratio for the observation and self movement conditions over the rest to zero showed statistically significant suppression from baseline in mu oscillations over both the left and the right hemisphere during both conditions. This finding supports our expectation and also known literature, in the point that each "version" of the motor action – executed or observed leads to decrease of the signal power, as a result of the mu rhythm desynchronisation, compared to the relax condition. Further more from this finding we can claim that through the mu rhythm desynchronisation we observed and recorded significant mirror neuron system activity.

6.2 Hemispherical differences significance

Within the second question we wanted to find out if the MNS is contralaterally differentiated for distinguishing left and right body side movement. To support this we expected to find contralateral correlations in mu rhythm strength according to left/right index finger in both nonrest conditions, and hemispheric differences in the mu power also in nonrest conditions.

Our equations for obtaining contalateral and ipsilateral hemispherical are detaily described in the chapter 5.7.2.2 of the statistical analysis. From the equations it is obvious that if we want to prove our thought about contralateral functional properties of MNS, the outputs μ_{Li}' , μ_{Lc}' , μ_{Ri}' , μ_{Rc}' shall significantly differ from each other. This means that we expected $\mu_{Li}' > 0$, $\mu_{Lc}' < 0$, $\mu_{Ri}' > 0$ and $\mu_{Rc}' < 0$ values.

The results for our second question are plotted in the following graphs (Figure 6.5 – Figure 6.8). Each graph compares mu power differences from ipsilateral and contralateral view according to left/right index finger movement or observation in the two mu typical (8

to 13 Hz and 15 to 25 Hz) bands to zero, which is the baseline for particular electrode couple.

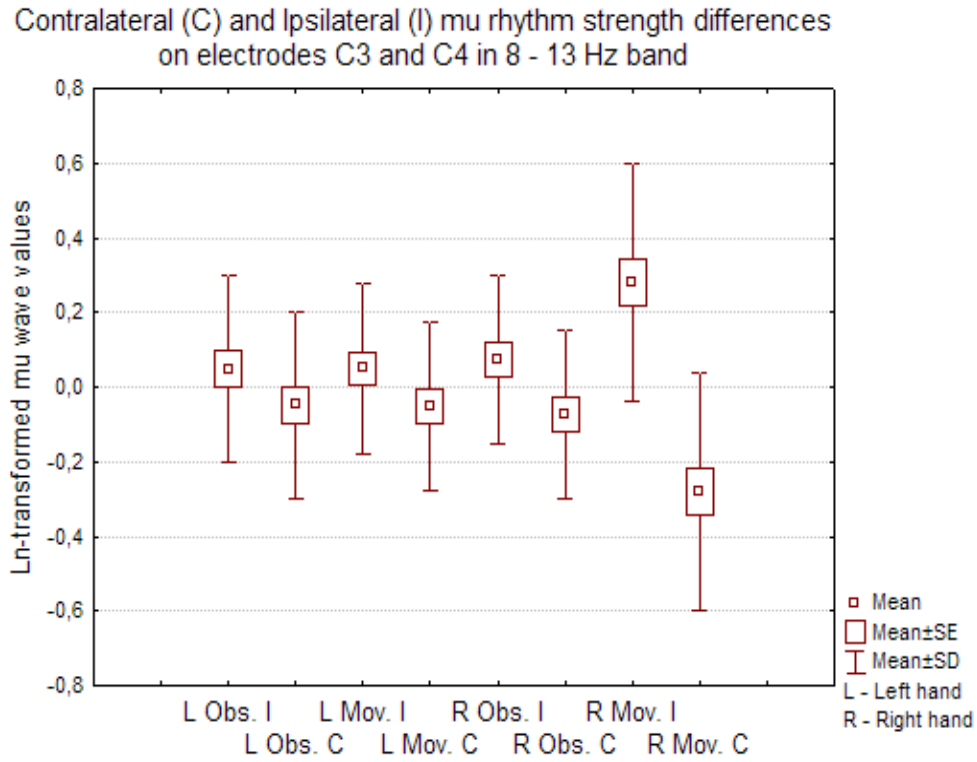


Figure 6.5: ***Contralateral and ipsilateral mu power differences on electrodes C3 and C4.***

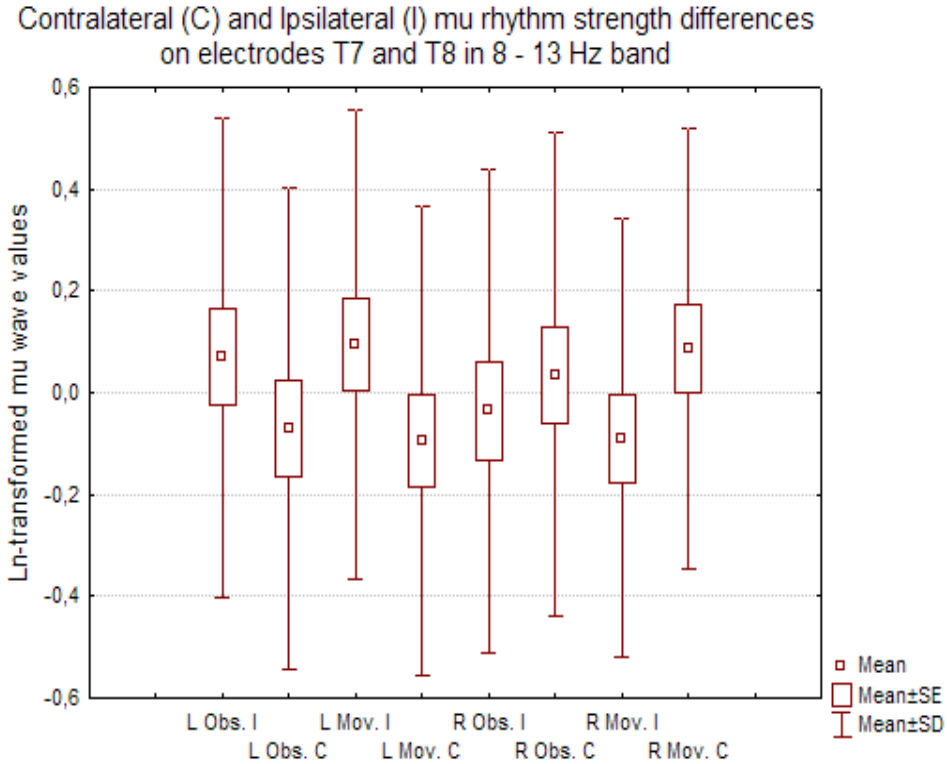


Figure 6.6: ***Contralateral and ipsilateral mu power differences on electrodes T7 and T8.***

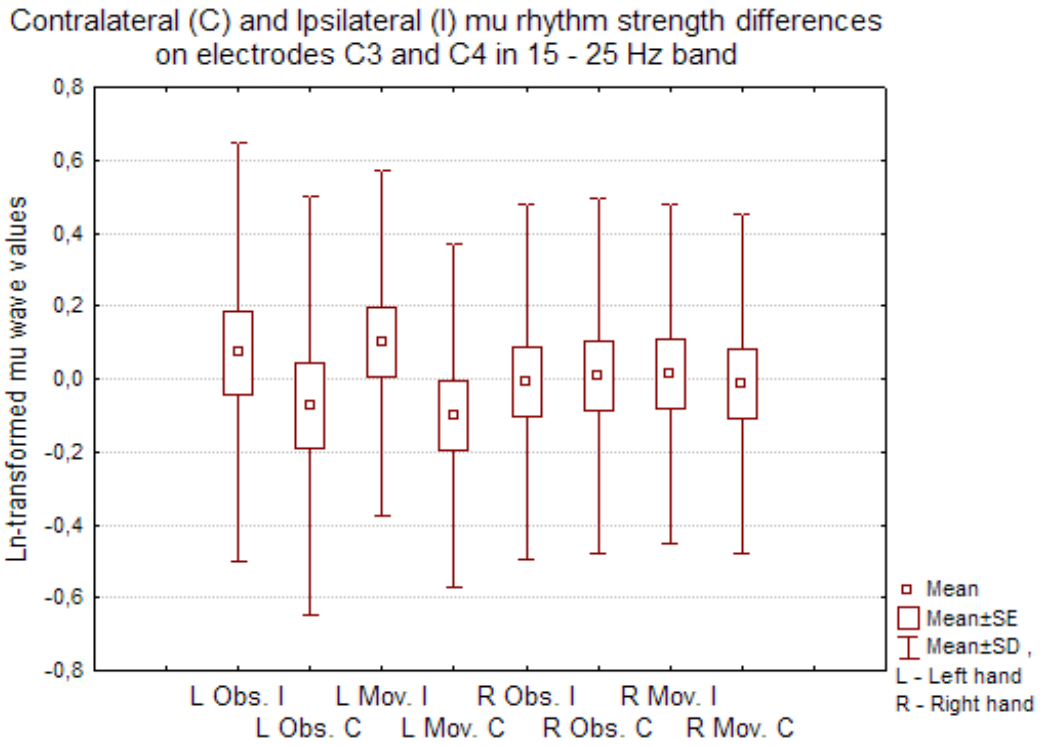


Figure 6.7: ***Contralateral and ipsilateral mu power differences on electrodes C3 and C4.***

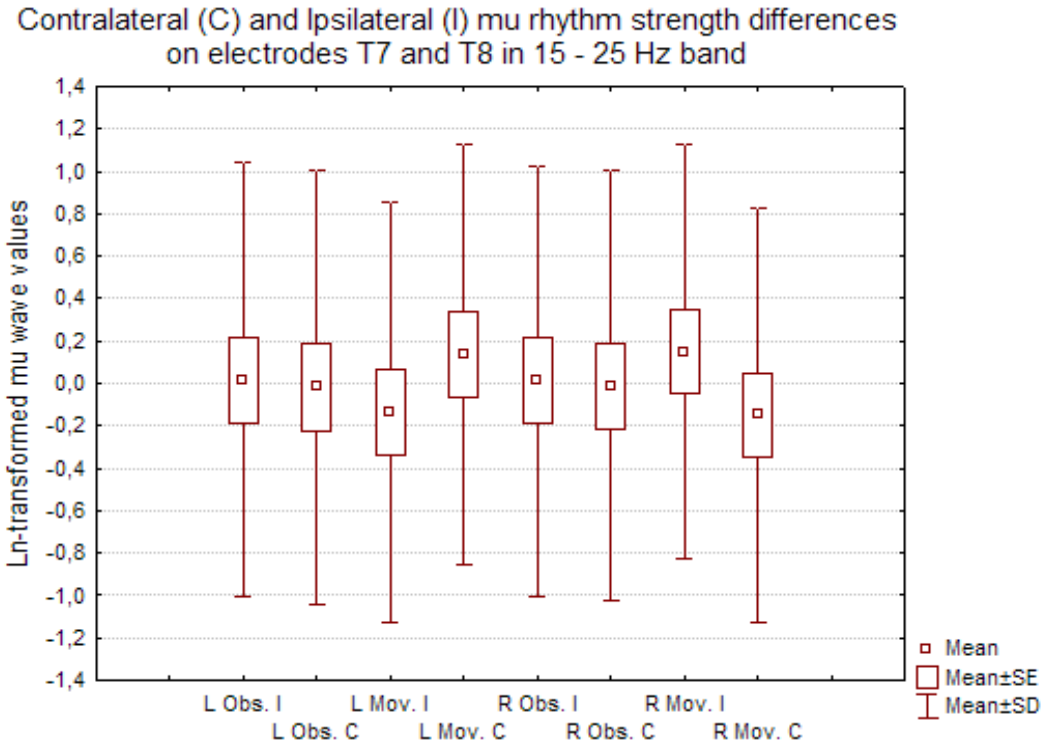


Figure 6.8: ***Contralateral and ipsilateral mu power differences on electrodes T7 and T8.***

In each of the graphs presented, the squares represent the mean log ratio of power in the frequency (8–13 Hz or 15-25 Hz) band during the observed (Obs.) and self (Mov.) motor movement conditions, for particular hand from ipsilateral and contralateral point of view. Rectangles represent the standard error of the mean. Error bars represent standard deviation of the mean.

T-tests comparing μ log ratio of ipsi- and contra-lateral views in both observation and movement conditions to zero showed statistically insignificant suppression from baseline in both hemispheres during both conditions. There was found only one exception case, which was statistically significant, within the right index finger self movement condition in 8 to 13 Hz bandpass over the C3 electrode (Figure 5.6: ipsilateral $t(24) = -4.43$, $p < 0.000175$; contralataeral $t(24) = 4.43$, $p < 0.000175$). Even though there are a slight patterns of hemispherical differences, these are too weak to be especially significant. These results are contradicting our expectation of significant hemispherical differences. However, from this finding we can assume that mirror neuron system activity is not hemispherically differentiated for distinguishing left and right movement. It is rather approximate to distinguish and understand any kind of movement.

Summary

We provided and overview of our master thesis focused on neuropsychological research of mirror neuron system (MNS) and the associated electrophysiological index called mu rhythm. The neurophysiological knowledge about MNS and mu rhythm properties was reviewed. We introduced and relatively in detail described core principles of electroencephalography and provided basic knowledge about different EEG rhythms. Based on the theory, we developed hypothesis examining the previous knowledge about motor resonance. Moreover we came up with another experimental question, testing properties of mu rhythm and therefore mirror neurons. For proving our claims and questions we designed an EEG psychological experiment.

In the data analysis section, we proposed a new principle of evaluating the power suppression or enhancement over the hemispheres, which was basically our second research question. The experiment proved our first claim, stating that mirror neuron activity modulates PSD of the specific EEG rhythm, that reflects their activity. The second question was confuted, because no statistically significant differences in hemispherical differences were proved. For this case we offered the explanation that mirror neuron system might not be hemispherically differentiated for distinguishing left and right body side movement. We think that it is rather approximate to distinguish and understand any kind of movement.

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