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Measurement of Event-related Potentials and Placebo

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Abstract: ERP is common abbreviation for event-related brain potentials, which are measured and used in clinical practice as well as in research practice. Contemporary studies of placebo effect are often based on functional neuro-magnetic resonance (fMRI), positron emission tomography (PET), and event related potentials (ERP). This paper considers an ERP instrumentation system used in experimental researches of placebo effect. This instrumentation system can be divided into four modules: electrodes and cables, conditioning module, digital measurement module, and PC module for stimulations, presentations, acquisition and data processing. The experimental oddball paradigm is supported by the software of the instrumentation.

Keywords: Biomedical measurements, Electroencephalography, Event-related potentials, Cognitive neuroscience, Placebo.

1 Introduction

Electroencephalography (EEG) is a neuro-physiological method frequently used in diagnostics for detecting neuro-physiological disorders of central nervous system (CNS). This method is based on measurement of variations of brain electrical activities. EEG signals are non-stationary and oscillatory signals, and their magnitudes are less than 300uV[1]. EEG signal frequency bands are usually divided into five areas: delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz) and gamma (≥30 Hz).

ERP is common abbreviation for event-related brain potentials. During the first researches of this potentials, only phenomena of specific electrical activities evoked by a stimulus were observed. These phenomena were named evoked potentials (EP), thus making distinction from spontaneous electrical activities of brain (the ones not evoked by external stimuli – and called just

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EEG potentials). After that, in 1969 Herb Vaughan used the name event-related potentials (ERP) for brain electrical activities he observed during intended movements and other psychological processes relatively independent from stimuli [2].

Nowadays, evoked potentials are standard diagnostic method in clinical tests and they are evoked by visual, audio, magnetic or electrical stimulation of sensory or motor pathways. This method procedures enables detection of defects in neural pathways before appearance of clinical signs of defects. For example, it is possible to detect defects of eye neural pathways before eyesight weakening or before changes at rear part of eye, analyzing records of visual evoked potentials.

Some of ERPs (P100, N100, P200, N200 and P300), which occur during simple tasks performed by subject, are used for detecting cognitive functions of respondents. Individual factors which influence parameters of the measured ERP include respondent age, awakeness level and active attention. Important factors for clinical application of ERP measurements in clinical conditions are stability and repeatability of recorded parameters.

Recent researches are often directed towards the application of ERP in Brain Computer Interface (BCI) systems which provide paralytic patients with basic communication capabilities so that they can communicate their intends to caregivers, operate switches, operate word processing programs, and move artificial limbs [3 – 5]. Also, ERP measurements found to be very useful for improving technological concepts intended for human-machine interaction [6].

Generally, it is very difficult to detect accurate record of ERP after presenting only one stimulus, because ERP is superposed with spontaneous EEG potential which has enough high intensity to cover ERP. The method for achieving accurate ERP record is based on presentation of many individual stimuli (tens or hundreds) followed by recording individual potential records, and finally ended by averaging all the individual records which results in more accurate measurement. This method rejects spontaneous EEG potentials, enabling clear extraction of ERP, and it is still most frequently used in practice, although there are new researches on alternative methods (so called parametric methods) based on one stimulus presentation. [7 – 8]

Scientific interest in the nature and mechanisms of placebo effects started 1955 with Henry Beecher and his influential paper “The powerful placebo” [9]. During 1978 Levine, Gordon and Fields demonstrated that the opioid antagonist naloxone could block placebo painkillers, suggesting that endogenous opioids are involved, and after that, there was no doubt that placebo effect has neurobiological base [10]. A serious of studies of placebo based on functional neuro-magnetic resonance (fMRI), positron emission tomography (PET), and event related potentials (ERP) are performed [11 – 14].

Continuing from [11 – 14] new researches of placebo effect, based on measurements of ERP, are performed in Laboratory of experimental psychology Novi Sad [15]. Those new researches were to answer the two questions. First question was: "Are there ERP components which are correlates of placebo effect when respondents are stimulated by thermal stimuli?". Second question was: "Are there ERP components which are correlates of anti-placebo effect when respondents are stimulated by thermal stimuli?" Answer on first question was positive and it gave a new confirmation of correlation between ERP and placebo. Answer on second question is still being formulated by current researches, but regardless of the final answer, this is the first research of correlation between ERP and anti-placebo effects.

This paper considers an ERP instrumentation system used during the ERP/placebo researches from [15]. To best knowledge of the authors, this is the first one-PC based ERP instrumentation system designed for ERP/placebo experiments, and the first ERP instrumentation system with thermal stimuli module integrated with PC. This novel system is designed according to specific requirements formulated after analyzing plan of the ERP/placebo researches.

2 Instrumentation

The instrumentation system can be divided into four blocks (Fig. 1). First block is consisted of cables and electrodes connected to respondent. Electrodes are Ag/AgCl (silver/silver/chloride) surface disk electrodes (9 mm diameter), because this type of electrodes have relatively low impedance and stable offset potential. Cables are shielded and its shield is connected to virtual ground of amplifier circuit, for this techniques reduce capacitive and inductive coupling of cables with the environmental electrical installations. The cables are not longer then 120cm for reducing inductive coupling, and they are thin and flexible for enabling more stable mechanical positioning and easier manipulation of cables.

The system is one-channel but scalable so it can be extended with more measurement channels. One channel requires one pair of cables and its electrodes can be positioned for both unipolar and bipolar measurements. There is also one DRL (Driven Right Leg) cable, included for additional reducing of capacitive coupling effect between respondent and environmental electrical installations. The name DRL is used because this is usual name for this technique since it was applied first time in electrocardiography measurements [16 – 17].

The amplifier, based on modular EEG design [18], is a three-stage amplifying and filtering circuit, implemented on one PCB (Printed Circuit Board). At the input, there is an electrostatic discharge protection circuit and passive low-pass filter for rejecting high frequencies (> 1 kHz). First amplifying stage is a preamplifier based on instrumentation amplifier INA114P for

obtaining high input impedance and high CMRR (Common Mode Rejection Ratio). From this stage, inverted common mode voltage is driven to the DRL output of the amplifier and further to DRL cable and location of subject. This technique is often used when it is necessary to increase CMRR, and in this amplifier's CMRR is 102dB.

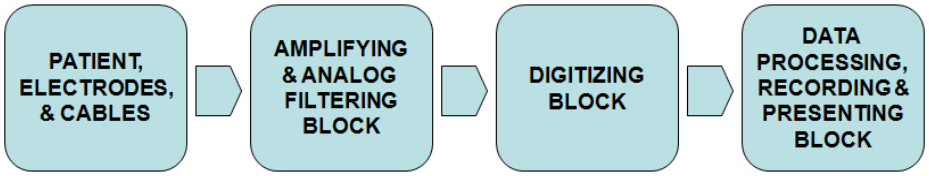


Fig. 1 – *Modular structure of the instrumentation system.*

Second amplifying stage has a central role for increasing the amplification gain. Also, before input and after output of this stage, there are high-pass filters with corner frequency of 0.15 Hz, thus providing rejection of offset voltage from amplifier input. Third stage has role of final amplification and also of anti-aliasing filtering. The supply of the amplifier is unipolar (5 V), and there is also virtual ground buffered from 2 V voltage source. This voltage source is implemented on another PCB (the one with microcontroller) and leaded to the amplifier PCB.

The role of digitizing block is digital measurement of the amplified voltage. This module is based on 8-bit microcontroller PIC18F4550 which has built-in A/D (analog-to-digital) converter with 10-bit resolution. Considering this resolution, the amplifier's amplification gain and A/D conversion reference voltage, the effective input resolution of the system is 0.5 μV.

PIC18F4550 is an USB V2.0 compliant microcontroller. It has three power managed modes: run (CPU on, peripherals on), idle (CPU off, peripherals on) and sleep (CPU off, peripherals off). The microcontroller can operate at four crystal modes, including high precision PLL for USB (there are two external clock modes, up to 48 MHz). Internal oscillator block enables 8 user-selectable frequencies, from 31 kHz to 8 MHz, which are also user-tunable to compensate for frequency drift. Secondary oscillator can be used by timer 1 at 32 kHz. Dual oscillator options allow microcontroller and USB module to run at different clock speeds, and fail-safe clock monitor allows for safe shutdown if any clock stops[19].

The Analog-to-Digital (A/D) converter module of the microcontroller has 13 inputs, and this module allows serial conversion of an analog input signal to a corresponding 10-bit digital number. The maximum recommended impedance for analog sources is 2.5 kΩ. The module has five registers [19]:

- A/D Result High Register (ADRESH),
- A/D Result Low Register (ADRESL),
- A/D Control Register 0 (ADCON0),
- A/D Control Register 1 (ADCON1),
- A/D Control Register 2 (ADCON2).

R/W-0	U-0	R/W-0	R/W-0	R/W-0	R/W-0	R/W-0	R/W-0
ADFM	—	ACQT2	ACQT1	ACQT0	ADCS2	ADCS1	ADCS0
bit 7							bit 0

Fig. 2 – *ADCON2: Register for controlling the microcontroller's A/D module* [19].

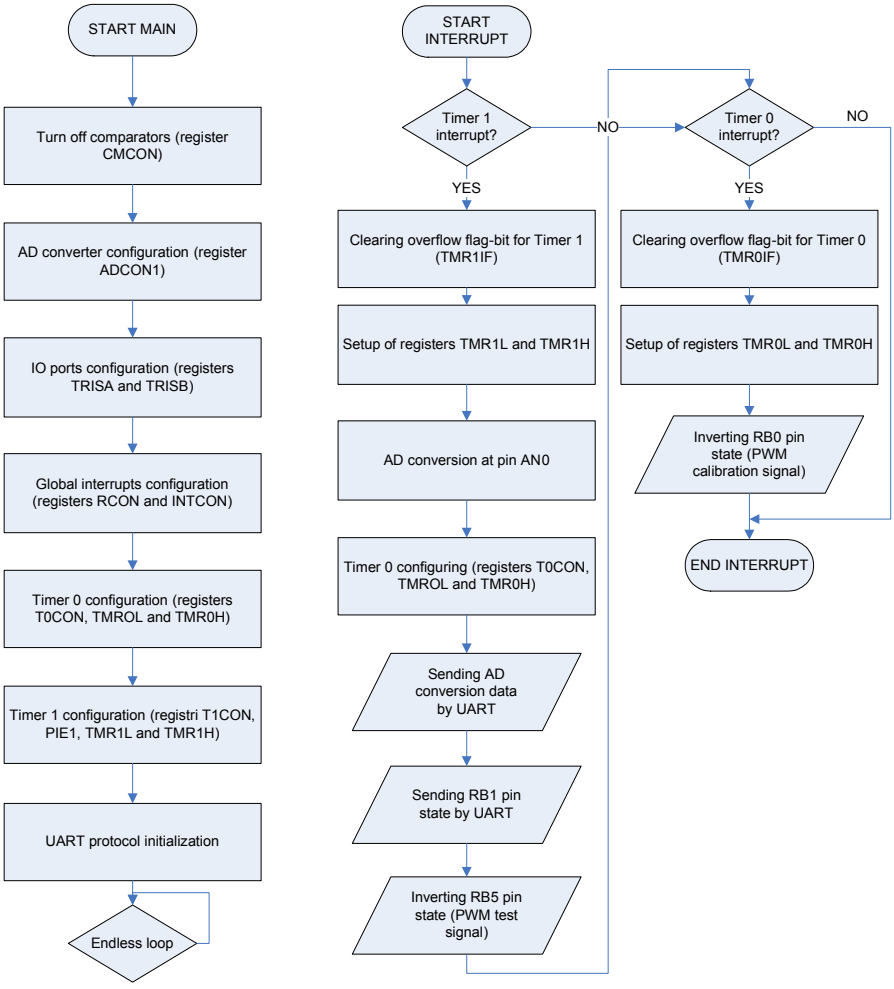


Fig. 3 – *Control flow diagram of the implemented firmware.*

The ADCON0 register, controls the operation of the A/D module, the ADCON1 register, configures the functions of the port pins, and the ADCON2 register (Fig. 2) configures the A/D clock source, programmed acquisition time and justification. ADCON2 register is significant, because of time management requests for ERP acquisition. Roles of its configuration bits are explained subsequently[19]:

In the implemented system, used microcontroller's clock is 8 MHz, so period of $T_{OSC} = 0.125$ μs is the basis for calculation if enough number of firmware instructions ($N_{ISR\text{-}INS}$) can be performed during interrupt service routine. 256 Hz is the most common sampling frequency for EEG (electro-encephalography) signal acquisition, so duration of interrupt service routine T_{ISR} should be less than 3.9 ms. Therefore, maximum $N_{ISR\text{-}INS}$ is 7778, allowing enough space inside interrupt service routine for implementing acquisitions, basic data processing, timers management and communication handling.

At Fig. 3 block diagram of the implemented firmware is presented. Output of the amplifier is lead to pin AN0, which is the input for the microcontroller's A/D module. Timer 0 is used for generating calibration signal, and timer 1 for performing acquisitions, basic data processing and communication handling. Chosen sampling frequency of A/D conversion is 256 Hz. RB0 pin is the output of PWM calibration signal, RB1 pin is the input for signal from reaction taster (which is controlled by respondent during specific cognitive tasks regarding recognition of stimuli), and RB5 is the controlling output intended for measurement by oscilloscope.

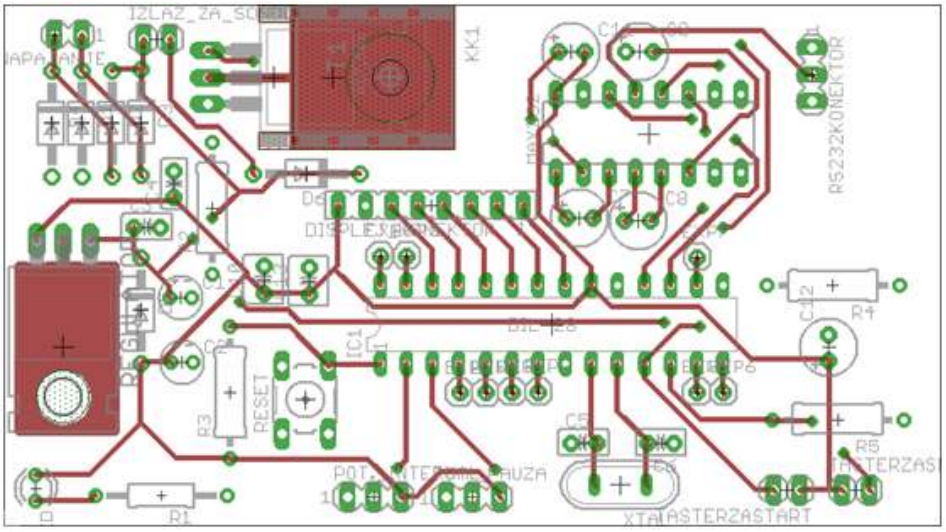


Fig. 4 – *PCB design of thermal stimuli module.*

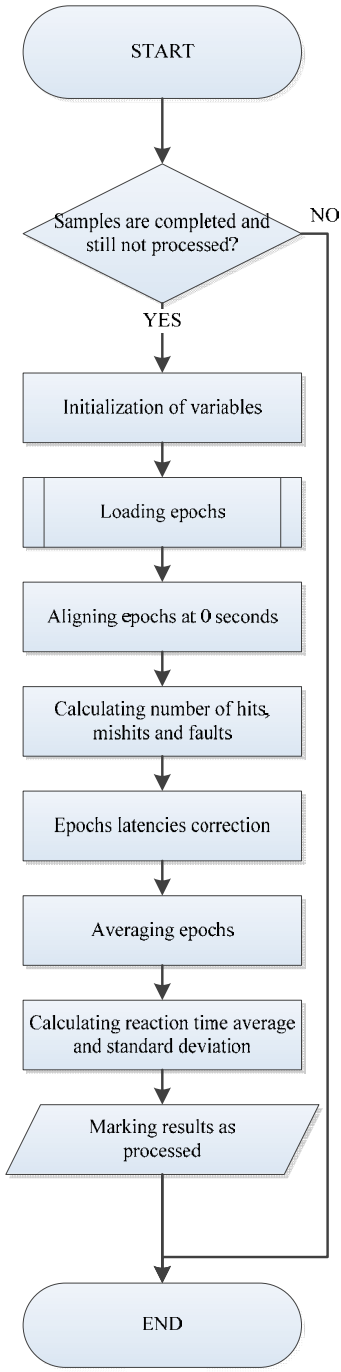


Fig. 5 – *Control flow diagram of the main software unit.*

Data are being sent to last block of the instrumentation system by UART protocol. Digital outputs of microcontroller (RC6/TX and RC7/RX) are used for this digital communication. Before connecting these outputs to MAX232 (digital chip for serial communication), there are digital optocouplers implemented for achieving necessary galvanic isolation of the system.

In the last block from Fig. 1, PC is used for hosting software application which triggers appropriate stimuli to subject, acquire and process measured data which results in ERP records. PC monitor is used for presenting visual stimuli to subject, and earphones are used for presenting sound stimuli to subject. Except those usual functionalities of PC and PC monitor in ERP instrumentation, PC is also integrated with thermal stimuli module and software application is extended by software module for control and synchronization of the thermal stimuli module.

The thermal stimuli module is based on thermal probe made of isolated resistors, and controlled by microcontroller PIC18F2550 and power transistor TIP31C. PCB design of the thermal stimuli module is presented at Fig. 4.

All firmware units are developed by compiler MicroC Pro for PIC, and all software units are developed in Delphi7 development environment. Control flow diagram of the main software application unit is given at Fig. 5.

3 Results

In this section, at first, the results regarding testing of the instrumentation system during its development are presented. After that, the results of the placebo related experiments [15], performed by those instrumentation system, are summarized and commented.

During the development of the instrumentation, various test scenarios were performed for verification of electrodes and cables, conditioning (amplifying and filtering) circuits (Fig. 6), digital measurement circuits, firmware and software. Thermal stimuli module's probe is calibrated for achieving 44^0C which is considered as the temperature of pain threshold.

After these verifications, the instrumentation is validated through various typical oddball paradigm experiments, for obtaining expected P300 responses. This experimental paradigm is a technique used in ERP research in which trains of stimuli that are usually auditory or visual are used to assess the neural reactions to unpredictable but recognizable events. In measurement session setup (Fig. 7a), main electrode was connected to subject's C_Z and referent electrode to mastoid bone. The subject was asked to react by button pressing incidences of target stimuli that are hidden as rare occurrences (deviant stimuli) amongst a series of more common stimuli (standard stimuli). At Fig. 7b, some of averaged latencies of ERP P300 components, obtained during these experiments, are presented.

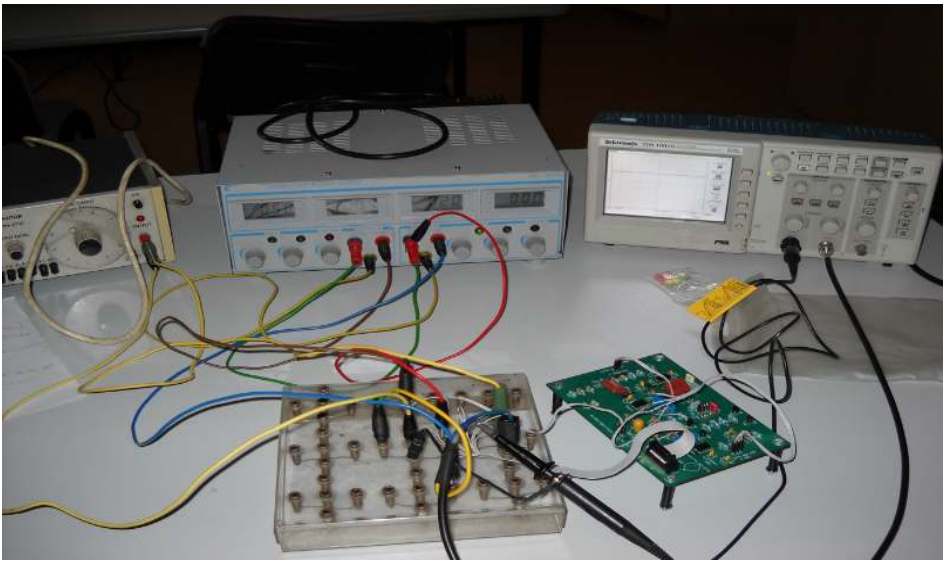
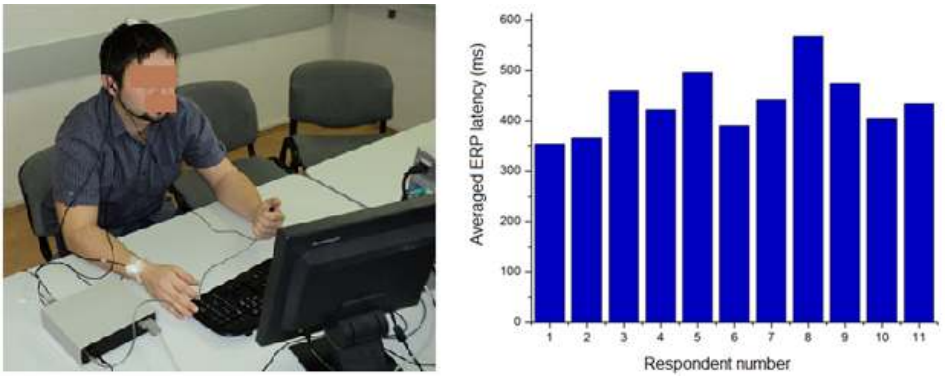


Fig. 6 – *Testing of an amplifying PCB during development of instrumentation.*



(a) (b)

Fig. 7 – (*a*) *Measurement session setup;* (*b*) *Measured ERP P300 latencies.*

In the last phase, the instrumentation is applied in placebo related experiments. In those experiments, the design of experiments enabled relations between measured parameters and placebo effects.

Experimental results for one respondent are shown at Fig. 8, and experimental oddball paradigm was followed. Vertical axe show ERP voltage in microseconds and horizontal axis show time in milliseconds. Green line is the average of standard epochs, and red line is the average of deviant epochs. In this experiment total number of epochs is 100, and total number of deviant epochs is 20.

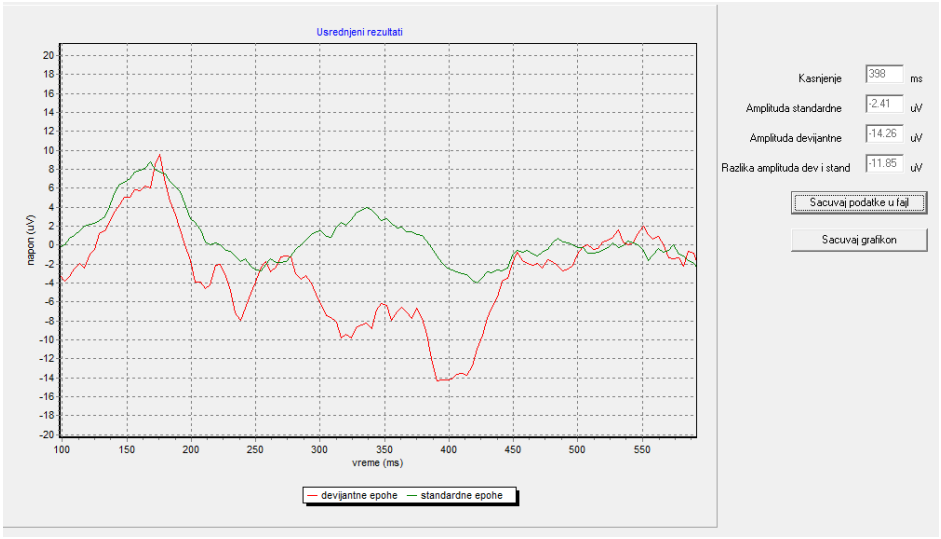


Fig. 8 – *Waveforms of acquainted and processed epoch samples. Red line waveform is calculated from samples acquainted during deviant stimuli presentation, and green line waveform is calculated from samples acquainted during standard stimuli presentation.*

Deviant stimuli were pseudo randomly presented by the algorithm based on Delphi built-in random functions and additional limitation of avoiding subsequential deviant stimuli. Moreover, there were at least two standard stimuli between subsequential deviant stimuli. This limitation was implemented for avoiding subject's expectations, which are supposed to be greater if there is no this limitation. Results were being saved into file system as CSV(comma separated values) files, but also graphically presented by the application.

Details on the design of these experiments, and details on findings of those placebo effect researches are presented in [15]. In the next paragraphs of this section we comment the main findings of those researches.

The first and foremost conclusion in [15] is that distinct neural correlates of analgesia/expectancy based on the different profiles of the ERP waveforms are registered. All three experimental situations with analgesia showed different ERP early effects in comparison to the condition with no analgesia. This result is also correspondent with the subjective judgment of the respondents. Interestingly, the results showed the placebo effect does not differ from the pharmaco-analgesia. These results are interpreted as early expectancies that any analgesic should produce some effect in comparison to no-analgesic condition.

In the subjective judgment data effects of "anti-placebo" were not observed. However, in the medium ERP effects prominently distinct "anti-placebo" and no-analgesia effects in comparison to the analgesia and placebo

effects were observed. However, neural correlates, or their unconsciousness responses revealed that the duration of the effects in the absence of analgesia and in the anti-placebo situation are identical. This result led the authors of [15] to the conclusion, that on the neural level, suggestion of "bad medicament" (similar to situation of no-medicament) caused such a metal activity which, figuratively, can be interpreted as a worry-response – "is this still going to hurt me?" This effect is the one which calls into a question doctors' recommendations (which contain negative connotation) of medicaments, as they could potentially and unconsciously diminish a valuable impact that the placebo could have during a treatment.

4 Conclusion

Contemporary studies of placebo are often based on functional neuro-magnetic resonance (fMRI), positron emission tomography (PET), and event related potentials (ERP). Following those studies, experiments related to researching placebo by measurements of ERP, were performed in Laboratory of experimental psychology Novi Sad.

This paper considers a novel ERP instrumentation system used in those experiments, and gives the results of testing the instrumentation system during development, and comments the results of applying the instrumentation system in [15]. To best knowledge of the authors, this is the first one-PC based ERP instrumentation system designed for ERP/placebo experiments, and the first ERP instrumentation system with thermal stimuli module integrated with PC.

The instrumentation system can be divided into four modules: electrodes and cables, conditioning module, digital measurement module, and PC module for presentations, acquisition and data processing. The system is one-channel but scalable so it can be extended with more measurement channels. Considering A/D converter's resolution, the amplifier's amplification gain and A/D conversion reference voltage, the effective input resolution of the system is 0.5 μV. Chosen sampling frequency of A/D conversion is 256 Hz.

PC is used for hosting software application which triggers appropriate stimuli to subject, acquire and process measured data which results in ERP records. PC monitor is used for presenting visual stimuli to subject, and earphones are used for presenting sound stimuli to subject. Except those usual roles of PC in ERP instrumentation, PC is also integrated with thermal stimuli module and software application is extended by software module for control and synchronization of the thermal stimuli module.

It is important to emphasize the researches from [15], because they are the application of the presented instrumentation system, and those researches and the instrumentation system together are an example of successful interdisciplinary effort which lead to the novelties in two directions – development of new

instrumentation system and new insights into placebo effects. Researches from [15]: 1) gave a new confirmation of correlation between ERP and placebo, and 2) opened a new and interesting area of studying correlation between ERP and anti-placebo effects.

For these experimental researches purpose, in the instrumentation system oddball paradigm model is applied, for obtaining expected P300 responses. The oddball paradigm is a technique used in ERP research in which trains of stimuli that are usually auditory or visual are used to assess the neural reactions to unpredictable but recognizable events. In measurement session setup, main electrode was connected to subject's C_Z and referent electrode to mastoid bone. The subject was asked to react by button pressing incidences of target stimuli that are hidden as rare occurrences (deviant stimuli) amongst a series of more common stimuli (standard stimuli).

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