

Received: 11.10.2017

Accepted: 28.12.2017

A – Study Design
B – Data Collection
C – Statistical Analysis
D – Data Interpretation
E – Manuscript Preparation
F – Literature Search
G – Funds Collection

THE EEG EXAMINATION, TOGETHER WITH P300 POTENTIAL AS A METHOD FOR CAUSATIVE DIFFERENTIATION IN PATIENTS WITH A GENETIC AND ENVIRONMENTAL CONDITIONING TO ALCOHOL ADDICTION

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SUMMARY

The electrophysiological characteristics of alcoholics, such as the P300 amplitude of the Event-Related Potential (ERP), are related to high risk in their offspring, and are considered to be biological endophenotypes of a predisposition to develop alcohol use disorders. Contemporary knowledge justifies early diagnoses of the alcohol risk degree among adolescents, or even children, including their families, involving an examination of the P300 potential as an endophenotype, prior to achievement of an age of alcohol initiation. The results of such research approaches may be of importance not only cognitively, but also of prophylactically, in the early recognition of increased susceptibility to alcohol. The simplicity and non-invasiveness, and the exceptionally low costs of the methods described, should obtain for the present as well as in the future, a wider examination, one potentially even mass scope of in character and usefulness. The knowledge of such an endophenotype and genetically-related susceptibility, in the individual, family, and social dimension and transmission, and in the rearing of children and adolescents, could protect – not just individuals – but many from entering into the route of addiction, which is most frequently the effect of acting unaware and with negative life consequences, both generational and transgenerational for generations to come.

Keywords: alcoholism, evoked potentials P300, endophenotype

INTRODUCTION

Family alcoholism is the customarily, traditionally, and commonly adopted term for alcoholism occurring in people belonging to one family and their relatives. The justification for the genesis of this term, contemporarily more rarely applied, was supplemented by the results of studies concerning transgenerational transmission of those genes recognized to increase the predisposition for alcohol alcohol in humans (Dick et al., 2007; Bell et al., 2016). At present, the studies of the causes of alcoholism are biases towards a recognition of both the genetic conditioning, and the effect of the widely understood environment, as early as in children and adolescents, in combination with the effect of family, upbringing, the effect of the school education environment, and that of peers' and society around (Devor & Cloniger, 1989; Moss et al., 2007; Rangaswamy & Porjesz, 2008; Salvatore et al., 2015; Cservenka, 2016). The precise differentiation of the contribution of causative agents in the etiology of alcohol addiction, together with an introduction of the typology of the disease, caused difficulties and required research approaches integrated in nature, which combined the results of studies on the use of neurophysiological methods, experimental psychology, psychiatry and sociology, as well as genetics and epigenetics.

The utility of neurophysiological markers in the study of alcoholism

Electroencephalographic methods gained a new cognitive, as well as application importance in the results of studies of the etiology of alcohol use disorders, including evoked potentials (Sutton et al., 1965; Handy, 2005). Event-Related Potentials (ERP) – are changes in the form of waves in electroencephalographic recordings when the examined persons are exposed to the effect of a sudden stimulus defined as an *event*. The range of stimuli contemporarily applied in studies, previously called an event, covers their broad spectrum, including those of auditory, visual, motor stimuli, sensory stimulation, as well as the performance of cognitive tasks in psychological tests (Begleiter et al., 1984; Handy, 2005). The first evaluations and calling the stimulus an 'event' resulted from the occurrence of a new situation in the soundproof environment of the electroencephalographic registration, changed by an acoustic signal, the effect of which revealed itself in resting electroencephalographic registration (EEG). Thus, it was justifiable to ascribe the term 'event' to acoustic or visual signals and consequences causatively related with the triggering of its electroencephalographic registration, together with its conscious perception by the examined person (Sutton et al., 1965). It is noteworthy that the term 'event' in the context of three components of the discussed phenomenon – acoustic signal or light signal and its electroencephalographic registration and conscious perception – justified the ascribing to the phenomenon of the term 'event', which in conditions of experimental stimulation and the effect of natural stimuli, in a neurophysiological sense, was the triggering by a stimulus, and at the same time, by an event, remaining for longer or permanently in the form of a memory trace (Sutton et al., 1967).

Despite the detection long ago of the evoked P300 potential and the explanation of its role and function in cognitive and memory processes, attempts to satisfactorily explain the genesis and neuronal mechanisms conditioning the triggering and function of evoked potentials remained unsuccessful for many years (Sutton et al., 1965). Studies conducted in recent years enhanced the recognition of the genesis and the importance of these potentials in neurophysiological and neuropsychological cognitive processes. These studies even managed to precisely identify the frontal-to-temporal and parietal lobe activation patterns of these potentials in experimental conditions on the conscious perception of stimuli, inducing evoked potentials, as well as mechanisms of neurohormonal activity in the process of their development (Huang et al., 2015; Salvatore et al., 2015).

The registration and study of the evoked potentials is a non-invasive and safe method of recognizing psychophysiological cerebral processes. According to the electromagnetic approach, evoked potentials are the presentation of the accumulated value of postsynaptic potentials, many thousands or even millions of cerebral cortex pyramidal neurons, synchronically active at the same time. Evoked potentials are divided into early, also firstly in time occurring and defined as 'sensory', or exogenous. They occur within the first 100 milliseconds from the stimulus induced stimulation, which most often features physical stimulation. In turn, potentials generated after the elapse of 100 milliseconds from the effect of triggering a stimulus, are defined as 'cognitive', or endogenous, because they simultaneously present the cognitive value of the stimulus, i.e., evaluating closer its physiopsychological quality of meaning [Sur & Sinha, 2010; Huang et al., 2015).

The above-presented time division of evoked potentials ascribes a numerical value to the type of potential, which expresses the time of wave occurrence, counted from the time of triggering by a stimulus, defined as an event in the classic terminology. P50 and N100 potentials are classified as early potentials; the first of these being a positive wave, occurring between milliseconds 40 and 75, whereas N100 is a negative wave observed within the interval of approximately 100 milliseconds. Classified into the second group of evoked potentials are: positive potential P200, negative N200, negative N300, and positive P300, as well as the negative potential N400 and the positive P600. In order to simplify the descriptive presentation of individual potentials, null numbers accompanying letter-number symbols are frequently omitted, leaving the first number of the value expressing milliseconds. It should be added that the negative potentials N200, usually registered as N2, were additionally divided into 3 time and functional subgroups: N2a, N2b and N2c (Sur & Sinha, 2010).

The basic values and characteristics of evoked potentials are defined by their positive or negative polarity with respect to the output, or zero value, labelled with the letter P (positive), or N (negative). The subsequent characteristics are the amplitude of individual wave components, measured in microvolts and their duration in milliseconds. Subsequent values are the latency time, counted in milliseconds, which covers the time of the conduction of neuronal and interneuronal stimulation, from receptors triggered by the stimulus (event) to pyramidal neurons of

the cerebral cortex and the initiation of the analyzed potential. The final analysis covers the cortico-cranial presentation with maximum amplitude values and mapping its gradients with respect to the network of the distributed cranial electrodes conducting potentials [Handy, 2005; Kropotov, 2009; Pąchalska, Kaczmarek and Kropotov, 2014).

GENESIS OF EVOKED POTENTIALS

It should be mentioned that despite many studies into evoked potentials there is still a lack of full data concerning the genesis and the neurophysiological mechanisms of generating neuronal transmission by cortical pyramidal neuron activity. This limits a more comprehensive research approach with the use of evoked potentials in the domain of developmental and behavioural psychology, diagnostic and pharmacotherapeutic procedures, as well as in psychiatric behavioural therapies.

Within the scope of the genesis of evoked potentials, the generation of P300 was confirmed within the cerebral cortex frontal lobe, with the co-effect of the frontal lobe cortex with hippocampus and with the temporal-parietal cortex [Knight, 1996; Kirino et al., 2000]. Positive potential P300 occurs in the area of the parietal-central cortex, if the examined person consciously receives the test task stimuli. In turn, the use of the functional magnetic resonance (fMRI), together with the triggering of evoked potentials (ERP), enabled the confirmation of the existence of a pattern in the activity of the cortical evoked potential, based on the co-effect of the frontal lobe cortex with the temporal lobe cortex and parietal lobe cortex. Confirmation of the above-mentioned results was additionally provided by invasive, i.e., direct registration of evoked potentials from the cerebral cortex in humans, proving that the temporal-parietal junction, together with some structures of the hippocampus and parietal lobe and frontal lobe cortex, may be considered as structures responsible for the final stage of neuronal location and the generation of P300 potential in humans (Smith & Halgren, 1989; Tarkka et al., 1995).

The hitherto results of studies concerning the generation of evoked potentials and their neurotransmission within the structures of the central nervous system confirmed the engagement and activity of the dopaminergic and noradrenergic systems, both in the sub-cortical structures and in the cerebral cortex (Pineda, 1995; Frodl-Bauch et al., 1999).

Evoked potentials P300, located within the frontal cerebral cortex, present the dopaminergic mechanism of neurotransmission; while when registered in the parieto-temporal sphere – noradrenergic transmission (Nieuwenhuis et al., 2005). Studies of patients with Parkinson's disease, the pathomechanism of which consists in the deficit of dopamine in the subcortical centres (substantia nigra), provided additional evidence confirming the functional variation of the two neuronal systems. A deficit of dopamine was also found in the processes of evoking P300 potentials, located within the pyramidal cells of the frontal cerebral cortex, the amplitude of which was closely related with the concentration and synthesis of dopamine. The application of sulpiride, which is the blocker of the dopaminergic

receptors D2, increased low values of dopamine concentration, related with an increase in the P300 amplitude, was analyzed within the frontal cerebral cortex (Takeshita & Ogura, 1994). It should be emphasized that a more detailed description of the function and neuropsychological and pathological importance of potentials evoked by various types of stimuli, exceeds the framework of this work.

EVOKED POTENTIALS AS ELECTROPHYSIOLOGICAL ENDOPHENOTYPES

In introducing the concept of an endophenotype into psychopathology, it was defined as the factor which is possible to be measured quantitatively, existing, but invisible to the unaided eye, and located within the neuronal network between the distant genotype, and the phenotype presented as a change of function or characteristics typical of a disease or status, with new, previously inexistent properties (Gottesman & Goud, 2003). The criteria in effect concerning the consideration of the characteristics as an endophenotype are: (1) cause-effect relationship with the disease, (2) heritability of these characteristics, (3) its presence, irrespective of the presence of pathological symptoms, (4) occurrence of such characteristics in other patients in the family, and (5) occurrence of an increased frequency in relatives, despite the absence of the symptoms of the disease (Salvatore et al., 2015).

While providing a precise definition of endophenotype, it should be added that this concept is neither the same as a biomarker, nor an indirect phenotype, because these 2 concepts are not determinants of the necessary gene activity, have no precise localization, and also do not satisfy the established criteria of a qualification valid for phenotype. Despite this, many researchers apply these concepts interchangeably, due to the connection of some characteristics, as well as convergent goals in their presentation (Porjesz et al., 2005).

Evoked potentials (ERP), while remaining under genetic regulation and control, have been considered as electrophysiological phenotypes, presenting their dependence on genes with respect to the function of the neuronal structures of the cerebral cortex and the central nervous system. It was found that children from families burdened with alcohol use disorder, are characterized by a developmentally impaired morphology of the structures of the cerebral cortex and cerebellum, responsible for decision-making and control executive functions. Also, in the processes of adolescent development, evaluated by the characteristics of electrophysiological phenotypes, developmental delay was revealed within the subcortical nuclei with accompanying deficit and cognitive dysfunctions, and deviations in behaviours. Compared to the control values of families and offspring free from alcohol use disorder, the affective and behavioural reactions of the children of parents with alcohol addiction syndrome are characterized by the suppression and balance deficit, making wrong decisions in research tests and life, as well as an impaired capability for making peer social relations (Be-

gleiter et al., 1984; Euser et al., 2012; Kamarajan et al., 2015). Neurophysiological dysfunctions and developmental disorders in the neuronal structures, with accompanying cognitive difficulties and anti-social behaviours in children from families burdened with alcohol use disorder, simultaneously indicate an increased risk of addiction to alcohol in adolescent development, as well as to the necessity to undertake more effective prophylactic and protective actions in the process of education, social development and upbringing (Cservenka & Nagel, 2012; Hill & O'Brien, 2015).

P300 potential

The P300 response, which is a positive potential triggered by a light or acoustic signal of a high frequency of 600 Hz or 1600 Hz, and registered in an electroencephalographic recording within the range of delta and theta waves after the elapse of 300 milliseconds from the effect of a light or acoustic signal, has been considered as a phenotype with an increased susceptibility to alcohol and alcohol use disorder when its amplitude was decreased. The value of the amplitude of P300 potential remains under genetic control, and its decreased value is genetically and family connected with the existing sensitivity to alcohol and tendency towards its abuse (O'Donnell et al., 1987). It is noteworthy that the amplitude of P300 has been considered as a phenotype presenting the presence of the genes of susceptibility, and even metaphorically considered as a 'electrophysiological signature' identifying this characteristics with an active alcohol use disorder, or unrevealed susceptibility to its development (Frodl-Bauch et al., 1999; Dick et al., 2006).

Evoked P300 potentials are connected with the neurochemical activity of the nerve cells and type of neuronal transmission. They are a resultant of the accumulated values of postsynaptic stimulatory and inhibitory potentials determined by 3 groups of neurotransmitters, represented by glutamate, gamma-aminobutyric acid (GABA) and acetylcholine (ACH) (Jones et al., 2006). The contribution of the dopaminergic, serotonergic and noradrenergic systems in the shaping of P300 is indirect, and considerably lower than that of glutamate and acetylcholine. The role and participation of gamma-aminobutyric acid (GABA) consists in the modulatory effect of inhibition, and the stimulatory effects of glutamate. In turn, the effect of acetylcholine as a neurotransmitter consists in a synergistic effect with glutamate, and antagonist effect with respect to gamma-aminobutyric acid (GABA) (Apergis-Schoute et al., 2007; Philpot et al., 2009; Hodgkinson et al., 2010; Hill et al., 2013).

The decisive effect on the value of P300 is caused by the allelic version of the *CHRM2* gene encoding the synthesis of muscarinic type 2 receptor proteins, which is connected with the effect of acetylcholine in pyramidal neurons of the cerebral cortex. Cholinergic muscarinic receptor, the protein of which is encoded by the *CHRM2* gene variant located on chromosome 7, is characterized by an increased reactivity to acetylcholine, and its presence is related with alcoholism and depressive disorders. It should also be mentioned that the scope of these

disorders, related with a decreased amplitude of P300, covers a decreased inhibition syndrome, abuse of psychoactive substances, disturbed and antisocial behaviours, Attention-Deficit Disorder (ADD) and the Attention-Deficit Hyperactivity Disorder (ADHD). In the EEG records of persons addicted to alcohol who are carriers of this gene, stimulation of muscarinic acetylcholine receptors M2 also changes the patterns of registration of slow waves delta and theta, with the effect of reduced values of induced oscillations, which have also been considered as an endophenotype (Wang et al., 2004; Jones et al., 2004; Jones et al., 2006; Porjesz & Rangaswamy, 2007; Rangaswamy & Porjesz, 2008).

Data from international reports indicating the presence of genetic factors related to a susceptibility for alcohol use disorder, and the lack of such studies in Poland, justify undertaking the evaluation of P300 potential connections, as an endophenotype, with alcohol use disorder in a population of young and adult alcoholics.

CONCLUSIONS

The EEG examination, together with P300, may be the method for the causative differentiation of already existing alcoholism in young people, as well as in the application of an effective therapy, differing in management and methods valid in the treatment of patients with a genetic and environmental conditioning of alcohol addiction. Current research also justifies early diagnoses of the degree of alcoholism risk among adolescents, or even children, including their families, consisting in the examination of P300 potential as an endophenotype, prior to the achievement of the age of alcohol initiation. The results of such research approaches may be not only of cognitive, but also of prophylactic importance, in the early recognition of increased susceptibility to alcohol. Thus, the examination of P300 may be an opportunity for a more comprehensive diagnosis; however, non-invasive and screening diagnostics of the genetic conditioning of an increased susceptibility to alcohol in children and adolescents; therefore, at the age before alcohol initiation. The simplicity and non-invasiveness, and the low costs of the EEG and P300 methods, should result now and in the future, in a wider, or even mass scope application of such an examination. It should be emphasized that knowledge concerning the possession of such an endophenotype and genetically-related susceptibility, in the individual, family, and social dimension and transmission, and in the upbringing of children and adolescents, could protect – not just one individual – but even many people, from entering into the route of addiction, which is most frequently the effect of acting unaware, with negative life consequences, both generational and transgenerational for generations to come.

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