SUB-COMPONENTS OF EVENT RELATED POTENTIALS (ERP) ASSOCIATED WITH POLYMORPHISMS IN GLUTAMATE, GABA AND DOPAMINE NEUROTRANSMITTER RECEPTORS

by

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ABSTRACT

SUB-COMPONENTS OF EVENT RELATED POTENTIALS (ERP) ASSOCIATED WITH POLYMORPHISMS IN GLUTAMATE, GABA AND DOPAMINE NEUROTRANSMITTER RECEPTORS

Event related potentials (ERPs) reflect perceptual and cognitive processes and therefore provide an electrophysiological window onto brain function during cognition. P300 component as well as spectral components of ERPs are highly heritable. Potential candidates for the genetic determinants of ERPs are genes encoding several most important neurotransmitter receptors. In this study, we aimed to identify associations of functional polymorphisms of genes encoding glutamate receptor 2A subunit, (NMDAR2A), GABA receptor gamma-2 subunit (GABRG2) and dopamine receptor D2 subunit (DRD2) with auditory ERPs. EEG recordings and genetic analysis of 72 Turkish male healthy volunteers were performed in this study. Groups were formed according to their polymorphism types for each of the three neurotransmitter receptors. Three cognitive paradigms were designed to generate auditory ERPs. ERP recordings of each polymorphic group were analyzed in the time domain by measuring P300 amplitude and latency, and furthermore, in the time-frequency domain by decomposition of ERP signals via using wavelet transform with analysis of variance (ANOVA). Results provide evidence of strong effect of GABRG2 polymorphism with ERP characteristics both in time domain and in time-frequency domain. The effects of NMDAR2A and DRD2 polymorphisms are less significant on P300 wave. However, time-frequency decomposition of ERP data showed other effects could be observed in specific frequency bands of all three polymorphisms that were not reflected in the time-domain representation of the data. The results of this study show that extended analyses on the correlations of genetic differences among normal population on electrophysiological parameters may extend our view on the genetic basis of cognitive activities.

Keywords: Event Related Potential (ERP), P300, Polymorphism, Receptor, NMDAR2A, GABRG2, DRD2, Time-Frequency Domain Analysis, Wavelet Transform.

ÖZET

GLUTAMAT, GABA VE DOPAMİN NÖROTRANSMİTTER RESEPTÖRLERİNDEKİ POLİMORFİZMLERLE BAĞLANTILI OLAYA İLİŞKİN POTANSİYELLER'İN (OİP) ALT BİLEŞENLERİ

Duyumsal ve bilişsel süreçleri yansıtan olaya ilişkin potansiyeller (OİP), beynin yüksek bilişsel süreçlerde nasıl çalıştığına dair elekrofizyolojiksel ışık tutmaktadır. OİP'lerin spektral bileşenleri ve P300 alt bileşeni bireyler arası değişkenliğe sahip olduğu kadar yüksek oranda kalıtsal olma özelliğine de sahiptir. OİP'lerin bu kalısallık özelliğine atfedilen adaylar, bazı önemli neurotransmitter reseptörlerini kodlayan genlerdir. Bu çalışmada, glutamat reseptör 2A alt birimi (NMDAR2A), GABA reseptör gamma-2 alt birimi (GABRG2), ve dopamin reseptör D2 alt birimini kodlayan genlerdeki fonksiyonel polimorfizmlerin işitsel OİP'lerle olan ilişkileri incelendi. Bunun için 72 sağlıklı erkek gönüllünün EEG kayıtları alındı ve genetik analizleri yapıldı. İstatistiksel çalışma için, her üç polimorfizm tipine göre ayrı gruplar oluşturuldu. Üç farklı bilişsel paradigma uygulanarak işitsel OİP'ler elde edildi. ERP kayıtları P300 latans ve genliği ölçülmek suretiyle zaman alanında, ve daha sonra dalgacık dönüşümü yöntemi kullanılarak zaman-frekans alanında varyans analizi (ANOVA) ile incelendi. Alınan sonuçlar, GABRG2 polimorfizminin hem zaman hem de zaman-frekans alanında OİP'ler üzerinde önemli bir etkisi olduğunu gösterdi. Diğer iki polimorfizmin P300 üzerindeki etkilerinin ise daha az olduğu tespit edildi. Bununla beraber, spesifik frekans bantlarının zaman-frekans alanındaki analizleri, bu üç polimorfizmin OİP'ler üzerinde sadece zaman alanındaki analizlerinde farkedilemeyen daha başka etkileri de olduğunu gösterdi. Bu çalışma, normal populasyondaki genetik farklılıkların elektrofizyolojik parametrelerle ilişkilerini göstererek, bilişsel aktivitelerin genetik temelleri üzerindeki bilgilerimizi genişletmiştir.

Anahtar Sözcükler: Olaya İlişkin Potansiyel (OİP), P300, Polimorfizm, Reseptör, NMDAR2A, GABRG2, DRD2, Zaman Alanı, Zaman-Frekans Alanı, Dalgacık Dönüşümü.

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5HT2A	Serotonin 2A Receptor
ACC	Anterior Cingulate Cortex
ANOVA	Analysis of Variance
CHRNA7	Acetylcholine Alpha 7 Nicotinic Receptor
CNS	Central Nervous System
CNV	Contingent Negative Variation
COMT	Catechol-O-Methyltransferase
DAT	Dopamine Transporter
dNTPs	Deoxynucleotidetriphosphates
DRD2	Dopamine Receptor Type 2
DRD4	Dopamine Receptor Type 4
EPSP	Excitatory Postsynaptic Potentials
ERPs	Event Related Potentials
GABA	Gamma Aminobutyric Acid
GABRG2	GABA type A receptor gamma-2 subunit
IPSP	Inhibitory Postsynaptic Potentials
MMN	Mismatch Negativity
NMDA	N-methyl-D-aspartate
NMDAR2A	NMDA Receptor 2A subunit
PCR	Polymerase Chain Reaction
RFLPs	Restriction Fragment Length Polymorphisms
S1	Prior Warning Stimulus
S2	Stimulus
SAP	Shrimp Alkaline Phophatase
SNPs	Single Nucleotide Polymorphisms
SPL	Sound Pressure Level
STRs	Short Tandem Repeats
VNTR	Variable Number of Tandem Repeats
WT	Wavelet Transform

1. INTRODUCTION

1.1 Background

Event Related Potentials (ERP) were first described by Sutton [1]. ERP's are voltage changes recorded from the human scalp that are time-locked to a sensory, motor, or cognitive process and therefore provide an electrophysiological window onto brain function during cognition.

It is now widely accepted that ERPs result from intracortical currents induced by excitatory and inhibitory postsynaptic potentials (EPSPs, IPSPs), which are triggered by the release of neurotransmitters. Therefore, ERPs reflect postsynaptic effects of neurotransmitters like glutamate and GABA and indirect modulating effects from neuromodulators like acetylcholine, noradrenaline, dopamine or serotonin [2].

Not only the human electroencephalogram (EEG) and ERPs are dynamic and dependent on the physiological state of the brain, but also, they show trait characteristics and are genetically determined with intraclass correlations in monozygotic twins of between 0,5-0,9 [3]. Family studies suggest that several genetic factors contribute to the phenotypic variance of the human EEG/ERPs because EEG/ERP similarity rapidly declines with increasing genetic distance between family members [4]. Potential candidates for the genetic determinants of EEG/ERPs are genes encoding several most important neurotransmitter receptors that were mentioned and their transporters.

Since its amplitude and latency vary systematically as a function of task parameters, P300 has been widely used in psychophysiological studies of information processing. P300 amplitude appears to be diminished in a variety of neuropsychiatric disorders, most notably schizophrenia [5], alcoholism [6, 7]. Because the same deficit has also been found in clinically nonaffected relatives of these individuals with alcoholism and schizophrenia, reduced P300 components are often implicated in genetically transmitted neurobiological

liability to these disorders and considered to be a neurophysiological marker of genetic risk [8].

Conventional ERP analyses, as amplitude and latency measurement in averaged potentials is limited because ERP peaks are assumed descriptors of the unique neural activities, but ERPs are not homogeneous phenomena. For example, the complex component structure of P300 was revealed in several studies in terms of wave shape and scalp topography, source of generation, and functional reactivity [9, 10]. In addition, analysis of EEG / ERP in the frequency domain showed that different spontaneous or evoked EEG rhythms are functionally related to information processing and behavior [11, 12, and 13].

Traditional analysis techniques in the time and space domains do not adequately represent the dynamical time and space structure of the ERP signals. However, the wavelet transform (WT) assumes that different functional neuronal ensembles of the brain show different time-frequency characteristics in specific time windows.

Wavelet representation provides precise measurement of when and to what degree transient functional components occur in the ERP signal, and when and how the frequency content of a waveform changes over time. Therefore, WT is an efficient time-frequency decomposition method for transient ERP waveforms [12, 13].

1.2 Objectives

Objective of this study is to identify the effects of receptor polymorphisms of three major neurotransmitter (Glutamate, GABA and Dopamine) systems on the gross electrical activity of the brain by using event related potentials. Further, we aimed to identify specific ERP time-frequency components in relation with three major neurotransmitter system polymorphisms.

In this study our aim is to find possible associations between N-methyl-D-aspartate receptor 2A subunit (NR2A or GRIN2A), gamma-aminobutyric acid A subtype γ2 subunit

(GABAA γ 2), and dopamine D2 receptor (DRD2) polymorphisms and auditory ERPs. After identification of functional polymorphisms, the way they produced effects on ERPs will be further examined through the time-frequency analysis of ERP data in a population of 73 subjects.

To date, several studies have investigated the effects of polymorphisms on ERPs in normal individuals of different ethnic origins, but this study is distinct by studying a new population, investigating different polymorphisms, and using techniques like WT.

1.3 Outline of the Study

This study consists of seven chapters. The first chapter reviews a brief background and objectives of our study. Chapter 2 explains Event Related Potentials, heritability and influences of neurotransmitter systems, closer insight to the polymorphisms that were studied, overview of genetic and electrophysiological methods used in this study and the used paradigm types. Chapter 3 covers the information about both genetical and electrophysiological methods. In the Chapter 4, results of both genetical and electrophysiological analyses are given. The results are discussed in the Chapter 5. Conclusions are given in the sixth chapter of this study. The last chapter contains references.

2. EVENT RELATED POTENTIALS AND GENETIC INFLUENCES

2.1 Introduction

2.1.1 Event Related Potentials and P300

EEG recordings measure overall brain activity and arousal; however, when a person encounters a stimulus, a minute fluctuation occurs in the EEG. If, a few seconds later, the person encounters the same stimulus, a very similar minute fluctuation occurs. If this process of repeated stimulation is continued, a distinct pattern develops in the EEG as a response to the stimulus.

From the EEG recording, one can extract the distinct patterns of fluctuation. These distinct patterns are known as event-related potentials (ERP), and as the name suggests, ERPs are recordings of the neuroelectrical responses to events (stimulus). ERPs are obtained from EEG recordings by averaging the distinct patterns of the EEG to create a singular waveform.

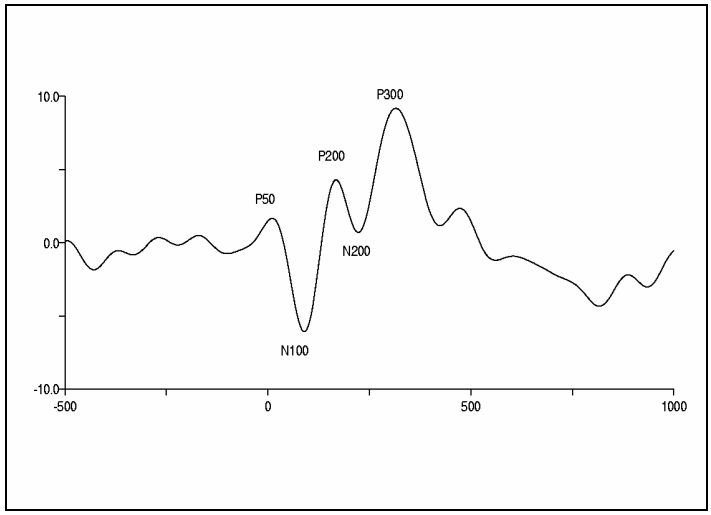


Figure 2.1 Basic ERP Components

The ERP is generally too small to be detected in the ongoing EEG and requires computer averaging over many stimulus presentations to achieve adequate signal/noise ratios. There are mainly four components of ERP; early brainstem responses (waves I-IV), the midlatency components (No, Po, Na, Pa, Nb), the "vertex potential" waves (P1, N1, P2), and task-related endogenous components (Nd, N2, P300 and slow wave) (Figure 2.1). All features of ERP can be measured by determining corresponding wave amplitude (in μV) and latency (in ms).

More than 40 years have passed since Sutton et al. first described positive event-related potential, commonly referred as P300 [1].

P300 can be obtained by the "oddball" paradigm in which two different stimuli are presented in a random order, and the subject is required to discriminate between an infrequent target stimulus and frequent nontarget or standard stimulus by responding covertly or overtly to the target. P300, also called as "P3b" is elicited with the target stimulus, occurs at about 300ms after the stimulus onset and is maximum over parietal scalp regions [14].

One of the most prominent theories regarding the cognitive basis of the P300 is that it indexes updating of working memory [15]. Stimuli enter the system and a memory comparison process is engaged whether the current stimulus is the same as the previous one or not. If a new stimulus is processed, cognitive system engages attentional mechanisms to "update" the neural representation of the stimulus context and P300 is elicited which can be affected by a number of task conditions such as the probability of the stimulus and task difficulty [15].

Several studies have tried to identify neural origins of P300, but precise neural origins have not been identified, yet. While, recordings from depth electrodes from neurological patients [16] suggested hippocampal areas in medial temporal lobe in the generation of P300, scalp recordings on individuals after temporal lobectomy [17] suggested hippocampal formation does not contribute directly to the generation of P300.

Three stimulus paradigm is a modification of the oddball task in which "distractor" stimuli are inserted into the sequence of target and standard stimuli. These novel stimuli can be dog barks, bell rings in an auditory task, and color forms or different letters in a

visual task. This novel and unexpected stimuli within the usual "oddball" task elicit "novelty P300" which is called as "P3a" [18] and is more frontally distributed compared to P3b. Since P3a exhibits frontal/central scalp distribution, and relatively short peak latency, it is thought to reflect frontal lobe function. P3a was named as that way because of its latency being relatively shorter than P3b, although it was identified after P3b, chronologically. ERP studies on humans with frontal lobe lesions have demonstrated that P3a requires frontal lobe attentional mechanisms [19]. Frontal lobe activity is not the only neural source for generation of P3a, hippocampal formation [10] as well as anterior cingulate [20] is associated with ERP processing of novelty.

As a summary, neuroelectric events that underlie P300 generation stem from the interaction between frontal lobe and hippocampal/temporal-parietal functions [10].

2.1.2 Individual Differences and Heritability of ERP

The P300 component is most commonly considered to be a manifestation of the central nervous system (CNS) activity that reflects attention to incoming stimulus information when memory representation of environment is updated [9].

Since its amplitude and latency vary systematically as a function of task parameters, i.e. attention [21, 22], stimulus probability, stimulus relevance, and the amount of processing resources available, such as in single versus dual tasks [23], with stimulus complexity [24], the quality of selection [25], and attention allocation [26], P300 has been widely used in psychophysiological studies of information processing.

P300 amplitude appears to be diminished in a variety of neuropsychiatric disorders, most notably in schizophrenia [5], alcoholism [6, 7]. Because the same deficit has also been found in clinically nonaffected relatives of these individuals with alcoholism and schizophrenia, reduced P300 components are often implicated in genetically transmitted neurobiological liability to these disorders and considered to be a neurophysiological marker of genetic risk [8, 27], P300 amplitude shows test-retest correlation of 0.6 [28, 29].

Twin and family studies have demonstrated significant genetic influences on P300, with heritability estimates ranging from 30% to 70%, depending on task conditions like modality and complexity [30, 31, 32, 33, and 34].

EEG spectral power measures have been demonstrated as highly heritable [35, 36, and 37]. Human P300 potential waveform is determined by the superimposition of EEG oscillatory responses at different frequency ranges [38] delta and theta frequency band evoked oscillations are known as primary constituents of P300 component [39, 40]. Specifically, delta and theta oscillations, taken together, explained most of the variance in the P300 component [37], considering that, in this study, in addition to time domain analysis of ERPs, also examining the responses generated at delta, theta, alpha, beta, and gamma frequencies by using time-frequency analysis was aimed.

2.1.3 ERP and Influence of Neurotransmitters

ERPs result from intracortical currents induced by excitatory and inhibitory postsynaptic potentials (EPSPs, IPSPs), which are triggered by the release of neurotransmitters. It is claimed that the ERP waveform corresponds to the summation of individual neuronal action potentials [41]. Experimental work and simulation studies rather suggests that scalp recorded ERPs primarily depend on synchronized synaptic discharges, mainly on apical dendrites of distributed cortical neurons which are in part under thalamic control [42].

ERPs reflect postsynaptic effects of neurotransmitters like glutamate and GABA and indirect modulating effects from neuromodulators like acetylcholine, noradrenalin, dopamine or serotonin [2]. Studies on the effects of neurotransmitter receptor agonists/antagonists have shown that they affect event related potentials both changing amplitude and latency of P300 (Figure 2.2).

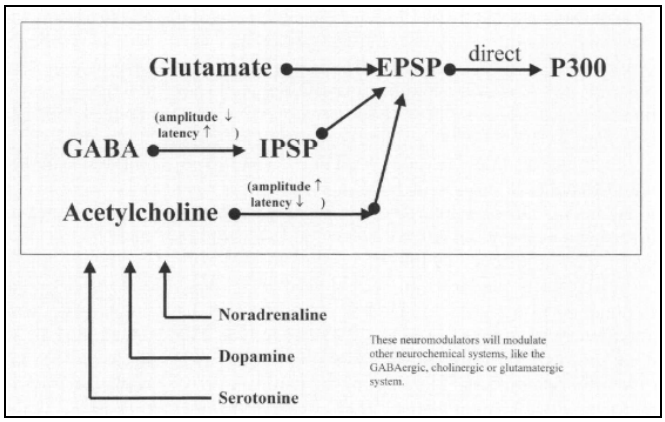


Figure 2.2 The effects of major neurotransmitters on ERPs [2].

2.1.3.1 Glutamatergic Influences

The glutamatergic system is the most important excitatory neurotransmitter system and plays an important role in the electrogenesis of P300 potentials [43]. The role of the glutamate receptors, especially NMDA receptor activity on ERPs has been shown in animal experiments with intracranial recordings [44]. Also, the mismatch negativity (MMN), a late cognitive ERP, was reduced after administration of NMDA channel blocker. The parallel functional similarities between P300 and NMDA receptor, and the direct excitatory postsynaptic effects of glutamate made glutamatergic system the primary candidate for the generation of P300 [2].

2.1.3.2 GABAergic Influences

GABA is the most important inhibitory neurotransmitter and inhibitory postsynaptic potentials (IPSPs) triggered by GABAergic effects could be responsible for late ERPs such as P300. There are two hypotheses on the way it affects ERPs. First, it may act by indirect influences on GABAergic IPSPs such that it could generate positive potentials by reducing the negativity of some cortical brain regions. Second, it may act by direct effects on P300, such that GABAergic influences decrease EPSPs [45] and reduce P300. The results indicate that GABA may have a significant effect on ERPs most probably by indirect effects influencing glutamatergic EPSPs.

2.1.3.3 Dopaminergic Influences

The results of the studies on dopaminergic system on the generation of ERPs are somehow contradictory. Stanzione et. al, suggested that dopaminergic neurotransmission has a physiological role in the generation of P300 in patients with Parkinson's disease whereas the results in non-demented patients concerning P300 latency and amplitude were inconsistent [46]. Another study in which dopaminergic fibers were damaged by toxic lesions speaks against an important role of the dopaminergic system in P300 generation [2].

2.1.3.4 Other Neurotransmitter Systems

The cholinergic neurotransmitter system has indirectly modulating effects in widely distributed neuronal networks and acetylcholine has been found to be involved in the generation of P300 [47]. Memory performance and P300 amplitude is increased after the administration of cholinergic substances and is reduced after the administration of anticholinergic substances [48]. In summary, cholinergic system is also an important neuromodulator of the ERPs.

There are inconsistent findings in humans on adrenergic effects on P300 [49, 50]. However, animal studies suggest that the noradrenergic system has a role in P300 generation [51]. A model proposes that noradrenaline has an inhibitory control on GABAergic activity. Serotonergic system effects are needed further investigations. Studies have shown that serotonergic drugs only when used with anticholinergic drugs have effects on P300 latency [52]. As a summary, dopamine, noradrenaline and serotonin seems to have effects on P300 by modulating GABAergic, cholinergic and glutamatergic systems.

2.1.4 Polymorphisms and Cognitive Processes

To date, many studies tried to show how genetic differences among individuals could be linked to individual differences in neuromodulators and thus, cognitive and

behavioral activities. The most used strategy was to identify candidate genes and their corresponding functional polymorphisms.

An allele is a variant of a gene, they are alternative forms of a genetic locus; a single allele for each locus is inherited separately from each parent. Using allelic frequencies, a gene can be defined as polymorphic that is having two or more alleles in substantial frequency. If the frequency of the most common allele is 0.99 or greater this allele can be considered as monomorphic, otherwise it is polymorphic. Single nucleotide polymorphisms (SNPs) are variants in a single nucleotide at a specific location in genomic sequence that may or may not have functional consequences for the protein. Around 6 million SNPs are believed to characterize the genetic variability among populations and it is likely that minority of these variations will be functional in nature. Most of these functional polymorphisms will affect either the regulation of transcription, via so called promoter polymorphisms, the organization of transcription, via so called splice site polymorphisms, or variations in the protein coding sequences, which themselves can result in changes that range from synonymous, in which the function of the protein is unchanged, to non-synonymous or missense, in which the function of the protein may be altered [53]. Restriction fragment length polymorphisms (RFLPs) are single base pair alterations in the DNA sequence at a particular locus on the genome. They are called as RFLPs because the alteration in sequence is such that the recognition site for a particular restriction enzyme is added or eliminated. Another type is variable number of tandem repeats (VNTR). VNTRs are polymorphisms where a particular sequence is repeated at a locus numerous times. Short tandem repeats (STRs) are repetitive sequences that are usually less than 20 base pairs.

In the search for genetic links between single genes and cognition, some of the candidate genes that used in these studies are alpha 7 nicotinic receptor (CHRNA7), brain derived neurotrophic factor (BDNF), catechol-O-methyltransferase (COMT), dopamine receptor type 4 (DRD4), dopamine transporter (DAT), monoamine oxidase A (MAOA), and serotonin 2A receptor (5HT2A) [53]. DRD4, DAT1, COMT and MAOA that are among the most widely studied and repeatedly associated with various psychiatric disorders like schizophrenia, attention deficit/hyperactivity disorder (ADHD), Alzheimer's Disease where attention is found to be disrupted.

Fosella et. al., has found modest associations of several polymorphisms with the efficiency of executive attention but not with overall performance measures such as reaction time [54]. Genetic variations in MAOA and DRD4 genes have been associated with behavioral performance in a study using attention network test [55]. Although sample size is small for a polymorphism study, Fan, have shown greater conflict effect in anterior cingulate cortex (ACC) by using event related functional magnetic resonance imaging (fMRI) techniques. Leonard, reported evidence in humans that a variety of polymorphisms in the promoter region of CHRNA7, which have been shown to affect CHRNA7 transcription, were associated with a failure to inhibit the P50 auditory evoked potential response in both normal controls and schizophrenic subjects [56]. This finding suggests that variations in the CHRNA7 promoter sequence affect the abundance of CHNRA7 protein and thereby affect nicotinic processing in human hippocampus and related cortices, which in turn affects processing in this cognitive paradigm. A variant in the COMT gene appears to account for significant variance in prefrontal cognitive function. SNP in exon 4 results an aminoacid substitution of Valine (Val) for Methionine (Met). These two different aminoacids have different enzymatic activity and ability to catabolize dopamine. Furthermore, individuals with two Met alleles performed best and individuals with no Met alleles performed worst in a Wisconsin Card Sorting Test in a sample of schizophrenics and controls [57, 58].

Several studies investigated possible involvements of neurotransmitter transporter polymorphisms in amyotrophic lateral sclerosis (ALS) [59] and epilepsy [60, 61]. Genetic variation of DAT gene has been associated with idiopathic absence epilepsy, while giving no evidence for an allelic association of EAAT2 gene or regulatory promoter polymorphism of the SERT gene [60].

Schizophrenia is of special interest in genetic studies. Dopaminergic, serotonergic, GABAergic and glutamatergic neurotransmitter systems have been proposed to be involved in the pathology of schizophrenia [62]. Changes in dopaminergic system [63], changes in the interactions between GABAergic, dopaminergic, and glutamatergic systems [64] changes, and altered interaction between GABAergic and serotonergic neurons have been indicated in the pathology of schizophrenia [65]. Schizophrenia is highly heritable disease [66], and there are many susceptible genes. That is the reason why polymorphisms, which might affect neurotransmitter systems that are thought to involve in the pathogenesis

of schizophrenia, have been studied densely in both disease studies and cognitive studies which try to identify individual cognitive and behavioral differences.

2.2 NMDAR2A, GABRG2 and DRD2 Polymorphisms

2.2.1 NMDAR2A Polymorphism

The NMDA receptors are composed of a common glutamate receptor, an ionotrophic NMDA1 (GRIN1) subunit and one of four NMDA2 (GRIN2) subunits (GRIN2A-GRIN2D) combined in an undetermined ratio to make up the receptor complex. Functional and non-functional polymorphisms were identified in NMDAR1 [67, 68], NMDAR2A [69], NMDAR2B [70, 71, 72] subunits in several studies. GRIN1 G101C promoter polymorphism has been associated with schizophrenia [73] in Italian sample. GRIN2A promoter GT repeat polymorphism has been associated with schizophrenia in two following studies in Japanese samples [69, 74], but not in Chinese [75]. Association of the repeat polymorphism was also tested for bipolar affective disorder [76] in which larger sample size is needed. GRIN2B, GRIK2, GRIK3, GRIK4, GRIK5 and GRIN2D are the other candidate polymorphisms that were studied but still needed replication. [77] has proposed combined effects of GRIN1 and GRIN2B in the etiology of schizophrenia. Most of the studies that investigated possible associations of NMDA receptors with alcohol dependence failed to show any evidence [78, 79].

The fact that expression of GRIN2A starts around puberty [80] and this corresponds to the period of onset of schizophrenia made GRIN2A a compelling candidate among all other NMDA receptors and subunits, and therefore became a candidate for molecular analysis. The GRIN2A gene consists of 14 exons spanning at least 131 kb of the 16p13.3 region (Figure 2.3)

Itokawa et. al., have found a variable $(GT)_n$ repeat polymorphism in the 5'-regulatory region of GRIN2A and showed this repeat sequence repressed transcriptional

activity in a length dependent manner, such that the longer the repeat, the greater the repression of promoter activity [69]. Associations of this polymorphism, with schizophrenia have been studied and gave strong evidence for hypoglutamatergic hypothesis in the etiology of this disease [69, 74].

In this study polymorphic $(GT)_n$ repeat in the 5' regulatory region of the NMDAR2A gene on 16p13.3, is selected because this polymorphism was found to be both functional and has been associated with a disease like schizophrenia which is known to be both heritable and causes cognitive deficits.

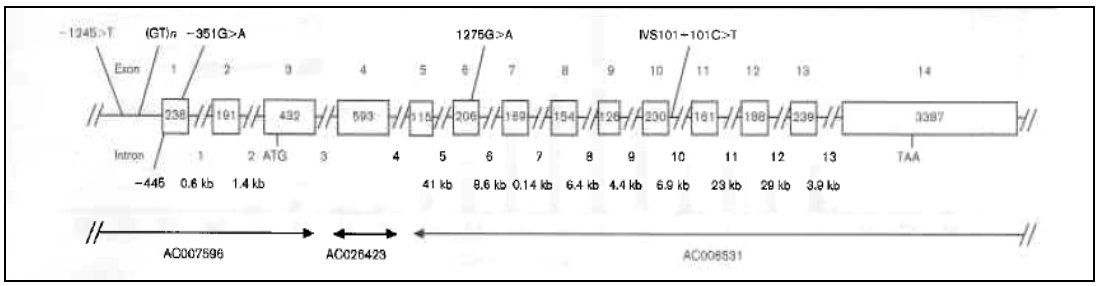


Figure 2.3 Genomic structure and location of polymorphic sites of GRIN2A gene [69].

2.2.2 GABRG2 Polymorphism

GABA is a major inhibitory neurotransmitter in the brain. This ligand-gated chloride ion channel is modulated by barbiturates, benzodiazepines, and ethanol. Two major types of receptors for GABA have been described which have been classified according to their pharmacological properties [81]. $GABA_A$ receptors are ionotrophic, they act through an intrinsic ion channel and antagonised by convulsant alkaloid bicuculline; $GABA_B$ receptors are insensitive to bicuculline and are metabotrophic, which act through G proteins.

An alternation of GABAergic neurotransmission has been implicated in epileptogenesis [82], mood disorder [83], and alcohol dependence.

A potential candidate for the genetic determinants of EEG/ERPs are genes encoding subunits of the gamma-amino butyric acid $(GABA)_A$ receptor. Activation of $GABA_A$-receptors initiates a current flow of chloride ions that peaks within 5 ms and lasts for less than 100ms [84]. This $GABA_A$-receptor mediated action is short lasting and may play a crucial role in generating synchronous EEG oscillations via local circuit neurons that mediate intrinsic inhibition [85].

Molecular cloning studies have revealed the existence of 13 mammalian subunits (α1-6, β1-3, γ1-3, and δ) of ionotropic $GABA_A$ receptors that can assemble in pentameric combinations. After Cheng, et al, have identified that G→A nucleotide exchange at nucleotide position 3145 in the intronic sequence of the $GABA_A$ γ2 (GABRG2) gene creates an artificial NciI RFLP site [86], this RFLP provided a useful DNA marker for allelic association or linkage analyses of the role of $GABA_A$ receptors in predisposition of alcoholism or other neuropsychiatric disorders [87, 88]. $GABA_A$ γ2, (GABRG2) subunit gene is assigned to chromosomal segment 5q33. The attention that is paid on this polymorphism is not surprising since its close proximity to a putative regulatory element that controls the splicing of the alternative eight amino acid exon of the γ_{2L} subunit. Alternative form of the γ2 subunit, γ2L, which contains an additionally spliced 8-aminoacid exon with a phosphorylation site may mediate action of ethanol [89].

In a study in which 95 psychiatry healthy subjects of German descent were physiologically phenotyped with four EEG/ERP parameters: EEG activation, anterior and posterior EEG synchronization, and event related activity (N100/P200-complex), they have found strong genotypic association with $GABA_A$ receptor γ2 subunit polymorphism and the event related N100/P200 complex, while no genotypic association was found for the another polymorphism in the $GABA_A$ receptor β subunit [90].

2.2.3 Dopamine D2 receptor (DRD2) Polymorphism

Dopamine neurons are involved in initiation and execution of movement, Parkinson's disease and schizophrenia are thought to be manifestations of imbalances

between dopamine receptors and dopamine [91]. Dopamine receptors are divided into two; D1 and D2 subtypes according to their G-protein coupling and ligand specificities.

The D2 dopamine receptor is a G protein-coupled receptor located on postsynaptic dopaminergic neurons that is centrally involved in reward-mediating mesocorticolimbic pathways. The D2 dopamine receptors have been of particular interest due to their affinity for antipsychotic drugs [92].

Arinami, et al have found two polymorphisms, the A-241G and -141C Ins/Del, by examination of 259 bp in the 5'-flanking region and 249 bp of exon 1 of DRD2 [93] and found -141C Ins/Del polymorphism is functional such that; reporter constructs containing the Del allele cloned into a luciferase reporter plasmid drove 21% and 43% expression compared with the Ins allele. Furthermore, in the same study an association with this functional polymorphism and schizophrenia was demonstrated [93]. The frequency of the Del allele was significantly decreased in the Japanese schizophrenic subjects compared with controls. The author suggested that; given that excess dopaminergic activity leads to the psychosis symptoms of schizophrenia, the low frequency of the Del allele seen in schizophrenics might contribute to disease psychosis.

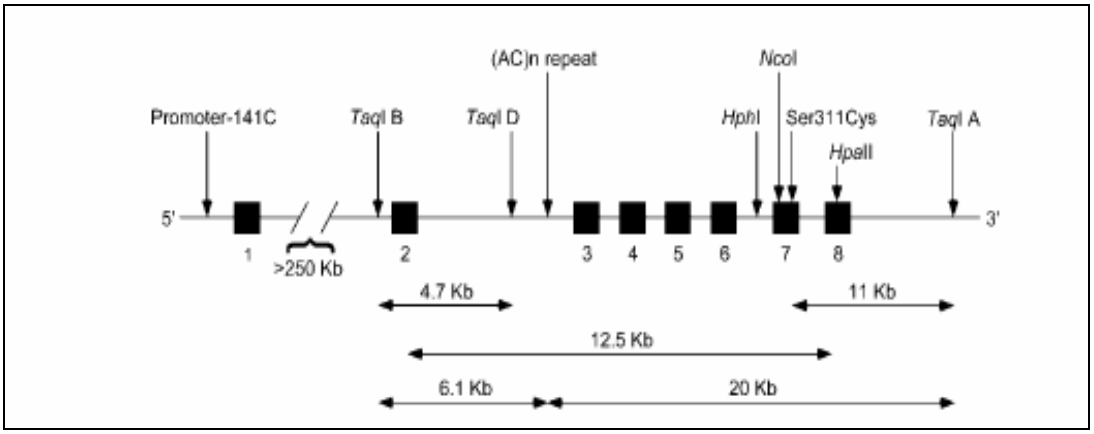


Figure 2.4 DRD2 gene and polymorphisms [95].

Because of its involvement in the reinforcing effects of rewarding behavior [94, 95], dopaminergic neurotransmission in addictions and effects of functional polymorphisms have been extensively investigated. Dopamine is released in response to a variety of drugs, including nicotine, cocaine and cannabis [96]. Although there are no confirmed

associations between dopamine receptor genes and addictions [97], -141C Ins/Del promoter polymorphism warrants further investigation because of its functional effects. Chinese population has been screened for associations of -141 C Ins/Del polymorphism with alcoholism and heroin abuse [98, 99] investigated both -141 C Ins/Del and VNTR polymorphism in the 3' UTR in DAT genes but could not associate these both polymorphisms with alcoholism and it was concluded that both DRD2 promoter region and DAT gene do not play a significant role in conferring vulnerability to alcoholism [98]. In a subsequent study, Li et al, could not find any significant difference of frequency of this allele between controls and heroin addicts [99]. What they could only find is an association between DRD2 promoter polymorphism and nasal inhalators but not with injectors, which still does not give any clear evidence of the involvement of DRD2 gene in the heroin abuse.

Since dopaminergic function is an important determinant for P300 components [100], several studies have investigated the association between DRD2 polymorphism and P300. However, in Chinese population, any significant difference regarding P300 components generated in response to auditory paradigms between the genotype groups of the TaqI polymorphism in normal young females [101] and –141 Ins/Del polymorphism in depressive patients [102] could not be demonstrated so far. This failure of any association attributed to the paradigms used in the study or the population that chose to be investigated by the authors, thus, a study demonstrated that association of the P300 component was more significant using visual paradigms in comparison to auditory paradigms for DRD2 receptor Taq1 polymorphism. Although, the results are contradictory, polymorphic region is located at the promoter region and could generate individual differences in EEG data in different ethnic groups with different experimental designs.

2.3 Overview of Genetic Analyses

2.3.1 Polymerase Chain Reaction

After Polymerase Chain Reaction (PCR) has been invented, a number of processes from DNA fingerprinting and mapping the human genome has become possible[103, 104]. PCR provides millions of copies of a defined region on the DNA molecule. Short pieces of DNA called primers hybridize to DNA sequences on either side which define the boundaries of the target section, and cause initiation of DNA synthesis in the presence of DNA polymerase enzyme and deoxynucleotidetriphosphates (dNTPs). dNTPs are named according to bases they contain. DNA has 4 different bases: Adenine, Guanine, Cytosine and Thymine which serve as the letters of genetical alphabet. The technique was made possible by the discovery of Taq polymerase, the DNA polymerase that is used by the bacterium Thermus Aquaticus that lives in hot springs. This DNA polymerase is stable at the high temperatures needed to separate the strands of DNA before each round of replication to perform the amplification. Figure 2.3.1 illustrates steps of PCR cycles. The reaction can be summarized as: First, the mixture is heated to denature the sides of the double- stranded DNA and then cooled to allow the primers to find and bind to their complementary sequences on the separated strands and the polymerase to extend the primers into new complementary strands. Repeated heating and cooling cycles multiply the target DNA exponentially, since each new double strand separates to become two templates for further synthesis. In about 1 hour, 20 PCR cycles can amplify the target by a million fold.

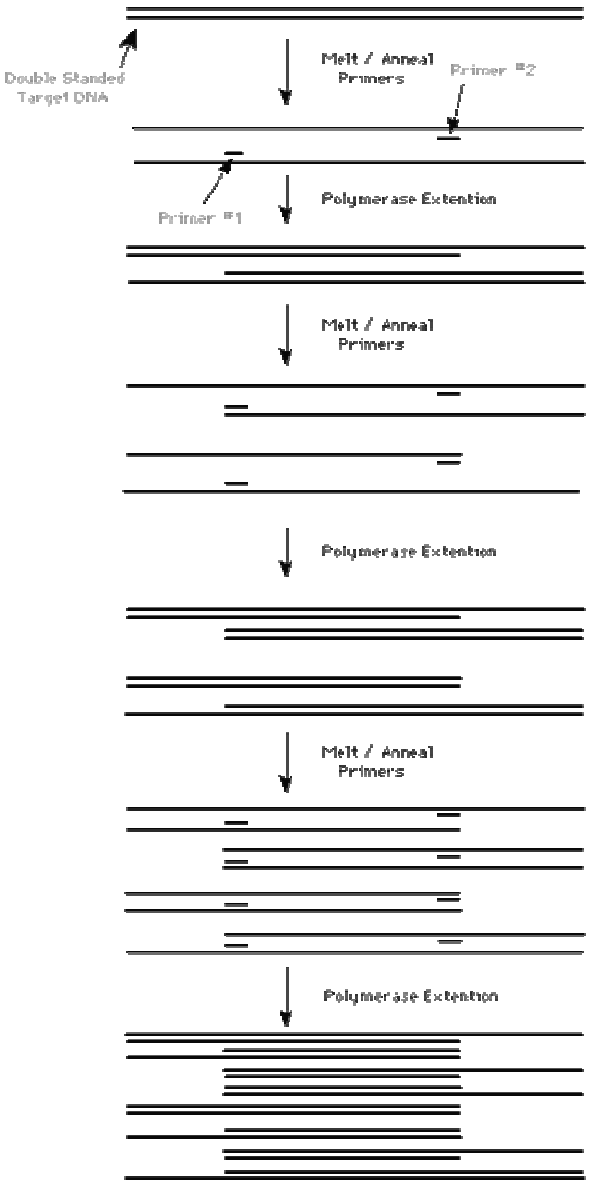
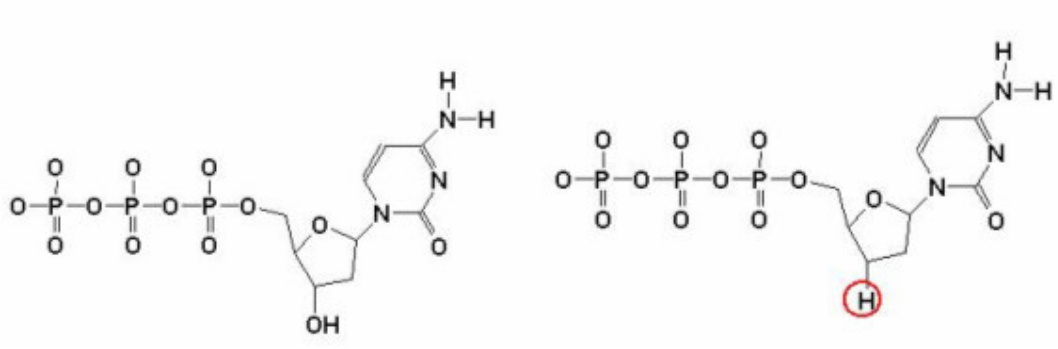


Figure 2.5 The first three cycles of Polymerase Chain Reaction (http://faculty.plattsburgh.edu).

2.3.2 Purification (Exo-SAP Reaction)

Before sequencing reaction, residual primers and dNTPs must be removed from the PCR products. For that reason Exo-SAP reaction was performed before sequencing

reaction. Shrimp alkaline phophatase (SAP) dephosphorylates residual deoxynucleotides preventing them from participating in sequencing reaction and ExonucleaseI degrades excess single stranded DNA (primers) from sample.



(a) **(b)**

Figure 2.6 Chemical structure of dNTP (a) and ddNTP (b)**.**

2.3.3 Sequencing Reaction

Sequencing reaction can be thought of as a special type of PCR reaction in which, primer is hybridized to the DNA, which was already amplified, this time, in the presence of chain terminators called dideoxynucleotides (ddNTPs). Each of four ddNTPs (ddA, ddC, ddG and ddT) are different from dNTPs (Figure 2.6.a) in that, they lack 3'-hydroxyl group (Figure 2.6.b) so that they can not form a phosphodiester bond with the following dNTP. Whenever a ddNTP is incorporated into a growing DNA chain, it stops. In the sanger method, DNA synthesis reaction is carried out in four different tubes containing only one of each ddNTPs, and radiolabeled dATPs. The result is a series of fragments of different lengths in each tube. If all reaction mixtures are electrophoresed in lanes next to each other in a high-resolution polyacrylamide gel, horizontal bands, which correspond to the sequence of the fragment starting from the bottom to the top, can be observed with autoradiography. Automated DNA sequencing method is based on Sanger's chain termination method [105]. In automated DNA sequencing, each of four dideoxynucleotides (ddA, ddC, ddG and ddT) is tagged with a different fluorescent molecule, so each will emit a different color fluorescence when exited by light (Figure 2.7). In this study an automated

sequencing method that is adapted to ABI Prism (Applied Biosystems) genetic analyzer was used because it is faster and more sensitive compared to manual sequencing methods.

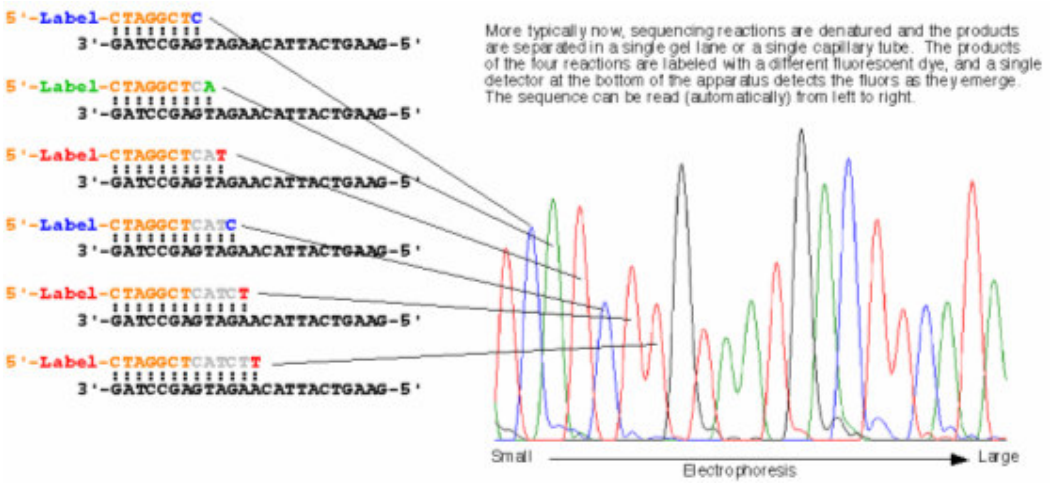


Figure 2.7 Sequencing method. Four different labelled ddNTPs are detected by the apparatus as they emerge (http://medstat.med.utah.edu/block2/biochem/Formosa/Figures).

2.3.4 Minisequencing Reaction

In minisequencing technique, primer extension reaction is performed, starting from a specific primer that is designed to anneal directly adjacent to the polymorphism or mutation site, by the incorporation of a single fluorescent dideoxynucleotide (ddNTP) which is complementary to the variant base in the template. Reaction mix does not include dNTP since incorporation occurs at a single site (Figure 2.8). This process is repeated in successive rounds of extension and termination to generate the fluorescently labeled fragment for analysis [106].

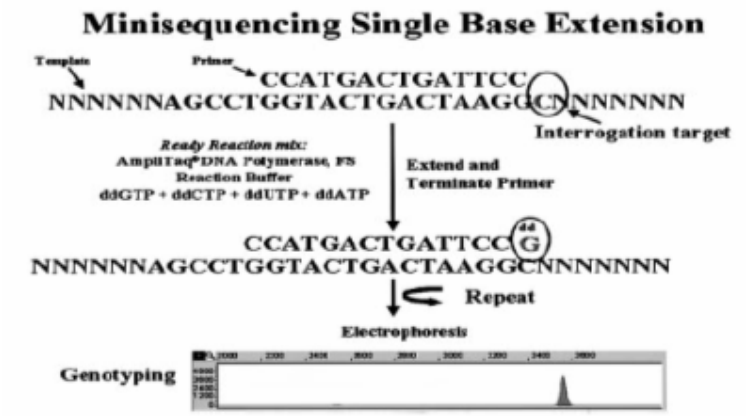


Figure 2.8 Minisequencing technique [106].

2.4 Paradigms

Almost all paradigms used in this study were modifications of *oddball* paradigm. In this paradigm, two stimuli are presented in a random order, with one occurring more frequent than the other. In the active condition, the subjects are required to discriminate the infrequent target stimulus by pressing a button or lifting their right index finger [14]. In this study Contingent Negative Variation (CNV) Paradigm, Go/No Go Paradigm, and Novelty Paradigm were used to generate auditory evoked potentials.

2.4.1 Go/Nogo Paradigm

In this paradigm two different auditory stimuli with 0.5 probabilities were used as Go and No-go stimuli. The subjects are required to discriminate the target (Go) stimulus by lifting their right index finger.

In some studies, different stimulus probabilities were examined, and it was found that less probable Go and No-go stimuli elicited larger P300s than more probable stimuli [107] It is thought that, high probability of the target (Go) stimuli produces a bias to button

press and subject has difficulty to inhibit finger movement for non-target (No-go) stimuli. This inhibition is associated with frontal cortex activity. Accordingly, the No-go P300 was found maximal at the frontocentral sites, whereas P300 in the Go condition was found maximal at the centroparietal sites [108, 109].

2.4.2 Novelty Paradigm

The "novelty" paradigm is a modification of oddball task; an additional infrequent-non-target stimulus is inserted into the sequence of infrequent target and frequent standard stimuli. Courchesne et. al., reported that unrecognizable (i.e. novel) visual stimuli which inserted into oddball task elicited a frontocentrally distributed P300 with shorter latency than the component elicited by the target stimulus [18]. This novelty P300 (P3a) was also reported for the auditory stimulus modality [110]

2.4.3 Contingent Negative Variation (CNV) Paradigm

In 1964 it was already known that the first few presentations of a novel stimulus produce an alerting response i.e., the alpha rhythm is blocked, and an orienting potential that rapidly falls as the stimulus is successively repeated may be generated at the vertex [111]. Walter et al, found that, when the response to a monotonously repeated stimulus has habituated, its initial amplitude could be restored if an association is established between the stimulus (S2) and a prior warning stimulus (S1). They noted that this restoration is particularly effective and long lasting if the subject is motivated to produce a motor response to the second stimulus (S2). In this situation, a slowly rising negative-going DC potential shift starts a little after the warning stimulus (S1) and terminates abruptly when the second stimulus S2 is delivered.

Walter et al, called the second stimulus the “imperative stimulus”, the motor response the “imperative response”, and the warning stimulus S1 the “conditional

stimulus". The negative shift preceding S2 was called "contingent negative variation" (CNV), purely as a descriptive term because it is contingent on the association between S1 and S2.

The paradigm involves the presentation of pairs of stimuli, separated by a time interval, and the establishment of a contingency between the stimuli. The CNV was originally described as an expectancy wave, although more recently it has been linked to motoric and non-motoric preparatory processes [112].

2.5 Time-Frequency Analysis of ERPs Using Wavelet Transform

ERP constitutes a mixture of multiple waves of various frequencies. ERP measures can be investigated in the frequency domain, which can yield insights into the functional cognitive correlations, by assessing specific frequencies [113].

Rhythmic activities found in human EEG consist of delta (0-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz), and gamma (30-80 Hz) oscillations. Evoked delta and theta oscillations represent the slow potentials in ERPs, such as P300 [114]. Alpha activity has been associated with a large number of cognitive processes, such as memory [115], attention [116]. Beta and gamma oscillations have also been associated with cognitive processes such as memory attention and higher cognitive functions such as consciousness [117].

There are several methods to extract oscillations of a specific frequency from ERP data. Examples are filtering, frequency analysis (Fourier Transformation, FFT), and time-frequency analysis (Wavelet Analysis). Since the time course of frequency information can be observed, wavelet transform is advantageous over the FFT. Wavelet transform (WT) uses shorter time windows for faster activity and longer time windows for slower ones. For this reasons, in this study, WT was used to decompose ERP signals into time-frequency components.

2.5.1 Wavelet Transform

To compute a wavelet transform, the original time series, x(t), is convolved with a scaled and translated version of a mother wavelet function, Ψ(t):

$$W_x^{\Psi}(b,a) = A_{\Psi} \cdot \int \Psi^* \left(\frac{t-b}{a} \right) \cdot x(t) \cdot dt \tag{2.1}$$

where Ψ^* denotes the complex conjugation of the wavelet function, b is the translation parameter, a is the wavelet's scaling parameter, and A_Ψ denotes a normalization parameter. The wavelet coefficients quantify the similarity between the original signal and the wavelet function at a specific scale a and latency b.

The mother wavelet is constructed in such a way that it has zero mean and is localized in both time and frequency space. This is in contrast to the Fourier transform where the harmonic basis functions extend over the whole time axis. Due to its localization in time the wavelet transform reflects the time-course of the frequency components of the signal, which is especially important when analyzing non-stationary signals.

In the case of Morlet's wavelets, the mother wavelet function is given by the formula

$$\Psi(t) = e^{j\omega_0 t} \cdot e^{-t^2/2} \tag{2.2}$$

where *j* denotes the imaginary unit, $(-1)^{1/2}$, and ω_0 is 2π times the frequency of the unshifted and uncompressed mother wavelet. The complex Morlet wavelets include a real and imaginary part consisting of a harmonic oscillation windowed in time by a Gaussian envelope as shown in Figure 2.9.

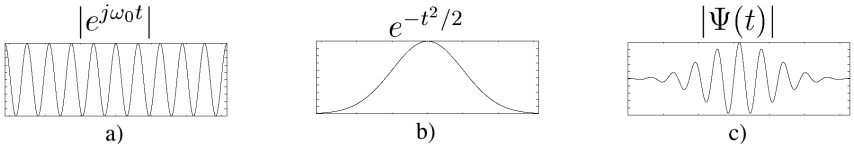


Figure 2.9 Wavelet Transform: Multiplying a sinusoidal function (a) and an envelope function (b) results in a wavelet (c).

The wavelet transform, $W_x(t,f)$, computed using the complex Morlet wavelets is a complex function, which can be divided into its real $\Re\{W_x\}$ and imaginary part, $\Im\{W_x\}$. The amplitude, $|Wx(t,f)|$ corresponds to the envelope of the filtered signal, xf(t).

To display the phase-locked activity in an ERP experiment, which is also called the evoked activity, first the EEG epochs are averaged in time to obtain the ERP, and the wavelet transform is computed on the averaged ERP signal:

$$\text{Evoked} = \left| A_\Psi \int \Psi^* \left(\frac{t-b}{a} \right) \cdot \frac{1}{N} \sum_{i=1}^{N} eeg_i(t)\, dt \right| \tag{2.3}$$

The absolute value of the transform displays the time-varying power of different frequency components. The frequency-specific baseline activity of the pre-stimulus period can be subtracted from the transform to obtain values that indicate the change in the oscillatory amplitude relative to baseline. This TF representation of the ERP contains only that part of the activity that is phase-locked to the stimulus onset.

To compute the activity that is not phase-locked to stimulus onset and is cancelled out in the average, each single trial is at first transformed into the TF plane and the absolute values of the TF are averaged subsequently:

$$\text{Total} = \frac{1}{N} \sum_{i=1}^{N} \left| A_\Psi \int \Psi^* \left(\frac{t-b}{a} \right) \cdot eeg_i(t)\, dt \right| . \tag{2.4}$$

The corresponding TF representation contains all activity of one frequency that occurred after stimulus onset, no matter whether it was phase-locked to the stimulus or not (Figure 2.10). As above, the activity in a pre-stimulus interval can be subtracted to obtain a relative measure.

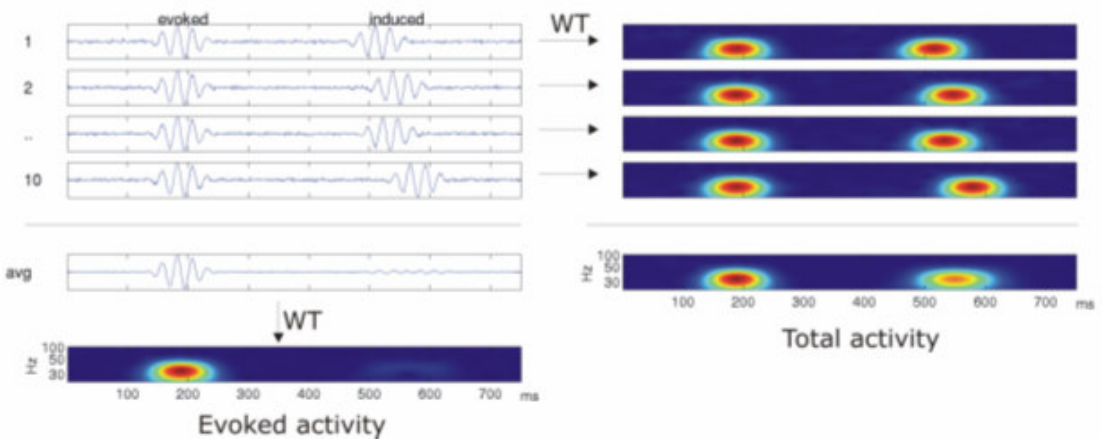


Figure 2.10 Evoked and Total Activity

3. MATERIALS AND METHODS

All genetic analyses were done in the molecular genetics department of the Reproductive Genetics Laboratory, Memorial Hospital, Istanbul.

EEG recordings of subjects and analysis of the EEG data were performed in the Cognitive Electrophysiology Laboratory of the Istanbul Faculty of Medicine.

3.1 Genetic Analysis

3.1.1 DNA Isolation

DNA isolation was performed using a commercial kit according to manufacturer's instructions (QIAamp DNA Mini Kit (Qiagen Co., Hilden, Germany).

Kit contains (QIAamp DNA Mini Kits, catalog no.51304), QIAamp spin columns, collection tubes, buffer AL, buffer ATL, buffer AW1, buffer AW2, buffer AE, and proteinase K.

The procedure was conducted according to the instruction manual as follows:

1. 20 µl Proteinase K was pipetted into the bottom of a 1.5ml centrifuge tube.
2. 200 µl of blood was added,
3. 200 µl of buffer AL was added and mixed by vortexing for 15s, and incubated at 56°C
4. Briefly centrifugation was performed to the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.

5. 200 µl ethanol (96-100%) was added to the sample and mixed by vortexing for 15 seconds. After mixing, briefly centrifugation was done to the tube to remove drops from inside the lid.
6. The sample were transfrerred into the QIAamp spin column (in a 2 ml collection tube) and centrifuged at 8000 rpm for 1 min. Then, QIAamp spin column was placed in a clean 2 ml collection tube (provided) and the tube containig the filtrate was discarded.
7. 500 µl buffer AW1 was added to QIAamp spin column and centrifuged at 8000 rpm for 1 min. Then, QIAamp spin column was placed in a clean 2 ml collection tube (provided) and the tube containig the filtrate was discarded.
8. 500 µl buffer AW2 was added to QIAamp spin column and centrifuged at 14000 rpm for 3 min.
9. QIAamp spin column was placed in a clean 1.5 ml microcentrifuge tube, and the collection tube containing the filtrate was discarded. 200 µl buffer AE was added. Incubation was made at room temperature for 5 min and then tube was centrifuged at 8000 rpm for 1 min. The fluid containing DNA was collected to the tube.
10. DNA was stored at –20°C.

3.1.2 Polymerase Chain Reactions

Primers were diluted first to 100µM concentration according to manufacturer's instructions (Qiagen Co., Hilden, Germany). 100µM concentration primer was further diluted to 12,5µM to use in the PCR reaction according to the equation: M1xV1=M2xV2. None of the primers but the NMDAR2A forward primer were fluorescently labeled because $(GT)_n$ repeat number was analyzed in the NMDAR2A polymorphism with fragment analysis procedure.

3.1.2.1 NMDAR2A Polymorphism

PCR mixture contained 8µl of (1,25 mM each), 5µl of 10X gold buffer, 3 µl of MgCl2 (25mM), 1 µl of forward (FAM-Labeled: 5'- GAA GGA AGC ATG TGG GAA ATG CAG-3'), 1 µl of reverse (5'-GTT TCT TGC TGG GTA CAG TTA TCC CCC T-3')

primers (Figure 3.1), 0.25 µl of Taq Gold Polymerase (5U/1µl), 1 µl of genomic DNA, and 30,75 µl dH2O. PCR conditions were; initial denaturation at 95 °C for 15 minutes, total of 35 PCR cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 1 minute, extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes.

gctcacctcttctggtagcttagaagtggcagcctcagggaggcttctgtttggtagaacttagaaggaagcatgtgggaaatgcag
atgtctttgcttttaggattctggctccctgagatcagaggagtgggttcccgtacgggggtggcgaggcacagggagaagcattg
gagcagactct**gtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgtgt**cggcgcgcctgtgcgtggaggg
ggataactgtacccagcgcgaggcactgggggaggaggaagcggtagcagagatctgaataattgattcttcagccgcaccaga
cgcccgggagcgggcgga

Figure 3.1 : NMDA receptor subtype 2A gene (NR2A) gene, 5' UTR.
Accession No: AF443855, PubMed, showing a genotype having 26 repeats as bold characters to indicate (GT)n repeat polymorphism. Forward and reverse primers are underlined.

3.1.2.2 GABRG2 Polymorphism

PCR mixture contained 8µl of dNTPs (1,25 mM each), 5µl of 10X Mg2-containing buffer, 1 µl of forward (5'-AGA AAT TTA CCA ACT GGT CTA GCC GG -3'), 1 µl of reverse (5'- AAA TCA AAT ATT GTG TCA TGC TTA GT -3') primers (Figure 3.2), 0.25 µl of Hot star Taq Polymerase (5U/µl), 2µl of genomic DNA, and 32,75 µl dH2O. PCR conditions were; initial denaturation at 95°C for 15 minutes, total of 35 PCR cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute, extension at 72°C for 1 minute, and final extension at 72°C for 10 minutes.

Figure 3.2 GABRG2 intronic sequence, showing primers as underlined, and 3145 A/G polymorphism with bold character (Accession No: AC091984, PubMed).

3.1.2.3 DRD2 Polymorphism Study

Since the primers for that polymorphism study have high annealing temperatures compared to the first two studies, DMSO was used in this reaction mixture and PCR conditions were modified to optimize the reaction and to avoid primer dimers. PCR mixture contained 5µl of dNTPs (2,5mM each), 5µl of 10x MgCl2 free buffer, 2µl of MgCl2, 1 µl of forward (5'-ACT GGC GAG CAG ACG GTG AGG-3'), 1 µl of reverse (5'-TGC GCG CGT GAG GCT GCC GGT-3') primers, as shown by Figure 3.3. 0.25 µl of IStar Taq Polymerase (5U/µl), 4µl of genomic DNA, 29,75 µl dH2O and DMSO in a final concentration of 4%. PCR conditions were; initial denaturation at 94°C for 3 minutes, total of 35 PCR cycles of denaturation at 95°C for 30 seconds, annealing at 66°C for 45 seconds, extension at 72°C for 45 seconds, and final extension at 72°C for 3 minutes.

ggtggtgcctcccgcaacccttggcttctgagtcctcaaaggagaagactggcgagcagacggtgaggacccagcctgcaatca
cagcttattactctgggtgtgggtgggagcgcagtgggcggtcgagggttgcgttcccgcctcaaaacaagggatggcggaatcc
cccaacccctcctacccgttccaggccggggatcgccgaggaggtacagctcctttggtgggggggcgggggcggggcctgtct
caggggcggggaccggggcacctccctcccgcgctccccgcgctcgggcgccgcagagctgtccagcttcagtgccgaaccg
gcagcctcacgcgcgcaccgcgccgcctccgccccgtccccgcgctccctcctgcccgcccgccccgcgcccggccccgccc
cgcc

Figure 3.3 Dopamine receptor D2 (DRD2) gene, 5'-flanking region. (Accessıon No:AF148806, PubMed). The CIns/Del polymorhism is shown as a bold letter to indicate there is an insertion of C. Forward and reverse primers are underlined.

Although polymorphisms on GABA and dopamine receptors are RFLP polymorphisms, that is, they can be identified by a restriction endonuclease reaction, these receptors were sequenced first, and minisequenced for the detection of SNPs.

3.1.3 Exo-SAP Reaction

PCR products of GABRG2 and DRD2 polymorphism studies were purified, before sequencing and minisequencing reactions. Reaction mixture contained 0,5µl of Exonuclease I and 1µl of SAP with 8 µl of PCR products. Reaction starts with initially 37°C for 70 minutes and then 72°C for 20 minutes.

3.1.4 Sequencing Reaction

This reaction mixture contained 4 µl buffer, 0,5 µl of mix (BigDye®Terminator v.1.1, Applied Biosystems), 2 µl of purified PCR product, 12,5 µl of dH_2O and 1 µl of forward or reverse primers. The same primers that were used for PCR were used in sequencing reactions (Figures 3.2 and 3.3) also, to identify the sequence of GABRG2 and DRD2 regions containing polymorphisms. Sequencing PCR reaction consisted of 25 cycles; denaturation at 96°C for 10s, annealing at 55°C for 5s and extension at 60°C for 4s.

3.1.5 Minisequencing Reaction

In minisequencing reaction, a new primer called as minisequencing primer (5'-AAT CCC CCA ACC CCT CCT ACC CGT TC-3') was designed for DRD2 polymorphism study (Figure 3.4). Since the forward primer (Figure 3.2) that was used in PCR in GABRG2 polymorphism study can be also used as a minisequencing primer, new primer was not necessary. Snapshot Multiplex Ready Reaction Kit (Applied Biosystems) was used in minisequencing reaction. Total reaction volume was 8 µl, including 1 µl of ready reaction premix, 1µl of minisequencing primer (12,5µM), 5 µl dH_2O, and 1 µl of purified PCR product (10ng). ddNTPs were labelled as follows: ddATP-dRGG™, ddCTP-dTAMRA™, ddUTP-dROX™, ddGTP-R110 ™. 1 drop of mineral oil was added onto the PCR mixtures to avoid evaporation. The conditions were 25 cycles of initial denaturation at 96°C for 10 seconds, annealing at 50°C for 7 seconds, and extension at 60°C for 30 seconds.

ggtggtgcctcccgcaacccttggcttctgagtcctcaaaggagaagactggcgagcagacggtgaggacccagcctgcaatca
cagcttattactctgggtgtgggtgggagcgcagtgggcggtcgagggttgcgttcccgcctcaaaacaagggatggcggaatcc
cccaacccctcctacccgttccaggccggggatcgccgaggaggtacagctcctttggtgggggggcgggggcggggcctgtct
caggggcggggaccggggcacctccctcccgcgctccccgcgctcgggcgccgcagagctgtccagcttcagtgccgaaccg
gcagcctcacgcgcgcaccgcgccgcctccgccccgtccccgcgctccctcctgcccgcccgccccgcgcccggccccgccc
cgcc

Figure 3.4 Minisequencing primer:The minisequencing primer was underlined. Again, the Cins/Del polymorphism is shown by bold character. Primer was designed such that it anneals along the sequence just one base before the polymorphic base.

3.1.6 ABI 3100 Capillary Electrophoresis

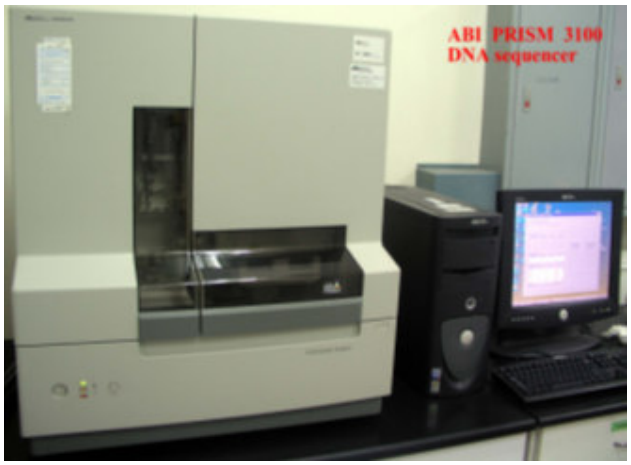


Figure 3.5 ABI Prism Sequencer Machine (Applied Biosystems)

After sequencing and minisequencing reactions, 1µl of product was mixed with 15µl of Hi-Di Formamide and denatured for 5 minutes at 94°C. After that, samples were left at -20°C for 5 minutes. The samples were then resolved and detected by capillary electrophoresis on automatic DNA sequencer ABI Prism (Applied Biosystems) 3100, using an appropriate polymer in the capillaries (Figure 3.5). The peak signals were analyzed with GeneScan® Analysis Software package (Applied Biosystems). Colors were assigned to individual ddNTPs as follows: green/A, black/C, blue/G, red/T. Fragment analysis, and minisequencing analysis were done with Gene Mapper v.3.5. software package (Applied Biosystems), sequence analysis was done with Sequence Navigator software package (Applied Biosystems).

3.2 Electrophysiological Phenotyping

3.2.1 ERP Recordings

The study included 72 healthy Turkish male subjects aged between 19 and 25 years (20.3 ±1.6). All subjects were university students consisted of mostly second year students of Istanbul Faculty of Medicine, Istanbul, whose total education durations were almost the same (14±1.45). All subjects were free of any neuropsychiatric disorders, including drug and alcohol addiction and do not use any other drugs that might interfere with the EEG, as established by a questionnaire. Written informed consent was obtained from all participants.

The subjects sat in an electrically shielded, sound-diminished, and dimly illuminated room. Recordings were taken with *Easy Cap* system with 32 channels placed on the fronto-polar (Fp1, Fp2), frontal (F3, Fz, F4, F7, F8), fronto-central (FC3, FCz, FC4), central (C3, Cz, C4), centro-parietal (CP3, CPz, CP4), parietal (P3, Pz, P4, P7, P8), temporal (T3, T4), fronto-temporal (FT7, FT8), temporo-parietal (TP7, TP8) and occipital (O1, Oz, O2) locations according to the international 10/20 system (Figure 3.6), referenced to linked earlobes. EOG was recorded for both horizontal and vertical eye movements with two electrodes placed at the outer canthus of the right eye and the nasion. Impedance of the electrodes was kept below 20 kΩ. Continous EEG recording was performed with a La Mont 32-channel digital EEG amplifier. The cut-off frequencies of the amplifier were set at 0.1 and 70 Hz. The EEG was sampled with a sampling frequency of 200 Hz and a quantification resolution of 12 bit.

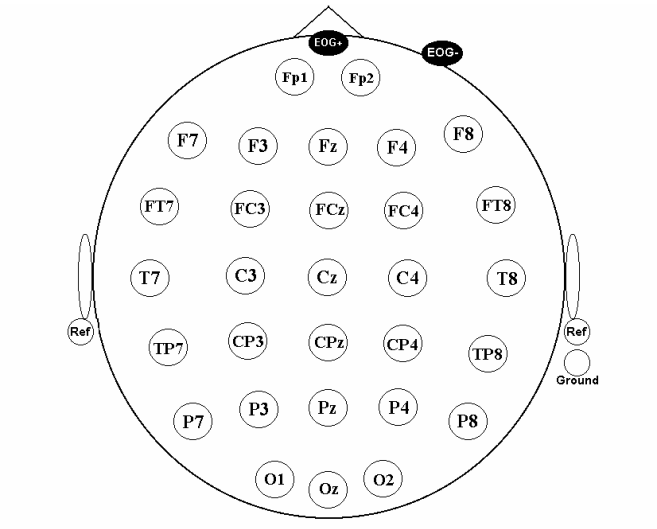


Figure 3.6 Electrode locations, according to international 10/20 system.

3.2.2 Experimental Design

The sound card of a separate computer, which was triggered by the recording computer via parallel port and presented by using a headphone, produced auditory stimuli. Sound pressure level (SPL) was 70dB and stimulus rise/fall time was 10 ms for all auditory stimuli. Table 3.1 shows experimental properties of paradigms including, tone, sweep number, probability, and duration.

Table 3.1
Experimental design with stimulus properties in the paradigms.

Paradigm	Stimulus type	Tone (Hz)	Probability (%)	Duration (ms)
CNV	Visual (S1)	-	100	200
	Auditory (S2)	1000	100	50
Go/Nogo	No-go	1500	50	50
	Go	1000	50	50
Novelty	Novel	-	15	500
	Target	2000	15	500
	Standart	1000	70	500

3.2.3 Analysis of the Data

ERP data were analyzed both in the time domain and in the time-frequency plane. At the very beginning, automatic artifact elimination (amplitude threshold: ±90μV) was applied before the analysis of the data. After event lists and individual epochs were generated, all single ERP epochs were reviewed if there was any residual artifacts due to eye movements. After the artifact elimination, single ERP trials were averaged off-line to obtain the individual averaged ERPs. All data analyses were carried out using the TAMERP software developed in the Matlab 6.5 (The MathWorks, Inc. – Natick, MA 01760, USA) environment.

3.2.4 ERP Analysis in the Time Domain

After the artifact elimination, in time domain analysis, ERPs were digitally filtered with cut-off frequencies 0.1–10 Hz for P300 potential, and 0.1-16 Hz for CNV. P300 amplitudes and latencies were measured for *go*, and *nogo* conditions in *go/nogo* paradigm, and *novel, target* conditions in the *novelty* paradigm. P300 potentials were measured as the most positive peak between 250–450 ms after the stimulus onset. Amplitudes and latencies of the P300 potentials were measured to the all type of stimuli in the paradigm set with a baseline of 200 ms pre-stimulus. For CNV paradigm, mean amplitudes between 0,5s-1s and mean amplitudes between 1 and 1.5 s were both measured according to 200ms pre-stimulus baseline correction.

3.2.5 ERP Analysis in the Time-Frequency Plane

For novelty paradigm, ERPs that were elicited with novel and target stimuli and for *go/nogo* paradigms, ERPs that were elicited with *go* and *nogo* stimuli were subjected to

time-frequency analysis including both evoked and total activities at delta, theta, alpha, beta and gamma bands were calculated in specific time intervals as shown by Table 3.2.

Table 3.2
Time windows for maximum power values: The time windows with maximum power values were obtained for each frequency component. Mean square amplitudes of these time windows were calculated and submitted to statistical analysis.

Frequency	**Paradigms**			
	GoNogo		**Novelty**	
	Evoked (ms)	Total (ms)	Evoked (ms)	Total (ms)
Delta	100-500	100-600	100-500	100-500
Theta	50-170	50-350	50-350	50-350
Alpha	50-170	50-170	50-350	50-200
Beta	30-100	30-120	50-150	50-200
Gamma	0-80	30-170	0-80	0-200

3.2.6 Statistical Analysis

Subjects were grouped according to their genotypes for each polymorphism distincly. The effects of polymorphisms on P300 amplitude and latency measurements (GoNogo and Novelty paradigms), and on early and late mean amplitude measurements (CNV paradigm) were statistically analyzed by an ANOVA design. Frontal (Fz, F3, F4), parietal (Pz, P3, P4), and central (Cz, C3, C4) channels were included to statistical analysis. Between subject factor was genotype and two within subject factors were Anterior-Posterior (AP) distribution (frontal, central, parietal) and lateral (LAT) distribution (left, midline, right)

Degrees of freedom (d.f.) were adjusted with the Greenhouse-Geisser epsilon coefficient for possible violations of the sphericity assumption and corrected P-values were reported.

4. RESULTS

4.1 Results of Genetical Studies

4.1.1 GABRG2 Polymorphism Study

GABAA γ2 Polymorphism Study revealed three types of genotypes in the G/A nucleotide change polymorphism at 3145 nucleotide position in the intronic sequence of GABRG2 receptor. Homozygote A (AA) genotype was determined in 21 (29%) subjects, homozygote G (GG) genotype was determined in 23 (32%) subjects and heterozygote (GA) genotype was determined in 28 (39%) subjects. Allele frequencies were 48,6% and 51,4% for A and G alleles respectively. Whole sequence of the multiplied region was determined by sequencing reaction, which revealed the same sequence (Figure 4.1) that was observed in the NIH web site.

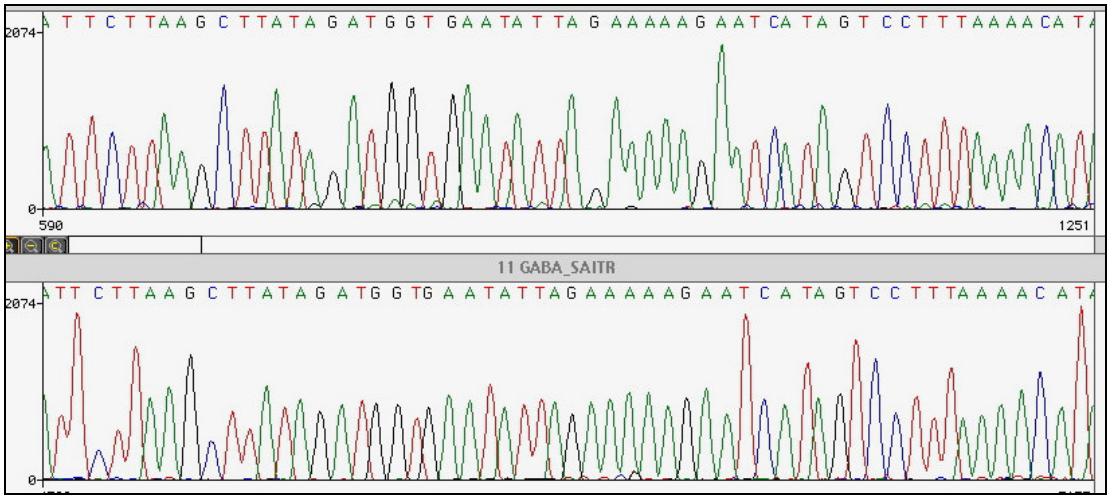


Figure 4.1 Figure shows sequencing reaction result of the intronic sequence of GABRG2

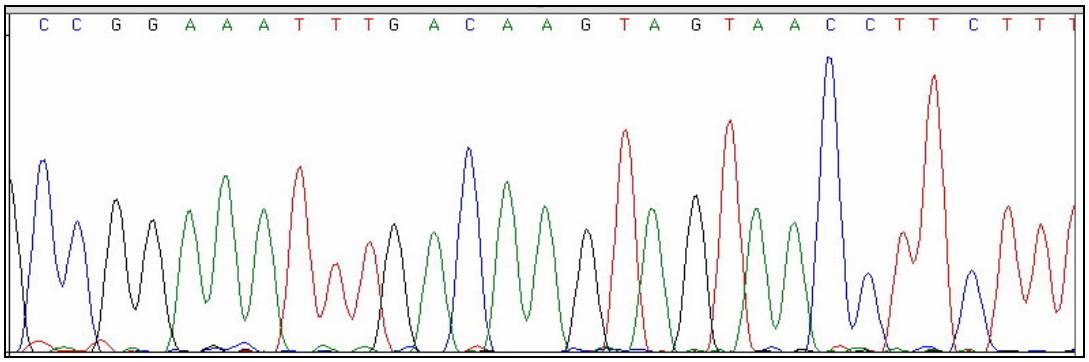


Figure 4.2 Sequencing reaction result of GABRG2 polymorphism

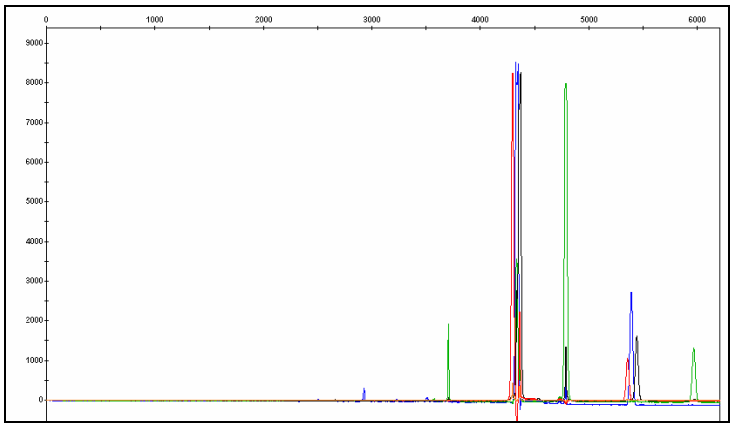


Figure 4.3 Minisequencing reaction result of GABRG2 polymorphism

In Figure 4.2 and 4.3 both sequencing and minisequencing results showing the subject was homozygote A for the GABRG2 polymorphism.

4.1.2 DRD2 Polymorphism Study

DRD2 polymorphism study identified three types of genotypes in the C Insertion/Deletion polymorphism at -141 nucleotide position in the 5' flanking region of DRD2. Homozygote C (Ins/Ins) genotype was determined in 60 (83,3%) subjects, homozygote Del (Del/Del) genotype was determined in only 1 (1,4%) subject and heterozygote (Ins/Del) genotype was determined in 11 (15,3%) subjects. Allele frequencies were 9% and 91% for Del and Ins alleles respectively. Whole sequence of the multiplied

region was determined by sequencing reaction, which revealed the same sequence that was observed in the NIH web site. Figure 4.4 shows a genotype that was homozygote for the polymorphism. Figure 4.5.a shows sequence of a subject who was heterozygote for C Ins/Del polymorphism. Since both alleles were shown in the computer monitor, one can see that the sequence is disrupted in a way because one of the alleles carrying an additional base due to an insertion of C in the marked point. Figure 4.5.b shows minisequencing reaction result of the same subject who was heterozygote for the insertional polymorphism.

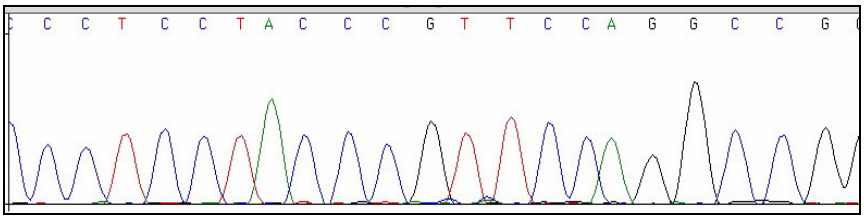
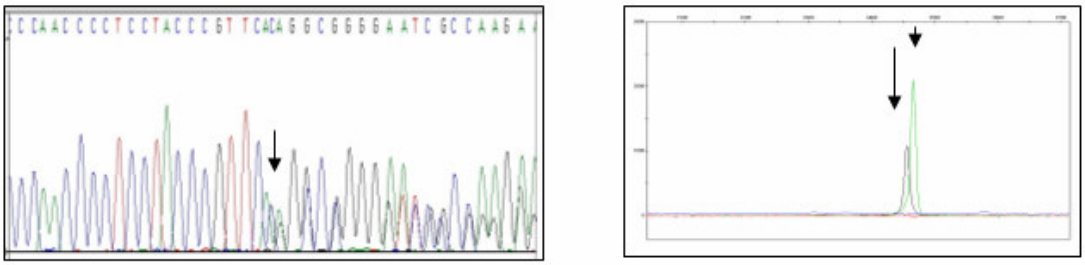


Figure 4.4 Sequencing reaction result for DRD polymorphism of a homozygote. Arrow indicates that there is an insertion for both alleles for this subject.



(a) **(b)**

Figure 4.5 Sequencing and minisequencing reaction result of a heterozygote for DRD2 polymorphism: a) Sequencing reaction result for DRD polymorphism of heterozygote carrying both insertion on one allele and a deletion on the other allele, b) minisequencing reaction result of the same subject.

4.1.3 NMDAR2A Polymorphism Study

The (GT)n repeat number ranged between 18 and 38. Due to high polymorphism rate in the examined region, the most prevalent group's frequency was 7% among all genotype groups. $(GT)_{26}$ allele was observed as the most common allele with a frequency of 22%. Average repeat number of alleles was 25.5. The allele frequency distribution was

shown in Figure 4.7. Since the exact in vivo activity of genotypes: $(GT)_{23}/(GT)_{26}$ and $(GT)_{24}/(GT)_{25}$ were not known even though their combined repeat lengths were the same, the tandem repeat alleles were divided into two classes according to their sum of repeats. 51 was chosen as a cut-off value. Group 1 contained 35 subjects having combined repeat lengths of less than 51; group 2 contained 37 subjects having combined repeats of which were equal or greater than 51.

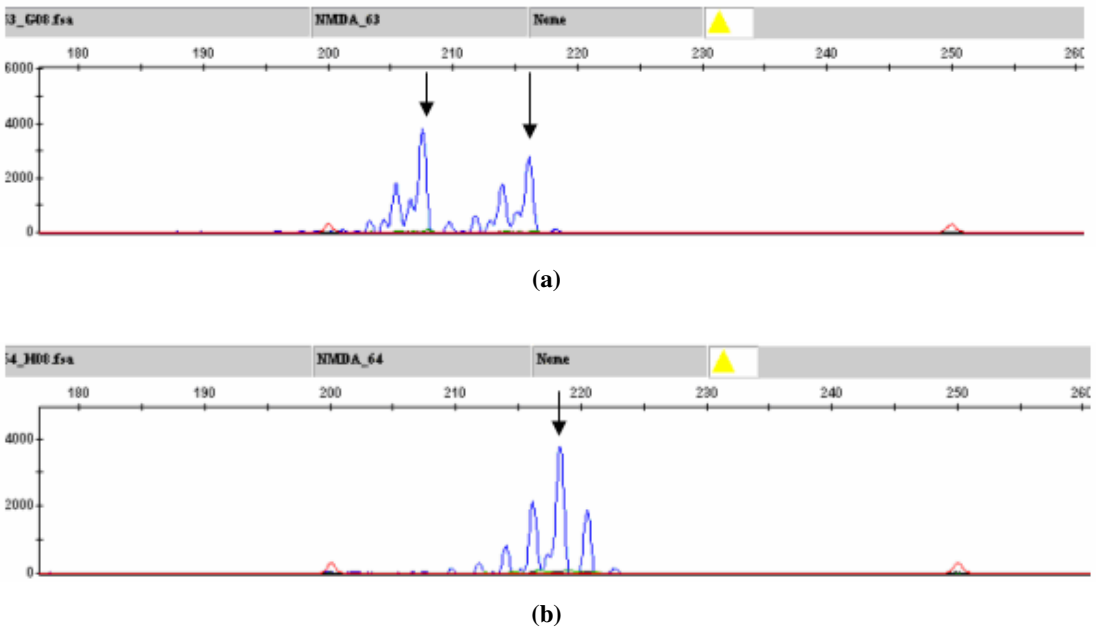


Figure 4.6 Fragment analysis result, showing a heterozygote carrying two different lengths of (GT)n repeat polymorphism (a), and a homozygote carrying the same number of (GT)n repeats (b).

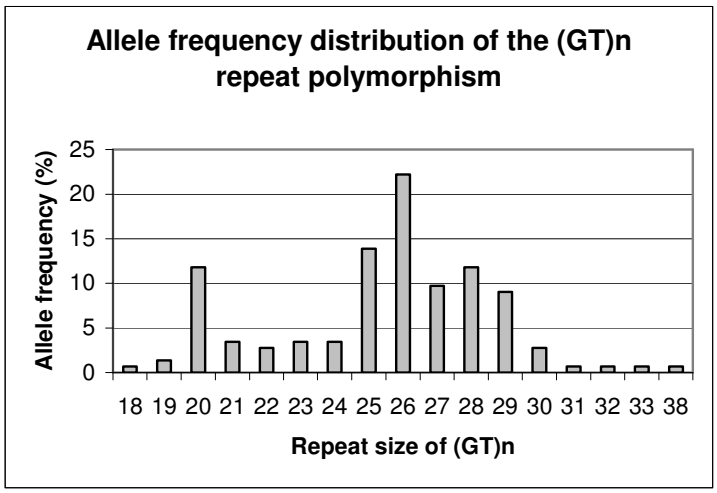


Figure 4.7 Allele frequency distribution of the repeat polymorphism, in our sample.

4.2 Results of Electrophysiological Studies

Before analyzing the EEG record, overall performance of each subject for all paradigms were controlled. The mean performance of the subjects in responding to target events under all conditions was 98.75%. The mean total reaction time of all subjects for CNV, *go/nogo*, and *novelty* paradigms were 0.3±0.08 s, 0.4±0.07 s, and 0.4±0.10 s respectively. After automatic artifact rejection, the trials, where the subjects missed target events or responded false positively were excluded from the averaging procedure. To obtain a reasonable signal/noise ratio in the averaged potentials, at least 30 sweeps for CNV paradigm and 40 sweeps for the other paradigms were left for analysis.

In both time and frequency domain 6 (CNV early, CNV late, Go, Nogo, Novel and Target) conditions were used for analysis using conventional ERP analysis parameters that were mean amplitudes for first two conditions and peak amplitudes and latencies of P300 for the last 4 conditions. Frontal (Fz, F3, F4), Parietal (Pz, P3, P4), and Central (Cz, C3, C4) electrodes were statistically analyzed.

4.2.1 Results of Time Domain Analysis of the ERPs

Time domain analysis of ERPs was restricted to the late positive potential P300 for go-nogo and novelty paradigms. For CNV mean amplitudes between 0.5 and 1 second (early component) and mean amplitude between 1 and 1.5 seconds (late component) after the warning stimulus (S1) were measured. To avoid type 2 errors in the ANOVA analysis, only a subset of main electrodes on two topographic axes (Antero-posterior axis (3 levels): Frontal, central and parietal; and Lateral axis (3 levels): Left, midline and right) were included in the statistical analyses, which correspond to F3, Fz, F4, C3, Cz, C4, P3, Pz and P4 electrodes.

For each polymorphism, grand-averaged waveforms of the 16 main channels including the occipital and temporal channels were visualized in a topographic manner. The waveforms for the two genotypic groups of each polymorphism were superimposed.

4.2.2 P300 Potential and Latency

In the whole group of subjects, Go P3 amplitude reached maximum amplitudes over the parietal regions ($p<0.001$), whereas Nogo P3 amplitudes were maximum over the centro-parietal regions ($p<0.001$).

Novelty P3 amplitude was higher over the centro-parietal regions ($p<0.001$), while target P3 had a parietal maximum for all subjects ($p<0.001$).

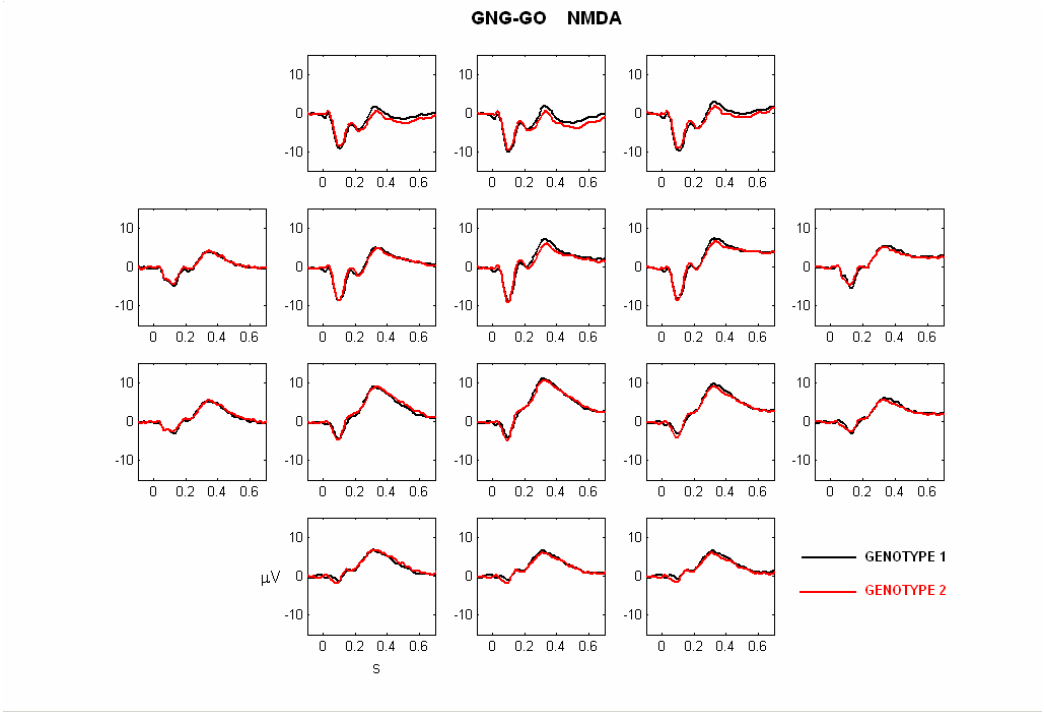
For the whole group of subjects, CNV amplitudes between 0.5-1.0 s time period were more negative over the frontal scalp region ($p<0.05$), whereas CNV amplitudes between 1.0- 1.5 s time period were more negative over the centro-parietal scalp regions ($p<0.05$).

NMDAR2A Polymorphism

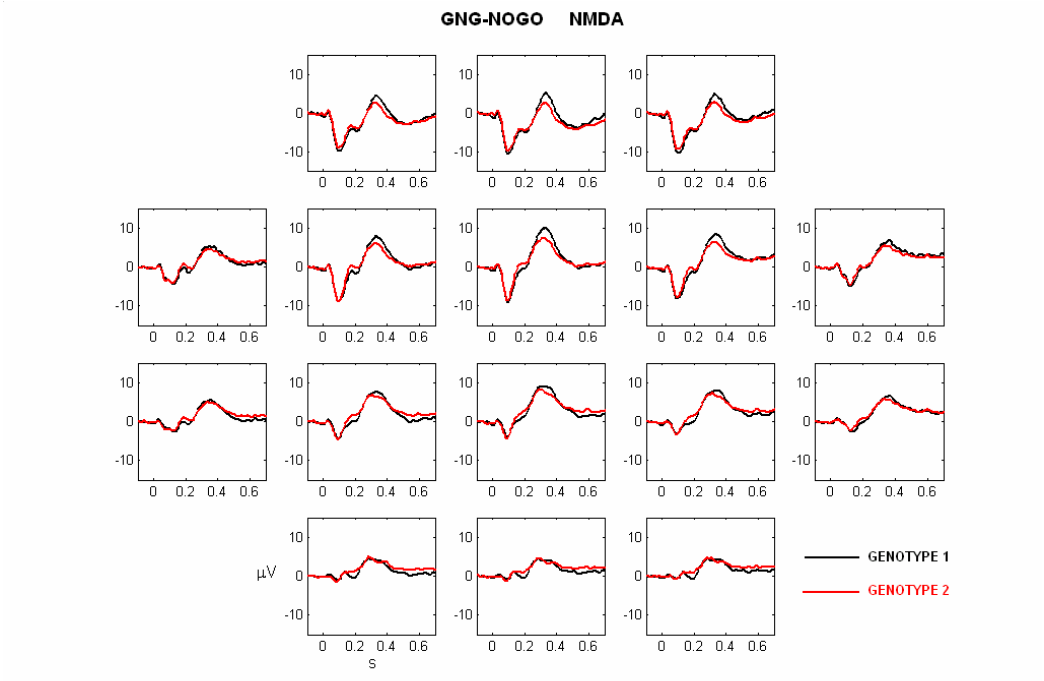
Go/Nogo Paradigm: P300 amplitudes and latencies for both go and nogo stimuli did not differ between genotype groups of the NMDA polymorphism (Figure 4.8).

Novelty Paradigm: For novel stimuli, neither the latency nor the amplitude of P300 differed between polymorphic groups. For targets, P300 amplitude difference was not observed between genotype groups. Latency differences between the genotype groups did not reach the statistical significance ($p=0.18$), but they differed in antero-posterior distribution (Group x AP: $F(2,138)=4.79$, $p=0.019$). Especially over the fronto-central scalp regions target P3 latency was shorter in genotype 2 compared to genotype 1 (Figure 4.9).

CNV Paradigm: There was no difference in the mean amplitudes for both early and late CNV between genotypic groups (Figure 4.10).

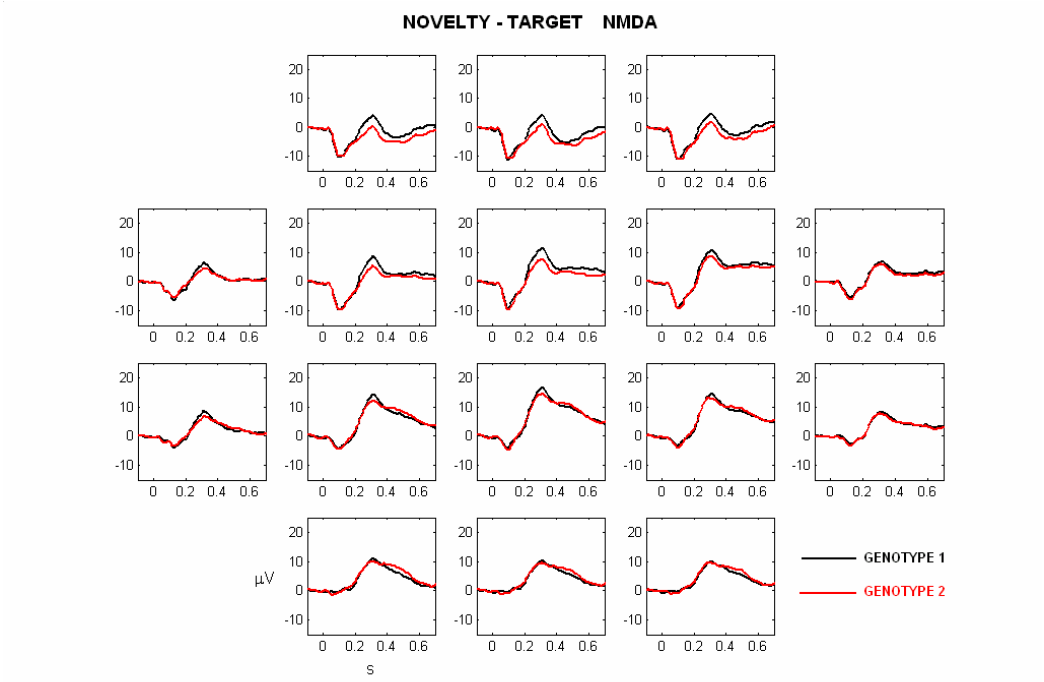


(a)

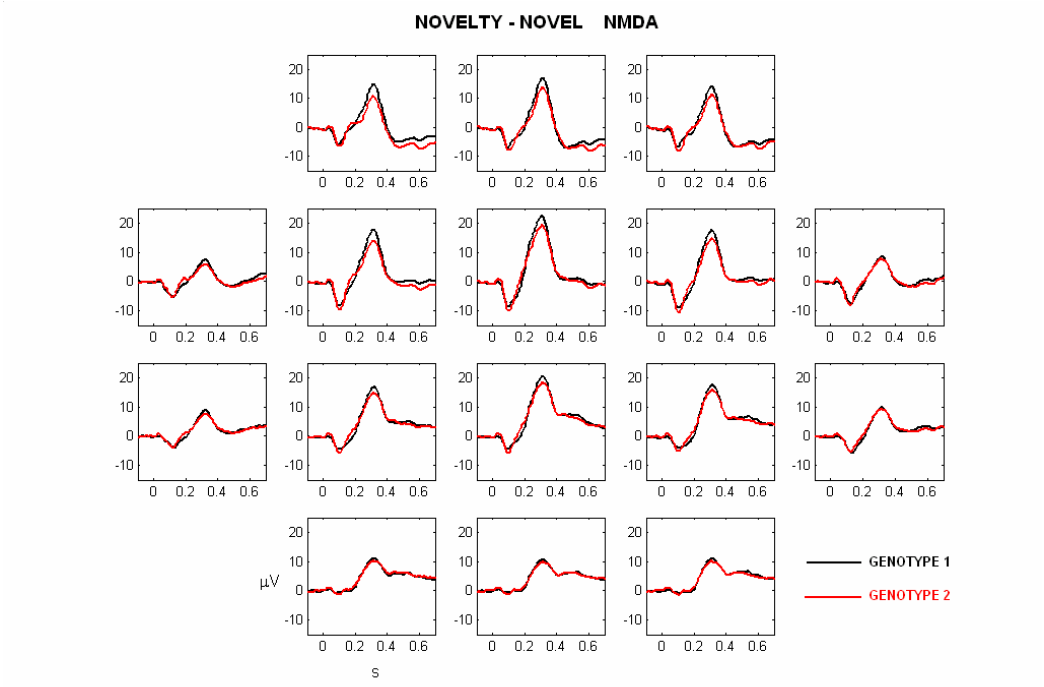


(b)

Figure 4.8 Grand averaged P300 amplitudes of the two genotype groups for NMDA polymorphism for the go stimuli (a), and nogo stimuli (b) in the Go/NoGo paradigm.



(a)



(b)

Figure 4.9 Grand averaged P300 amplitudes of the two genotype groups for NMDA polymorphism for the target stimuli (a), and the novel stimuli (b) in the novelty paradigm.

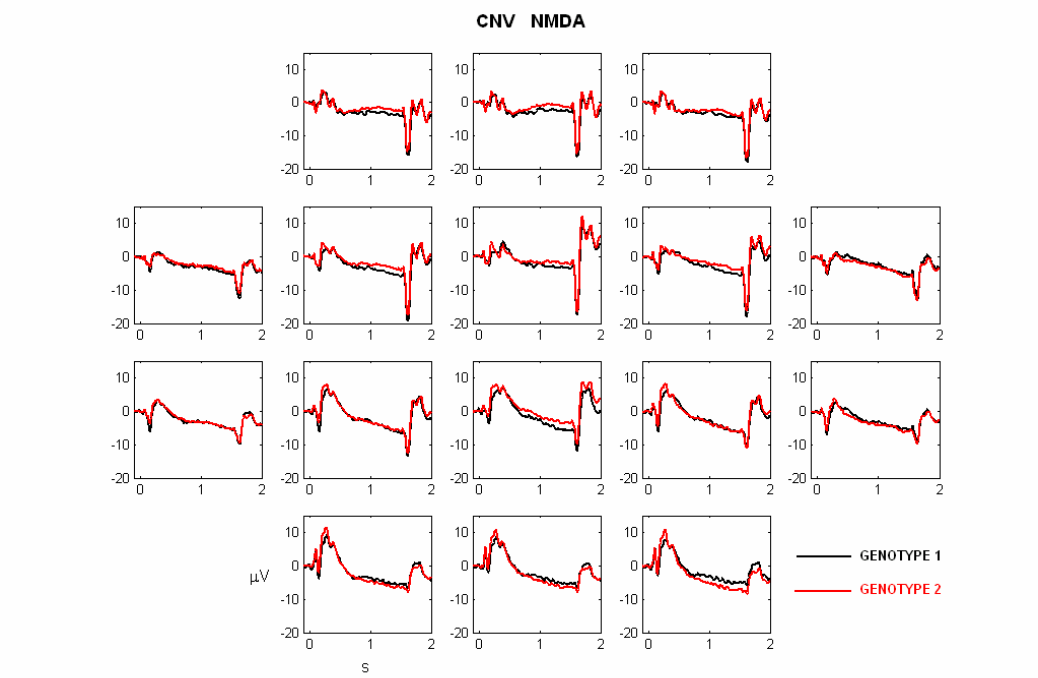


Figure 4.10 Grand averaged negativity of the two genotype groups for NMDA polymorphism, for the CNV paradigm.

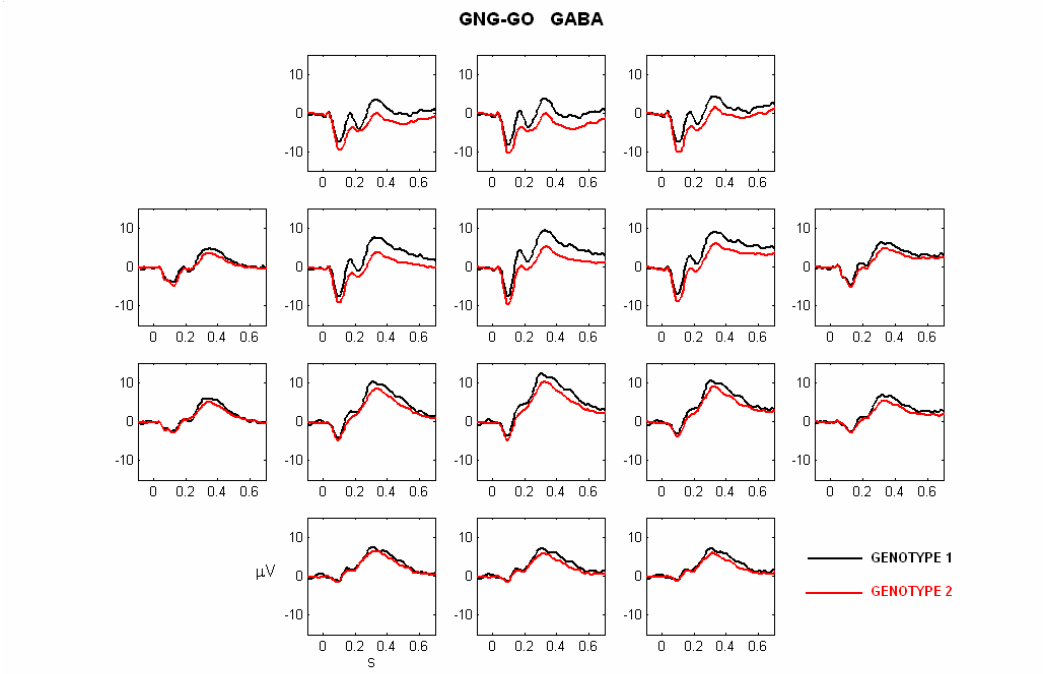
GABRG2 Polymorphism

Go/Nogo Paradigm:

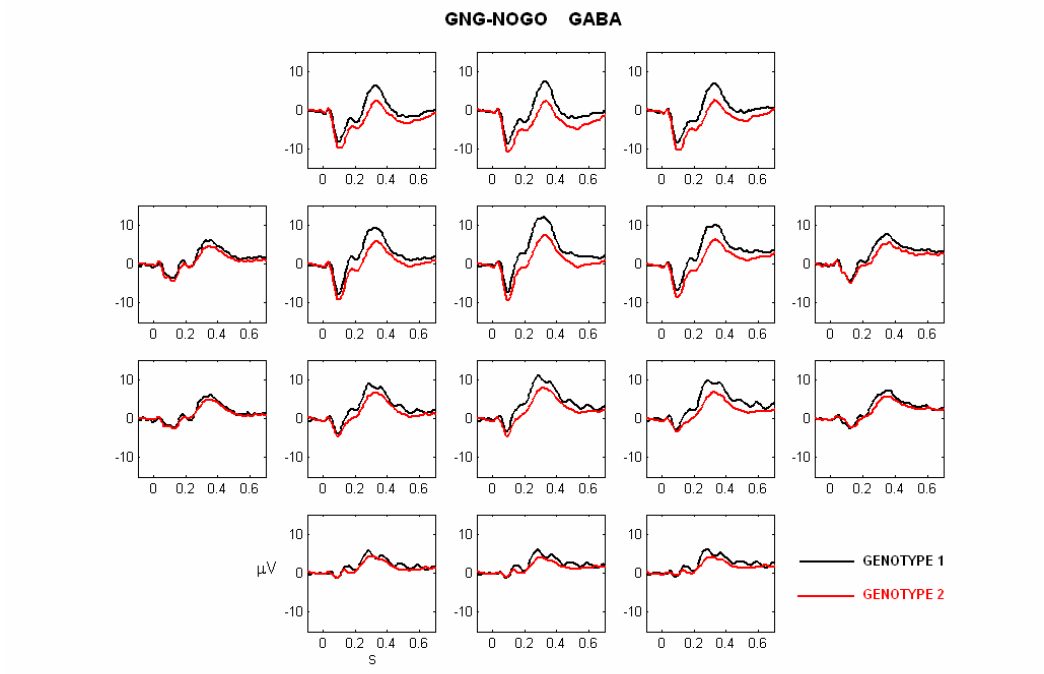
Go P3 amplitudes was significantly higher in AA genotype compared to other genotype group (Group: F(1,69)=5.29 p=0.025). Antero-posterior distribution of P3 amplitude was different between the two genotype groups (Group x AP: F(2,138)=2.65, p=0.099). NoGo P3 amplitudes were significantly higher in AA genotype compared to other genotype group (Group: F(1,69)= 8.33, p=0.005). However, P300 latency differences for both go and nogo stimuli did not reach statistical significance (Figure 4.11).

Novelty Paradigm: For novel stimuli, neither latency nor amplitude of P300 differed between the two genotype groups. However, P300 amplitude for target stimuli was higher for AA genotype than for the other group (Group: F(1,69)=3.73, p=0.058). This difference was much more remarkable for frontal and central scalp regions (Group x AP: F(2,138)=5.08, p=0.018). Target P300 latencies did not differ between the genotype groups (Figure 4.12).

CNV Paradigm: The mean amplitudes for both early and late CNV did not differ between the genotype groups. However, genotype groups differed over the centro-parietal regions (Group x AP: $F(2,140) = 2.76$, $p=0.071$) (Figure 4.13)

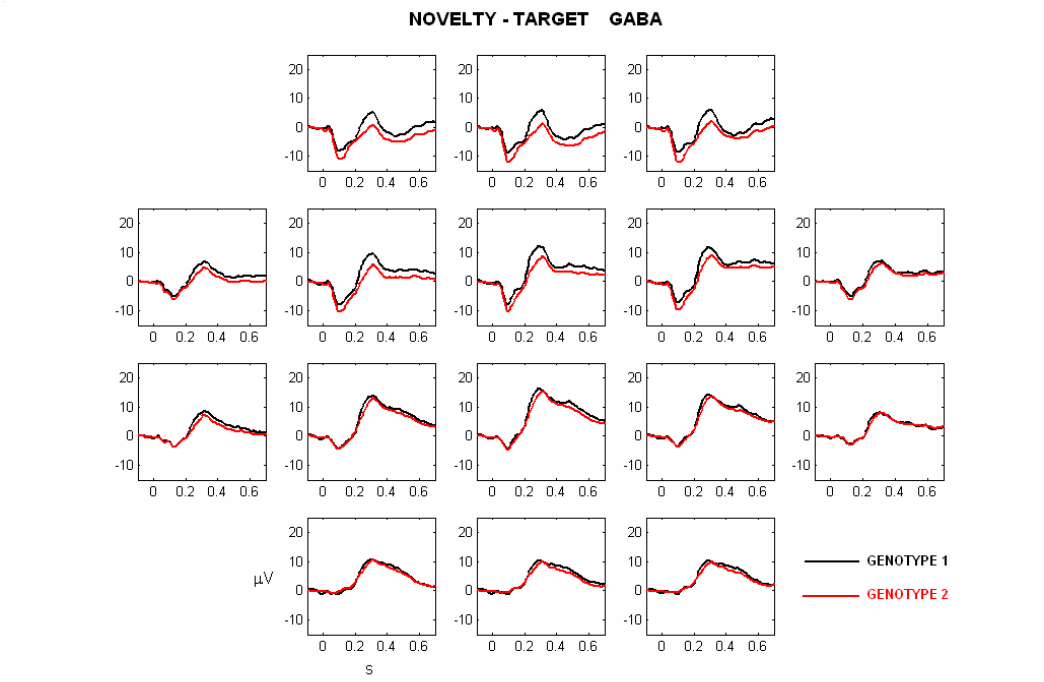


(a)

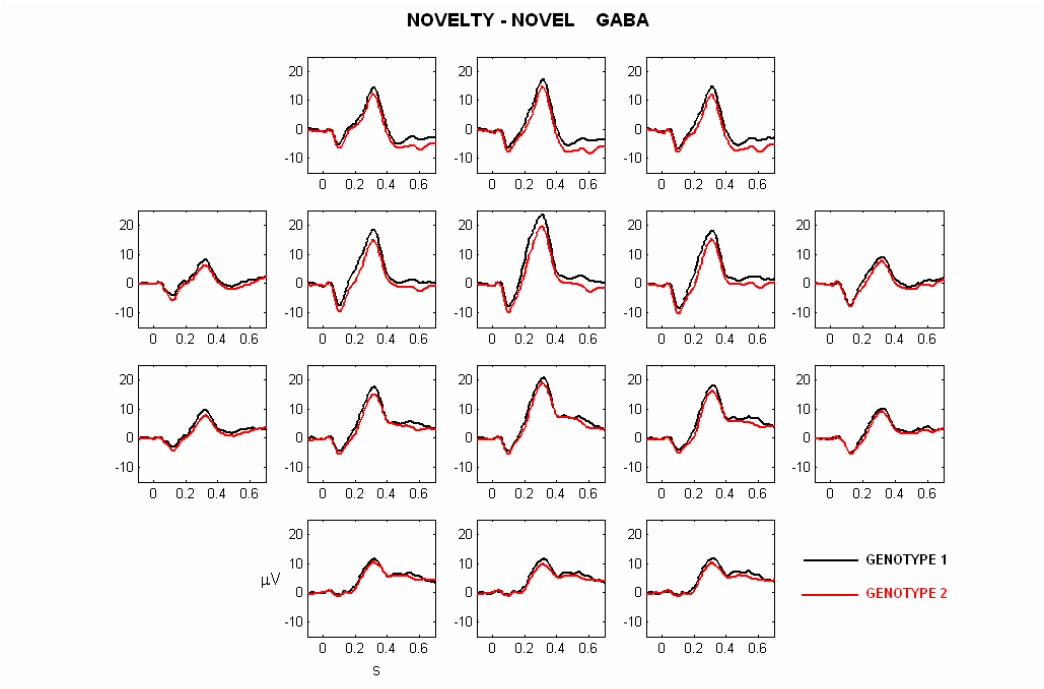


(b)

Figure 4.11 Grand averaged P300 amplitudes of the two genotype groups for GABA polymorphism for the go stimuli (a), and nogo stimuli (b) in the Go/NoGo paradigm.



(a)



(b)

Figure 4.12 Grand averaged P300 amplitudes of the two genotype groups for GABA polymorphism for the target stimuli (a), and novel stimuli (b) in the novelty paradigm.

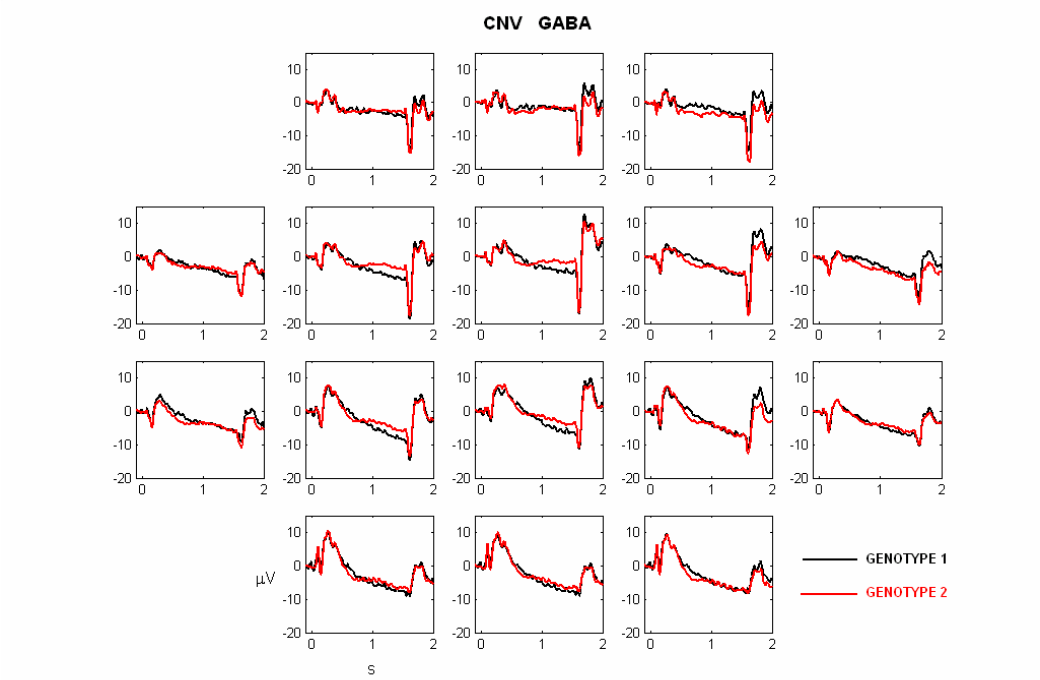


Figure 4.13 Grand averaged negativity of the two genotype groups for GABA polymorphism, for the CNV paradigm.

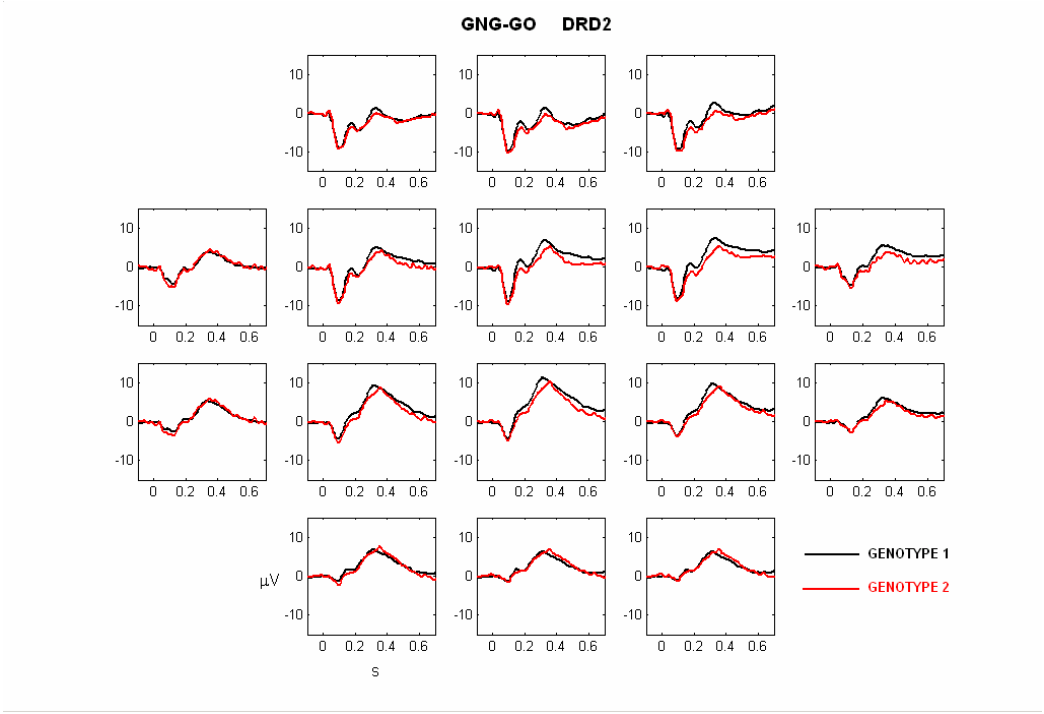
DRD2 Polymorphism

Go/Nogo Paradigm: For both go and nogo paradigms, P300 amplitudes did not differ between the genotype groups. While, Go P3 latency tend to be longer in genotype 2 compared to genotype 1 (Group: $F(1,69)=3.44$, $p=0.068$), this tendency was not obseved for P300 in the Go/NoGo paradigm. However a strong lateralization effect for NoGo P300 latency was observed (Group x LAT: $F(2,138)=3.049$, $p=0.024$) (Figure 4.14).

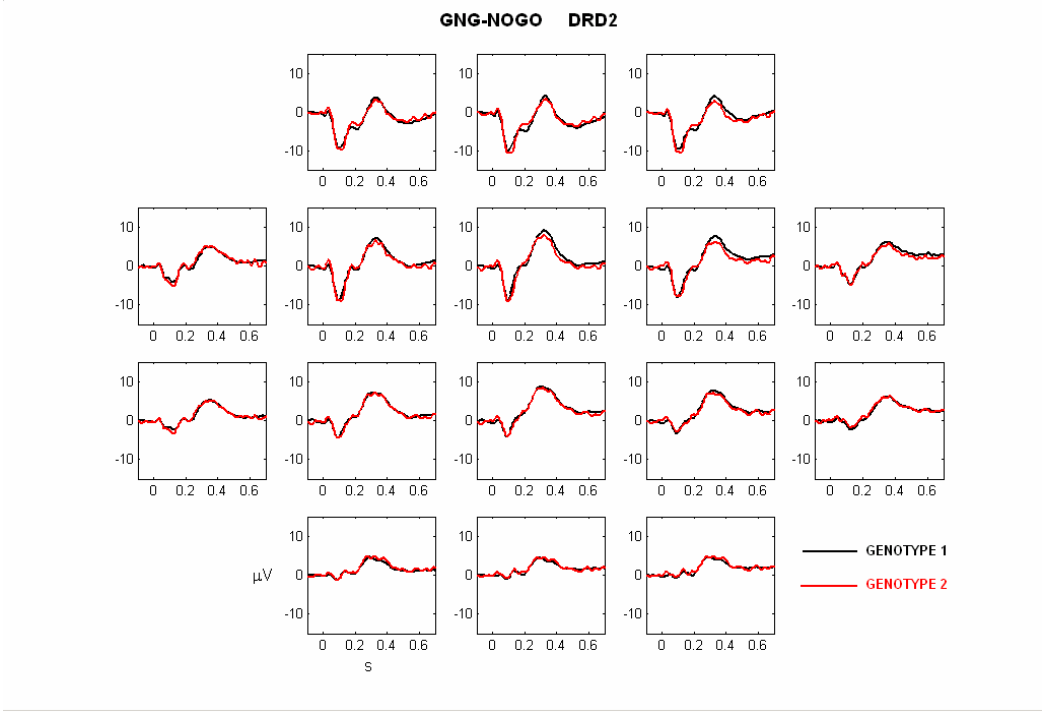
Novelty Paradigm: No difference was observed for novel stimuli in both amplitude and latency of P300. Target P300 amplitude and latencies did not differ either (Figure 4.15).

CNV Paradigm: There was no difference in the mean amplitudes of early and late CNV between genotype groups. However, late CNV amplitudes differed between the two groups in terms of their laterality (Group x LAT: $F(2,140)=6.12$, $p=0.003$). Genotype 2 showed homogenous distribution from left to the right side of the scalp, however a

dramatic amplitude difference was observed genotype 2 on the right side of the scalp (Figure 4.16)

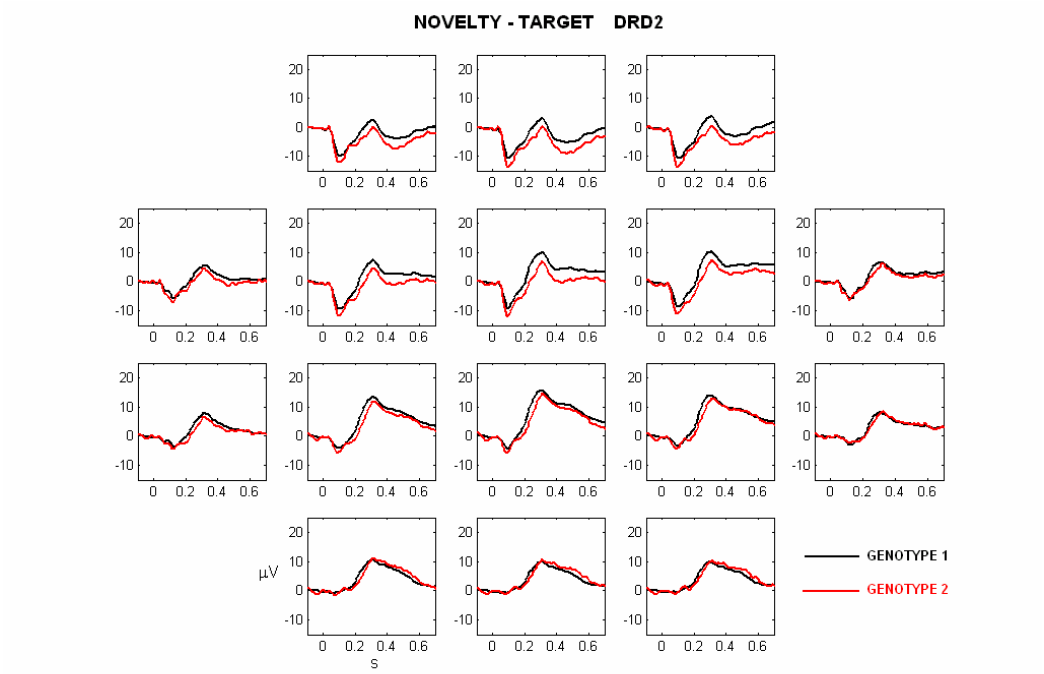


(a)

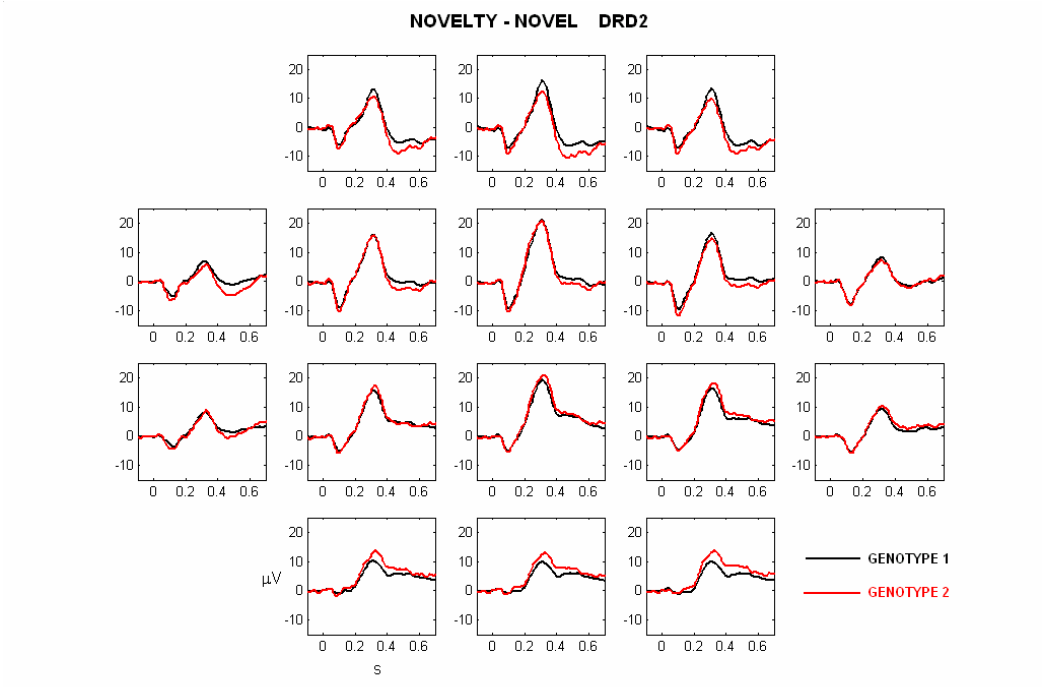


(b)

Figure 4.14 Grand averaged P300 amplitudes of the two genotype groups for DRD polymorphism for the go stimuli (a), and nogo stimuli (b) in the Go/NoGo paradigm.



(a)



(b)

Figure 4.15 Grand averaged P300 amplitudes of the two genotype groups for DRD polymorphism for the target stimuli (a), and novel stimuli (b) in the novelty paradigm.

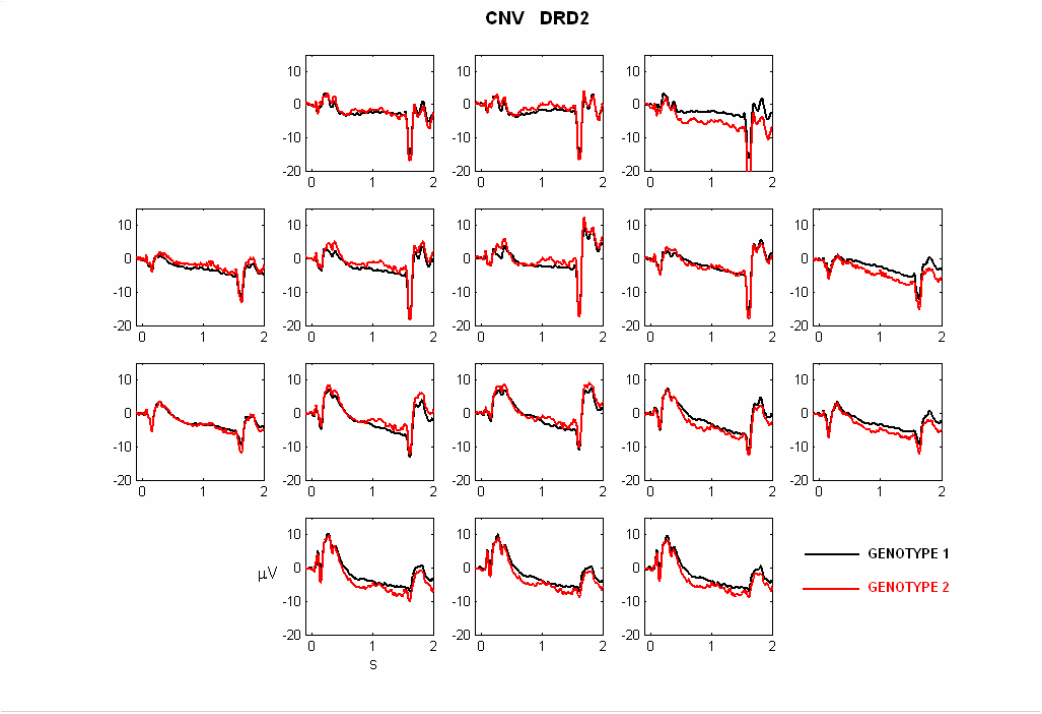


Figure 4.16 Grand averaged negativity of the two genotype groups for DRD polymorphism, for CNV paradigm.

Table 4.1
Summary of the analysis of variance performed on the P300 latencies and amplitudes for Go/Nogo paradigm.
(* p < .05, ** p < .01, *** p < .001)

Group	d.f.	GoNogo			
		Go		Nogo	
		Latency	Amplitude	Latency	Amplitude
GABA	1.69	-	5.287*	-	8.327**
AP	2.138	4.092*	167.541***	-	81.859***
LAT	2.138	2.802	20.348***	7.272***	26.166***
GABAxAP	2.138	2.9	2.650	-	-
GABAxLAT	2.138	-	-	-	3.057*
DRD	1.69	3.440	-	-	-
AP	2.138		107.740***	-	54.612***
LAT	2.138	4.491*	7.824***	5.927**	12.363***
DRDxAP	2.138	-	-	-	-
DRDxLAT	2.138	-	-	3.049**	-
NMDA	1.69	-	-	-	-
AP	2.138	-	209.108***	-	105.718***
LAT	2.138	5.694**	25.552***	8.368***	23.077***
NMDAxAP	2.138	-	-	-	
NMDAxLAT	2.138	-	-	-	-

Table 4.2
Summary of the analysis of variance performed on the P300 latencies and amplitudes for novelty paradigm.
(* p < .05, ** p < .01, *** p < .001)

Group	d.f.	Novelty			
		Novel		Target	
		Latency	Amplitude	Latency	Amplitude
GABA	1.69	-	-	-	3.731*
AP	2.138	6.8**	35.238***	4.877*	182.211***
LAT	2.138	19.351***	110.762***	7.471**	35.497***
GABAxAP	2.138	-	-	-	5.078*
GABAxLAT	2.138	-	-	-	-
DRD	1.69	-	-	-	-
AP	2.138	5.881**	23.171***	-	23.951***
LAT	2.138	10.114***	69.557***	3.534*	6.082**
DRDxAP	2.138	-	-	-	-
DRDxLAT	2.138	-	-	-	-
NMDA	1.69	-	-	-	-
AP	2.138	7.006**	41.427***	7.675**	232.182***
LAT	2.138	20.371***	135.351***	7.988***	43.173***
NMDAxAP	2.138	-	-	4.791**	-
NMDAxLAT	2.138	-	2.536	-	-

Table 4.3
Summary of the analysis of variance performed on the P300 latencies and amplitudes for CNV paradigm.
(* p < .05, ** p < .01, *** p < .001)

		CNV	
Group	**d.f.**	**Early Mean Amplitude**	**Late Mean Amplitude**
GABA	1.69	-	-
AP	2.138	4.586**	13.143***
LAT	2.138	5.837**	7.060**
GABAxAP	2.138	-	2.761
GABAxLAT	2.138	-	-
DRD	1.69	-	-
AP	2.138	3.564*	4.132**
LAT	2.138	6.201**	13.466***
DRDxAP	2.138	-	-
DRDxLAT	2.138	8.427***	-
NMDA	1.69	-	-
AP	2.138	7.914***	10.687***
LAT	2.138	5.169**	9.016***
NMDAxAP	2.138	-	-
NMDAxLAT	2.138	-	-

4.2.3 The Results of Time-Frequency Analysis of the ERPs

The time-frequency transforms of the midline channels are shown in the following figures for the two-genotype groups of each polymorphism. Only the comparisons with significant differences were displayed.

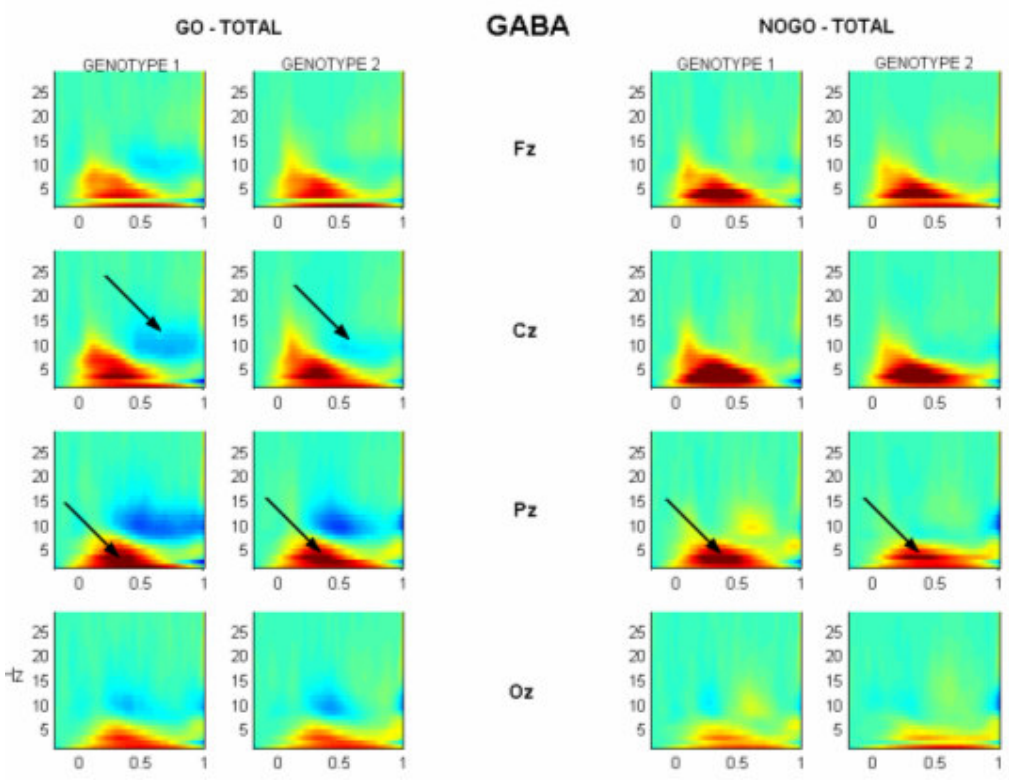


Figure 4.17 The total time-frequency powers to Go and NoGo stimuli of the Go/NoGo paradigm for the two GABA polymorphism genotype groups.

The time-frequency transforms of the Go and NoGo conditions of the Go/NoGo paradigm for the two-genotype groups of the GABA polymorphism are shown in Figure 4.17. The total powers that contain both phase-locked and non-phase-locked signal components were displayed. In the Go condition, a higher alpha desynchronization (upper left arrows) was observed in the genotype 1 in-line with a higher theta activity (lower left arrows). The theta difference is also present In the NoGo condition in terms of a higher theta response in the genotype 1, whereas the alpha desynchronization effect is absent.

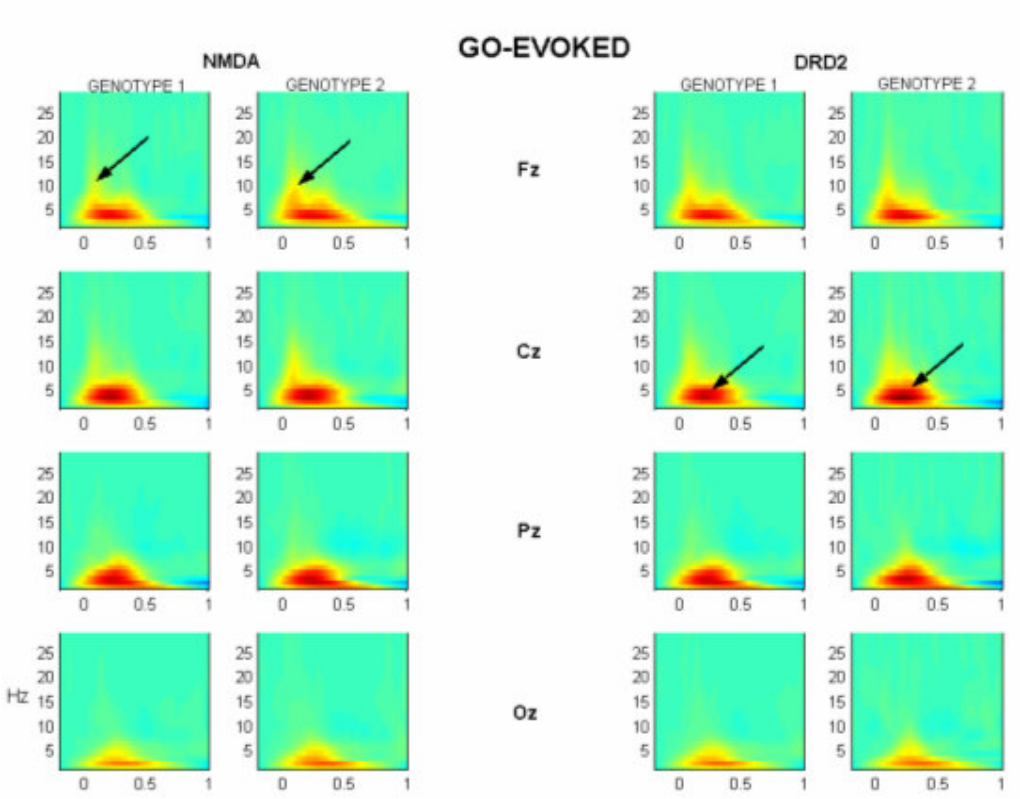


Figure 4.18 The evoked time-frequency powers to Go stimuli of the Go/NoGo paradigm of the two genotype groups of NMDA and DRD2 polymorphisms.

The evoked power of the Go condition of the Go/NoGo paradigm showed significant differences for the NMDA and DRD2 polymorphisms. The significant difference in the NMDA polymorphism was the higher power of the evoked alpha in fronto-central leads for the genotype 2. The significant difference for the DRD2 polymorphism was the increased evoked theta response in the genotype 2 (Figure 4.18).

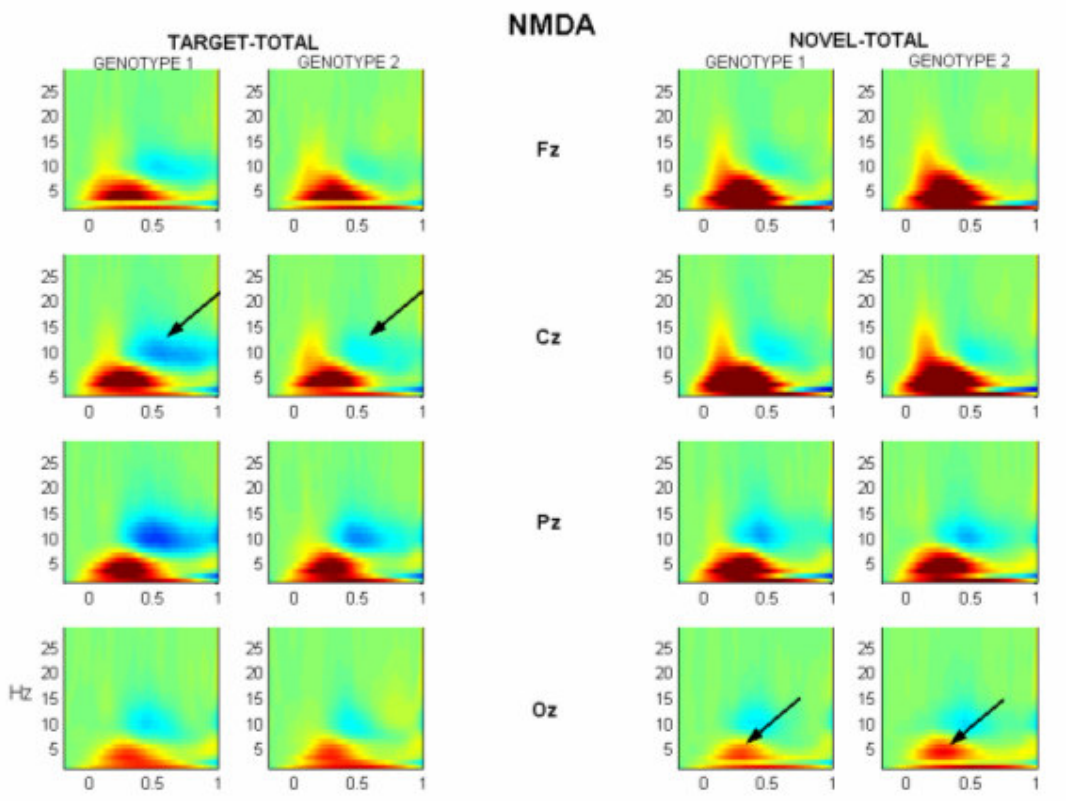


Figure 4.19 The total time-frequency powers of the responses to target and novel stimuli of the novelty paradigm for the two genotype groups of NMDA polymorphism.

In the novelty paradigm, the total powers of the responses to both target and novel stimuli showed significant differences for the NMDA polymorphism. The significant difference in the target condition was a higher alpha desynchronization in the genotype 1, whereas for the novel stimuli the total theta power was higher in the posterior leads for genotype 1 (Figure 4.19).

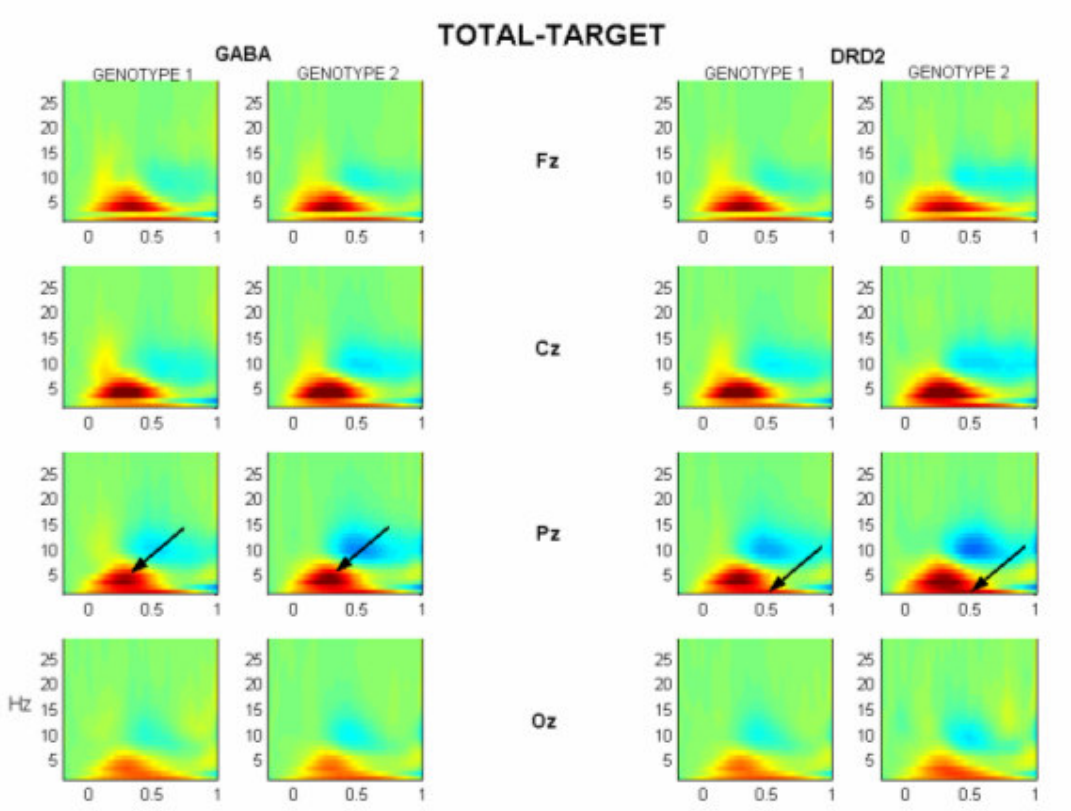


Figure 4.20 The total time-frequency powers of the responses to the target stimuli of the novelty paradigm for the two genotype groups of the GABA and DRD2 polymorphisms.

The total power of the responses to target stimuli of the novelty paradigm further showed increased parietal theta responses to genotype 2 of the GABA polymorphism and increased parietal delta responses to genotype 2 of the DRD2 polymorphism (Figure 4.20).

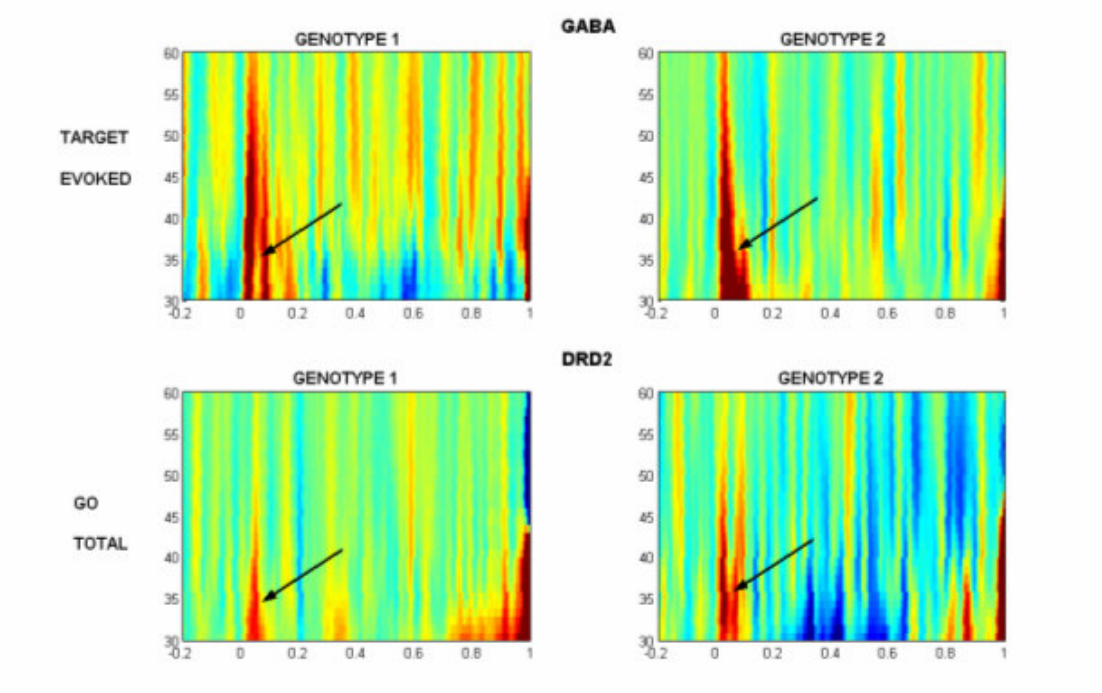


Figure 4.21 The evoked gamma responses to the target stimuli of the novelty paradigm for both genotype groups of the GABA polymorphism (uper row) and the total gamma responses to Go stimuli of the Go/NoGo paradigm for both genotype groups of the DRD2 polymorphism (lower row).

In the evoked gamma responses, the only significant difference was obtained for the GABA polymorphism. The genotype showed higher evoked gamma responses compared with the genotype 1. In the total gamma responses, the similar effect was obtained for the DRD2 polymorphism, where the genotype 2 showed a higher gamma power in the early time window compared with genotype 1. Only midline frontal leads are shown, because the gamma response is mainly observed in the frontal region (Figure 4.21).

Table 4.4
Evoked oscillations for go stimuli.
(* p < .05, ** p < .01, *** p < .001)

		Evoked oscillations for go				
	d.f.	**Delta**	**Theta**	**Alpha**	**Beta**	**Gamma**
NMDA	1,69	-	-	-	-	-
NMDA x AP	2,138	-	-	-	-	-
NMDA x LAT	2,138	3.87*	-	-	-	-
GABA	1,69	-	-	-	-	--
GABA x AP	2,138	-	-	-	-	-
GABA x LAT	2,138	-	-	-	-	-
DRD	1,69	-	-	-	-	-
DRD x AP	2,138	-	-	-	-	-
DRDx LAT	2,138	-	-	-	-	-

Table 4.5
Total oscillations for go stimuli.
(* p < .05, ** p < .01, *** p < .001)

		Total oscillations for go				
	d.f.	**Delta**	**Theta**	**Alpha**	**Beta**	**Gamma**
NMDA	1,69	-	-	-	-	-
NMDA x AP	2,138	-	-	-	-	-
NMDA x LAT	2,138	4.408*	-	-	-	-
GABA	1,69	-	-	-		-
GABA x AP	2,138	-	7.631**	4.383*	-	-
GABA x LAT	2,138	-	5.548**	-	-	-
DRD	1,69	-	-	-	-	-
DRD x AP	2,138	-	-	-	-	3.188*
DRDx LAT	2,138	-	-	-	-	-

Table 4.6
Evoked oscillations for nogo stimuli.
(* p < .05, ** p < .01, *** p < .001)

		Evoked oscillations for nogo				
	d.f.	**Delta**	**Theta**	**Alpha**	**Beta**	**Gamma**
NMDA	1,69	-	-	-	-	-
NMDA x AP	2,138	-	-	-	-	-
NMDA x LAT	2,138	-	-	-	-	-
GABA	1,69	-	-	-	-	-
GABA x AP	2,138	-	-	-	-	-
GABA x LAT	2,138	-	-	-	-	-
DRD	1,69	-	-	-	-	-
DRD x AP	2,138	-	-	-	-	-
DRDx LAT	2,138	-	-	-	-	-

Table 4.7
Total oscillations for nogo stimuli.
(* p < .05, ** p < .01, *** p < .001)

		Total oscillations for nogo				
	d.f.	**Delta**	**Theta**	**Alpha**	**Beta**	**Gamma**
NMDA	1,69	-	-	-	-	-
NMDA x AP	2,138	-	-	-	-	-
NMDA x LAT	2,138	-	-	-	6.733**	-
GABA	1,69	-	-	-	-	-
GABA x AP	2,138	-	7.870**	-	-	-
GABA x LAT	2,138	-	-	-	-	-
DRD	1,69	-	-	-	-	-
DRD x AP	2,138	-	-	-	-	-
DRDx LAT	2,138	-	-	-	-	4.971*

Table 4.8
Evoked oscillations for target stimuli.
(* p < .05, ** p < .01, *** p < .001)

		Evoked oscillations for target				
	d.f.	**Delta**	**Theta**	**Alpha**	**Beta**	**Gamma**
NMDA	1,69	-	-	3,829*	-	-
NMDA x AP	2,138	-	-	-	-	-
NMDA x LAT	2,138	-	-	-	-	-
GABA	1,69	-	-		-	5,003*
GABA x AP	2,138	-	-	-	-	-
GABA x LAT	2,138	-	-	-	-	-
DRD	1,69	-	3,754 *	-	-	-
DRD x AP	2,138	-	-	-	-	-
DRDx LAT	2,138	-	3,052 *	-	-	5,096*

Table 4.9
Total oscillations for target stimuli.
(* p < .05, ** p < .01, *** p < .001)

		Total oscillations for target				
	d.f.	**Delta**	**Theta**	**Alpha**	**Beta**	**Gamma**
NMDA	1.69	-	-	-	-	-
NMDA x AP	2.138	-	-	7.631**	-	-
NMDA x LAT	2.138	-	-	-	-	-
GABA	1.69			-	-	-
GABA x AP	2.138	-	3.866*	-	-	-
GABA x LAT	2.138	-	4.869**	-	-	-
DRD	1.69	4.064*	-	-	-	-
DRD x AP	2.138	-	-	-	-	-
DRDx LAT	2.138	-	-	-	-	-

Table 4.10
Evoked oscillations for novel stimuli.
(* p < .05, ** p < .01, *** p < .001)

		Evoked oscillations for novel				
	d.f.	**Delta**	**Theta**	**Alpha**	**Beta**	**Gamma**
NMDA	1.69	-	-	-	-	-
NMDA x AP	2.138	-	-	-	-	-
NMDA x LAT	2.138	3.308*	-	-	-	4.576**
GABA	1.69	-	-	-	-	-
GABA x AP	2.138	-	-	-	-	-
GABA x LAT	2.138	-	-	-	-	-
DRD	1.69	-	-	-	-	-
DRD x AP	2.138	-	-	-	-	-
DRDx LAT	2.138	6.798**	-	-	-	-

Table 4.11
Total oscillations for novel stimuli.
(* p < .05, ** p < .01, *** p < .001)

		Total oscillations for novel				
	d.f.	**Delta**	**Theta**	**Alpha**	**Beta**	**Gamma**
NMDA	1.69	-	4.447*	-	-	-
NMDA x AP	2.138	-	-	-	-	-
NMDA x LAT	2.138	-	-	4.119*	-	-
GABA	1.69	-	-	-	-	-
GABA x AP	2.138	-	-	-	-	-
GABA x LAT	2.138	-	-	-	-	-
DRD	1.69	-	-	-	-	-
DRD x AP	2.138	-	-	-	-	-
DRDx LAT	2.138	-	-	-	-	-

5. DISCUSSION

P300 component as well as spectral components of ERP are known to be highly heritable [32, 33, 34, 36, 37]. They serve as phenotypic markers among individuals in cognitive studies. In this study, polymorphisms in receptors on three major neurotransmitter systems that are thought to involve in the generation of P300 were investigated for possible associations between polymorphic genotypes and electrophysiological phenotypes. The possible effects of polymorphisms were discussed in two different sections of which contain first, the time-domain analysis and second, time-frequency domain analysis.

5.1 Time Domain Results

5.1.1 NMDAR2A Polymorphism Effects

For NMDA polymorphism, no significant amplitude and latency differences were observed. However, P300 latencies elicited to target stimuli deviated significantly in antero-posterior distribution (p: 0.019). Frontal P300 latency was significantly reduced in longer $(GT)_n$ repeat group compared to the shorter one. This was surprising such that; longer alleles have lower transcriptional activity of this excitatory neurotransmitter receptor and hence may reduce GRIN2A levels in the brain [69]. However, transgenic mice lacking the NMDA receptor ε 1 subunit, orthologous to human N2A, showed increases in both dopamine and serotonin metabolism in the frontal cortex [118]. This relationship between reduced levels of NMDAR2A and increased levels of dopaminergic metabolism might lead to the generation of shorter P300 latency that was observed only in frontal regions of longer repeat group.

5.1.2 GABRG2 Polymorphism Effects

According to genotyping results, allele frequency distributions of Turkish descent for the GABRG2 polymorphism were not different from German descent [90]. GABRG2 polymorphism heterozygotes G/A (39%) were more prominent than each of the homozygotes (A/A, G/G).

In time domain analysis, a significant genotypic association was found for the GABRG2 polymorphism and P300 amplitudes that were elicited to go and nogo stimuli. Furthermore, antero-posterior distribution difference for go stimuli and lateralization difference for nogo stimuli were observed between the two genotypic groups. Winterer et. al, has shown a strong association between G/A nucleotide exchange at position at 3145 in the intronic sequence of GABRG2 and N100/P200 complex, and found that this phenotypic difference was maximum at 132ms post stimulus [90]. In our study, the comparison of the A/A versus A/G and G/G genotypic groups gave significant results for the P300 potentials to Go (p: 0.025) and NoGo (p: 0.005) stimuli in the Go/NoGo paradigm, as well as P300 amplitudes elicited with novel (p: 0.05) stimuli, but not with target stimuli. The first group carrying A/A genotype had higher amplitudes than the other groups that were evident from Go/NoGo and novelty paradigms.

According to the initial hypotheses on the GABA effects on P300 we can consider that the higher GABAergic IPSPs in the A/A genotype could generate a higher positivity leading to a larger P300 amplitude. However, in the contrary direction it is also possible to consider that the reduced GABAergic influences on the glutamatergic system might increase the EPSPs that contribute to P300 generation. In this case, we have to expect that the A/A genotype has a reduced GABAergic activity. Unfortunately, there are no clear results in the literature on the effect of the different genotypes of this promoter polymorphism on the GABRG2 expression. Therefore, we cannot conclude for one of these possible explanations. However, it is a clear result that this polymorphism of the GABRG2 has a very strong effect on the P300 amplitude showing a significant direct or indirect contribution of the GABA activity on the generation of this potential.

In the late component of CNV paradigm, significant difference at anterior-posterior amplitude distribution was observed between groups. In A/A group CNV negativity at parietal scalp regions was greater compared to G carrying group. The results ensure that the trait-influencing allele of the GABRG2 polymorphism might be the presence of G.

5.1.3 DRD2 Polymorphism Effects

DRD2 genotyping revealed that the most common allele was C Ins (91%) and most common phenotype was C Ins/Ins genotype (83.3%) in 72 Turkish males. These results were significantly different from what Arinami et. al., have reported [93] in which the frequency of Ins allele was 78% in 312 normal Japanese subjects; but very close to what Chen et. al., has reported (90%) for Chinese population [102]. This difference might result from the relatively small sample size used in this study, or a real polymorphic difference between genotypes of Turkish and Japanese people. Arinami et. al., have reported decreased frequency of Del allele in schizophrenics, compared to normal individuals. He argued that this might contribute to elevation of D2 receptor density in schizophrenics [93].

In this study, no amplitude difference was observed in any of the paradigms used, however, a significant latency difference (p:0.06) was observed between Ins/Ins genotype group versus Del carrying genotype groups (Ins/Del and Del/Del) in P300 latencies that were elicited to go stimuli. Go P300 latency was significantly reduced in Ins/Ins genotype compared to Del/Del and Ins/Del genotypes. Del allele showed a decrease in promoter strength as compared with the fragment that contained the Ins allele [93] according to luciferase assays. Therefore, in individuals bearing Del allele, one can expect a decrease in promoter activity of DRD2 gene and hence a decrease of brain DRD2 density. Dopaminergic neurons are involved in initiation and execution of movements. It is not surprising that the latency difference was observed for *go* stimuli, which was the task-relevant stimulus that requested a button press. Relatively high density of D2 receptors may explain why Ins/Ins genotypes generated relatively earlier P300 response.

In summary, we can state that the most dramatic effects on the P300 and CNV amplitudes were obtained for the GABA-A receptor polymorphism, which shows an

important contribution of the GABAergic system in the generation of both ERP types. In contrast, the NMDA and DRD2 polymorphisms had slight effects on the P300 latency showing that their contribution to the global P300 wave is rather secondary.

5.2 Time-Frequency Results

5.2.1 NMDAR2A Polymorphism Effects

The low transcriptional activity variant of the NMDA receptor leads to an increased evoked alpha response in the Go condition of the Go/NoGo paradigm. In line with this result, in the targets of the novelty paradigm the alpha desynchronization following the stimulus is less strong in this genotype group with relatively long repeat numbers. In short, the low transcriptional activity variant of the NMDA receptor induces an increased alpha power both in the evoked and total activity compared with the short repeat variant. Therefore, we can consider that the effect of the NMDA receptor on alpha generation is rather in the limiting direction. This can be considered in accordance with the alpha desynchronization observed during information processing in the brain.

5.2.2 GABRG2 Polymorphism Effects

In both the Go and NoGo conditions of the Go/NoGo paradigm a significantly lower power is obtained in the total theta activity for the genotype 2 of the GABA-A polymorphism. This finding is in line with the results in the time domain, where the P300 amplitude is lower in the genotype 2. Considering that the theta activity is one of the main contributors of the P300 generation, this result in the time-frequency domain is mainly dependent on the GABA-A effects on the theta oscillations.

Additionally, a significant reduction of the alpha desynchronization is obtained for the Go responses of the Go/NoGo paradigm in the genotype 2. This is also in accordance with the time-domain result considering that the alpha desynchronization reflects a stronger cognitive activity state. The genotype that produces smaller P300 amplitude leads at the same time to a less strong alpha desynchronization.

In addition to the differences in lower frequency ranges, a significantly higher evoked gamma response is obtained for the genotype 2 of the GABA-A polymorphism. This result seems to be contradictory to the lower P300 amplitudes obtained in this genotype, because the increase in the early-evoked gamma response has been related to higher level of attention. However, as in the present study trait-effects but not state-effects are investigated, it is possible to consider that the genotype 1 group with lower amplitude evoked gamma response compensates this inefficient activity in the early sensory processing with a higher activation of the late P300 mechanisms.

5.2.3 DRD2 Polymorphism Effects

Although no clear amplitude effects were observed for the DRD2 polymorphism in the time domain, a range of significant changes was obtained in the time-frequency components. The evoked theta responses to the Go stimuli of the Go/NoGo paradigm were significantly higher for the genotype 2 that represents the lower promoter activity. In a similar direction, the total delta oscillations to target stimuli of the novelty paradigm were higher in the genotype 2.

As it has been shown in in-vitro studies that this genotype corresponds to a lower transcriptional activity, a possible explanation of these results is that decreased expression of DRD2, which acts mainly as an inhibitory receptor, is correlated with a higher synchronization of oscillatory activities in delta and theta ranges.

The topography of the gamma effect corresponds well to the distribution of DRD2 in the brain. Main gamma finding was observed in the frontal region and was in the same direction as the delta and theta effects, which means that increased total gamma amplitudes

were obtained for the lower promoter activity group. Hence, it seems that the level of DRD2 activity limits the synchronization of neuronal oscillation within all three frequency bands of delta, theta and gamma oscillations. This might be important in suppressing noisy states and selecting the stronger activation pattern out of a large set of representations.

6. CONCLUSION

Main results of this thesis are that the GABAergic activity has strongest effects on P300 generation. The effects of NMDA and DRD2 receptors are less significant on P300 wave. However, the time-frequency decomposition of the ERP data shows that a range of other effects can be observed for all three polymorphisms in specific frequency bands, which are not reflected in the raw time-domain representation of the data. The distinct functional meaning of these specific time-frequency differences cannot be conclusively stated at this level of analysis, although some tentative interpretation is possible. However, the results show that extended analyses on the correlations of genetic differences among normal population on electrophysiological parameters may extend our view on the genetic bases of cognitive activities.

7. REFERENCES

1. Sutton, S., M. Braren, J. Zubin, and E. R. John, "Evoked-potential correlates of stimulus uncertainty," *Science*, Vol. 150, pp. 1187-1188, Nov 1965.

2. Frodl-Bauch, T., R. Bottlender, and U. Hegerl, "Neurochemical substrates and neuranatomical generators of the event-related P300," *Neuropsychobiology*, Vol. 40, pp. 86– 94, 1999.

3. Van Beijsterveldt, C. E, and D. I. Boomsma, "Genetics of the human electroencephalogram (EEG) and event-related brain potentials (ERPs): a review, *Hum Genet.*, Vol. 94;4, pp. 319-330, Oct 1994.

4. Stassen, H. H., D. T. Lykken, P. Propping, G. Bomben, "Genetic determination of the human EEG: Survey of recent results on twins reared together and apart," *Hum Genet.*, Vol.80, pp. 165-176, Oct 1998.

5. Demiralp, T., A. Ucok, M. Devrim, Ü. Isoglu-Alkac, A. Tecer, and J. Polich, "N2 and P3 components of event-related potential in first episode schizophrenic patients: Scalp topography, medication, and latency effects," *Psychiatry Research*., Vol. 111, pp. 167-179, 2002.

6. Almasy, L., B. Porjesz, J. Blangero, A. Goate, H. J. Edenberg,, D. B. Chorlian, S. Kuperman, S. J. O'Connor, J. Rohrbaugh, L. O. Bauer, T. Foroud, J. P. Rice, T. Reich, and H. Begleiter, "Genetics of Event-Related Brain Potentials in Response to a Semantic Priming Paradigm in Families with a History of Alcoholism," *Am. J. Hum. Genet*, Vol. 68, pp. 128–135, 2001.

7. Porjesz , B., H. Begleiter, K. Wang, L. Almasy, D. B. Chorlian, A. T. Stimus, S. Kuperman, S. J. O'Connor, J. Rohrbaugh, L. O. Bauer, H. J. Edenberg, A. Goate, J. P. Rice, T. Reich, "Linkage and linkage disequilibrium mapping of ERP and EEG phenotypes," *Biological Psychology* , Vol. 61, pp. 229-248, 2002.

8. Carlson, S. R., J. Katsanis, W. G. Iacono, and A. K. Mertz, "Substance dependence and externalizing psychopathology in adolescent boys with small, average, or large P300 event-related potential amplitude," *Psychophysiology*, Vol. 36, pp. 583–590, 1999.

9. Polich, J., and A. Kok, "Cognitive and biological determinants of P300: An integrative review," *Biological Psychology*, Vol. 41, pp. 103–146, 1995.

10. Knight, R., "Contribution of human hipocampal region to novelty detection," *Nature*, Vol. 383, pp. 256-259, 1996.

11. Demiralp, T., A. Ademoğlu, M. Comerchero, and J. Polich, "Wavelet analysis of P3a and P3b," *Brain Topography,* Vol. 13, pp. 251-267, 2001.

12. Demiralp, T., and A. Ademoğlu b, "Decomposition of event-related brain potentials into multiple functional components using wavelet transform," *Clinical Electroencephalography,* Vol. 32, pp. 122-138, 2001.

13. Ademoglu A., T. Demiralp, J.Yordonova, V. Kolev, and M.Devrim, "Decomposition of event-related brain potentials into multicomponents using wavelet transform," *Applied Sig Process*, Vol. 5, pp. 142-151, 1998.

14. Polich, J., "P300 in clinical applications: Meaning, method, and measurements," in *Electroencephalography: Basic Principles, Clinical Applications, and Related Fields,* pp. 1073-1091, Baltimore: Williams & Wilkins, 1999.

15. Donchin, E., and M. G. H. Coles, "Is the P300 component a manifestation of context updating?," *Behav. Brain Sci.*, Vol. 11, pp. 355-372, 1988.

16. McCarthy, G., C. C. Wood, P. D. Williamson, and D. Spencer, "Task-dependent field potentials in human hippocampal formation," *Journal of Neuroscience*, Vol. 9, pp. 4235-4268, 1989.

17. Johnson, R., "Scalp-recorded P300 activity in patients following unilateral temporal lobectomy," *Brain,* Vol. 111, pp. 1517-1529, 1988.

18. Courchesne, E., S. A. Hillyard, R. Galambos, "Stimulus novelty, task relevance, and the visual evoked potential in man," *Electroencephalogr. Clin. Neurophysiol.*, Vol. 39, pp. 131-143, 1975.

19. Knight, R., M. Grabowecky, D. Scabini, "Role of human prefrontal cortex in attention control," *Advances in Neurology*, Vol. 66, pp. 21-34, 1995.

20. Desimone, R., E. K. Miller, L. Chelazzi, and A. Lueshchow, "Multiple memory stsrems in the visual cortex," in *The Cognitive Neurosciences*, pp. 475-486, Cambridge, MA: MIT, 1995.

21. Overtoom, C. C., M. N. Verbaten, C. Kemner, L. Kenemans, H. van Engeland, and J. Buitelaar, "Associations between event-related potentials and measures of attention and inhibition in the Continuous Performance Task in children with ADHD and normal controls," *Journal of the American Academy of Child and Adolescent Psychiatry*, Vol. 37, pp. 977–985, 1998.

22. Strandburg, R. J., J. T. Marsh, W. Brown, R. Asarnow, J. Higa, and R. Harper, "Continuous-processing-related event-related potentials in children with attention deficit hyperactivity disorder," *Biological Psychiatry*, Vol. 40, pp. 964–980, 1996.

23. Donchin, E., G. A. Miller, and L. A. Farwell, "The endogenous components of the event-related potential—A diagnostic tool?," *Progress in Brain Research*, Vol. 70, pp. 87–102, 1986.

24. McCarthy, G., and E. Donchin, "A metric for thought: A comparison of P300 latency and reaction time," *Science*, Vol. 211, pp. 77–80, Jan 1981.

25. Johnstone, S. J., R. J. Barry, J. W. Anderson, and S. F. Coyle, "Age-related changes in child and adolescent event-related potential component morphology, amplitude and latency to standard and target stimuli in an auditory oddball task," *International Journal of Psychophysiology*, Vol. 24, pp. 223–238, 1996.

26. Jonkman, L. M., C. Kemner, M. Verbaten, H. van Engeland, G. Camfferman, and J. Buitelaar, "Attentional capacity, a probe ERP study: Differences between children with attention-deficit hyperactivity disorder and normal control children and effects of methylphenidate," *Psychophysiology*, Vol. 37, pp. 334–346, 2000.

27. Begleiter, H., and B. Porjesz, "Neurophysiological phenotypic factors in the development of alcoholism," in *The Genetics of Alcoholism*, pp. 269–293, New York: Oxford Univ., 1995.

28. Segalowitz, S. J., and K. L. Barnes, "The reliability of ERP components in the auditory oddball paradigm," *Psychophysiology*, Vol. 30, pp. 451–459, 1993.

29. Winterer, G., M. Ziller, H. Dorn, K. Frick, C. Mulert, N. Dahhan, W.M. Herrmann, and R. Coppola, "Cortical activation, signal-to-noise ratio and stochastic resonance during information processing in man," *Clin. Neurophysiol*., Vol. 110, pp. 1193–1203, 1999.

30. O'Connor, S., S. Morzorati, J. C. Christian, and T. K. Li, "Heritable features of the auditory oddball event-related potential: Peaks, latencies, morphology and topography," *Electroencephalogr. Clin. Neurophysiol.*, Vol. 92, pp. 115–125, 1994.

31. Katsanis, J., W. G. Iacono, M. K. McGueand, and S. R. Carlson, "P300 event-related potential heritability in monozygotic and dizygotic twins," *Psychophysiology*, Vol. 34, pp. 47–58, 1997.

32. Van Beijsterveldt, C. E., P. C. Molenaar, E. J. de Geus, and D. I. Boomsma, "Individual differences in P300 amplitude: A genetic study in adolescent twins,". *Biol. Psychol.,* Vol. 47, pp. 97–120, 1998.

33. Almasy, L., B. Porjesz, J. Blangero, D. B. Chorlian, S. J. O'Connor, S. Kuperman, J. Rohrbaugh, L. O. Bauer, T. Reich, J. Polich, and H. Begleiter, "Heritability of event-related brain potentials in families with a history of alcoholism," *Am. J. Med. Genet*, Vol. 88, pp. 383–390, 1999.

34. Van Beijsterveldt, C.E.M., G. C. M. van Baal, "Twin and family studies of the human electroencephalogram: A review and a meta-analysis," *Biological Psychology*, Vol. 61, pp. 111-138, 2002.

35. Van Beijsterveldt, C. E., P. C. Molenaar, E. J. de Geus, and D. I. Boomsma, "Heritability of human brain functioning as assessed by electroencephalography," *Am. J. Hum. Genet.*, Vol. 58, pp. 562–573, 1996.

36. Hansell, N. K., M. J. Wright, G. M. Geffen, L. B. Geffen, G. A. Smith, and N. G. Martin, "Genetic influence on ERP slow wave measures of working memory," *Behavior. Genetics*, Vol. 31, pp. 604-614, Nov 2001.

37. Anokhin, A. P., G. C. M. van Baal, C. E. M. van Beijsterveldt, E. J. C. de Geus, J. Grant, and D. I. Boomsma, "Genetic Correlation Between the P300 Event-Related Brain Potential and the EEG Power Spectrum," *Behavior Genetics*, Vol. 31, pp. 545-554, Nov 2001.

38. Kolev, V., T. Demiralp, J. Yordanova, A. Ademoglu, and U. Isoglu-Alkaç, "Time–frequency analysis reveals multiple functional components during oddball P300," *NeuroReport*, Vol. 8, pp. 2061–2065, 1997.

39. Stampfer, H.G., and E. Basar, "Does frequency analysis lead to better understanding of human event-related potentials?," *Int. J. Neurosci.*, Vol. 26, pp. 181– 196, 1985.

40. Demiralp, T., A. Ademoglu, M. Schurmann, C. Basar-Eroglu, and E. Basar, "Detection of P300 waves in single trials by the wavelet transform (WT)," *Brain Lang*. Vol. 66, pp. 108–128, 1999.

41. Hendrickson, D .E., and A.E. Hendrickson, "The biological basis of individual differences in intelligence," *Person. Individ. Diff.,* Vol. 1, pp. 3–33, 1980.

42. Winterer, G., and D. Goldman, “Genetics of human prefrontal function,” *Brain Research Reviews*, Vol. 43, pp. 134–163, 2003.

43. McCarley, R. W., S. F. Faux, M. E. Shenton, P. G. Nestor, and J. Adams, “Event-related potentials in schizophrenia: their biological and clinical correlates and a new model of schizophrenic pathophysiology,” *Schizophr. Res.*, Vol. 4, pp. 209-231, Mar-Apr 1991.

44. Javitt, D. C., C. E. Schroeder, M. Steinschneider, J. C. Arezzo, W. Ritter, and H.G. Jr. Vaughan, “Cognitive event-related potentials in human and non-human primates: Implications for the PCP/NMDA model of schizophrenia,” *Electroencephalogr. Clin. Neurophysiol. Suppl.*, Vol. 44, pp. 161-175, 1995.

45. Timsit-Berthier, M., “Contingent negative variation psychiatry,” in *Cerebral Psychophysiology: Studies in Event Related Potentials,* pp 429-438*, Amsterdam*: Elsevier, 1986.

46. Stanzione P, F. Fattapposta, P. Giunti, C. D'Alessio, M. Tagliati, C. Affricano, and G. Amabile, “P300 variations in Parkinsonian patients before and during dopaminergic monotherapy: A suggested dopamine component in P300,” *Electroencephalogr. Clin. Neurophysiol.*, Vol. 80, pp. 446-453, Sep-Oct 1991.

47. Callaway, E., “The pharmacology of human information processing,” *Psychophysiology*, Vol. 20, pp. 359-370, Jul 1983.

48. Dierks T., L. Frolich, R. Ihl, and K. Maurer, "Event-related potentials and psychopharmacology: Cholinergic modulation of P300,” *Pharmacopsychiatry,* Vol. 27, pp. 72-74, Mar 1994.

49. Joseph, K.C., and N. Sitaram, “The effect of clonidine on auditory P300,” *Psychiatry Res.*, Vol. 28, pp. 255-262, 1989.

50. Jonkman, L. M., C. Kemmer, M. N. Verbaten, H. S. Koelega, G. Camfferman, R. J. Van der Gaag, J. K. Buitelaar, H. Van England, “Effects of methylphenidate on event-related potentials and performance of attention-deficit hyperactivity disorder children in auditory and visual selective attention tasks,” *Biol. Psychiatry*, Vol. 41, pp. 690-702, 1997.

51. Aston-Jones, G., S. L. Foote, F. E. Bloom, “Task relevant late positive component of the auditory event-related potential in monkeys resembles P300 in humans,” *Science*, Vol. 223, pp. 186-188, 1984.

52. Meador, K. J., “Cholinergic, serotonergic, and GABAergic effects on the ERP,” *Electroencephalogr. Clin. Neurophysiol. Suppl.*, Vol. 44, pp. 151–155, 1995.

53. Goldberg, T. E., and D. R. Weinberger, “Genes and the parsing of cognitive processes,” *TRENDS in Cognitive Sciences* Vol. 8, pp. 326-335, July 2004.

54. Fossella, J., T. Sommer, J. Fan, Y. Wu, J. M. Swanson, D. W. Pfaff and M. I. Posner, “Assessing the molecular genetics of attention networks,” *BMC Neuroscience*, Vol.3:14, pp. 1-13, 2002.

55. Fan, J., J. Fossella, T. Sommer, Y. Wu, and M. I. Posner, “Mapping the genetic variation of executive attention onto brain activity,” *PNAS*, Vol. 100, pp. 7406–7411, June 2003.

56. Leonard, S., "Association of promoter variants in the a7 nicotinic acetylcholine receptor subunit gene with an inhibitory deficit found in schizophrenia," *Arch. Gen. Psychiatry*, Vol. 59, pp. 1085–1096, 2002.

57. Egan, M. F., "Effect of COMT val108/158 met genotype on frontal lobe function and risk for schizophrenia," *Proc. Natl. Acad. Sci. U. S. A.,* Vol. 98, pp. 6917–6922, 2001.

58. Berman, K. F., "Physiological activation of a cortical network during performance of the Wisconsin Card Sorting Test: A positron emission tomography study," *Neuropsychologia*, Vol. 33, pp. 1027–1046, 1995.

59. Jackson, M., G. Steers, P. N., Leigh, and K. E. Morrison, "Polymorphisms in the glutamate transporter gene EAAT2 in European ALS patients," *J. Neurology,* Vol. 246, pp. 1140-1144, 1999.

60. Sander, T., W. Berlin , A. Ostapowicz, J. Samochowiec, N. Gscheidel, and M. R. Hoehe, "Variation of the genes encoding the human glutamate EAAT 2, serotonin and dopamine transporters and susceptibility to idiopathic generalized epilepsy," *Epilepsy Research*, Vol. 41, pp. 75–81, 2000.

61. Ito, M., I. Ohmoria, T. Nakahori, M. Ouchida, and Y. Ohtsuka, "Mutation screen of GABRA1, GABRB2 and GABRG2 genes in Japanese patients with absence seizures," *Neuroscience Letters*, Vol. 383, pp. 220–224, 2005.

62. Meltzer H. Y., "Biological studies in schizophrenia," *Schizophr. Bull.*, Vol. 13, pp. 77–107, 1987.

63. Knable, M. B., and D. R. Weinberger, "Dopamine, the prefrontal cortex and schizophrenia," *J. Psychopharmacol.*, Vol. 11, pp. 123–131, 1997.

64. Goldman-Rakic, P. S., and L. D. Selemon, "Functional and anatomical aspects of prefrontal pathology in schizophrenia," *Schizophr. Bull.,* Vol. 23, pp. 437–458, 1997.

65. Dean, B., T. Hussain, W. Hayes, E. Scarr, S. Kitsoulis, C. Hill, K. Opeskin, and D. L. Copolov, "Changes in serotonin2A and GABAA receptors in schizophrenia: Studies on the human dorsolateral prefrontal cortex," *J. Neurochem.*, Vol. 72, pp. 1593–1599, 1999.

66. Owen, M. J., M. O'Donovan, I. I. Gottesman, *Psychiatric Genetics and Genomics*, Oxford: Oxford University, 2003.

67. Tani A, R. Kikuta, and K. Itoh, "Polymorphism analysis of the upstream region of the human NMDA receptor subunit NR1 gene (GRIN1): Implications for schizophrenia," *Schizophr Res.,* Vol. 58;1, pp. 83-86, Nov 2002.

68. Mundo, E., S. Tharmalingham, and M. Neves-Pereira, "Evidence that the NMDA subunit 1 receptor gene (GRIN1) confers susceptibility to bipolar disorder," *Mol Psychiatry*., Vol. 8, pp. 241-245, Feb 2003.

69. Itokawa, M., K. Yamada, K. Yoshitsugu, T. Toyota, T. Suga, and H. Ohba, "A microsatellite repeat in the promoter of the N-methyl-D-aspartate receptor 2A subunit (GRIN2A) gene suppresses transcriptional activity and correlates with chronic outcome in schizophrenia," *Pharmacogenetics*, Vol. 13, pp. 271–278, 2003.

70. Hong, C. J., Y. W. Yu, C. H. Lin, C. Y. Cheng, and S. J. Tsai, "Association analysis for NMDA receptor subunit 2B (GRIN2B) genetic variants and psychopathology and clozapine response in schizophrenia," *Psychiatr. Genet*., Vol. 11, pp. 219-222, Dec 2001.

71. Wernicke, C., J. Samochowiec, L. G. Smchmidt, G. Winterer, "Polymorphisms in the NMDA receptor 1 and 2B subunits are associated with alcoholism-related traits," *Biol. Psychiatry*, Vol. 54;9, pp. 922-928, Nov 2003.

72. Chiu, H. J., Y. C. Wang, and Y. J. Liou, "Association analysis of the genetic variants of the NMDA receptor subunit 2b (NR2b) and treatment-refractory schizophrenia in the Chinese," *Neuropsychobiology*, Vol. 47, pp. 178-181, 2003.

73. Begni, S., s. Morashi, S. Bignotti, F. Fumagalli, L. Rillosi, "Association between the G1001C polymorphism in the GRIN1 gene promoter region and schizophrenia," *Biol Psychiatry*, Vol. 53, pp. 617-619, Apr 2003.

74. Iwayama-Shigeno, Y., K. Yamada, M. Itokawa, T. Toyota, J. M. A. Meerabux, Y. Minabe, N. Mori, T. Inada, and T. Yoshikawa, "Extended analyses support the association of a functional (GT)n polymorphism in the GRIN2A promoter with Japanese schizophrenia," *Neuroscience Letters* , Vol. 378, pp. 102–105, 2005.

75. Zhao, X., H. Li, Y. Shi, R. Tang, W. Chen, J. Liu, G. Feng, J. Shi, L. Yan, H. Liu, and L. He, "Significant association between the genetic variations in the 5' end of the N-Methyl-D-Aspartate receptor subunit gene GRIN1 and schizophrenia," *Biol. Psychiatry*, Vol. 59, pp. 747–753, 2006.

76. Itokawa, M., K. Yamada, and Y. Iwayama-Shigeno, "Genetic analysis of a functional GRIN2A promoter (GT)n repeat in bipolar disorder pedigrees in humans," *Neurosci. Lett.*, Vol. 10:345, pp. 53-56, Jul 2003.

77. Qin, S., X. Zhao, Y. Pan, J. Liu, G. Feng, J. Fu, J. Bao, "An association study of the N-methyl-D-aspartate receptor NR1 subunit gene (GRIN1) and NR2B subunit gene (GRIN2B) in schizophrenia with universal DNA microarray," *Eur. J. Hum. Genet*., Vol. 13, pp. 807–814, Jul 2005.

78. Foley, P. F., E. W. Loh, D. J. Innes, S. M. Williams, A. E. Tannenberg, "Association studies of neurotransmitter gene polymorphisms in alcoholic Caucasians," *Ann. N. Y. Acad. Sci*., Vol. 1025, pp. 39–46, Oct 2004.

79. Samochowiec J., A. Grzywacz, J. Kucharska-Mazur, A. Samochowiec, J. Horodnicki, J. Pelka-Wysiecka, S. Syrek, "Family-based and case-control association studies of glutamate receptor GRIK3 Ser310Ala polymorphism in Polish patients and families with alcohol dependence," *Neurosci. Lett.,* Vol. 396, pp. 159–162, Mar 2006.

80. Watanabe, M., Y. Inoue, K. Sakimura, , and M. Mishina, "Distinct spatio-temporal distributions of the NMDA receptor channel subunit mRNAs in the brain," *Annal. N.Y. Acad. Sci.*, Vol. 707, pp. 463-466, 1993.

81. Johnston, G.A., "GABA receptors: As complex as ABC? Australian society for clinical and experimental pharmacologists and toxicologists," *Clinical and Experimental Pharmacology and Physiology*, Vol. 21, pp. 521-526, 1994.

82. McDonald, R. L., and R. W. Olsen, "GABAA receptor channels," *Annu. Rev. Neurosci.,* Vol. 17, pp. 569–602, 1994.

83. Massat, I., D. Souery, G. N. Papadimitriou, and J. Mendlewicz, "The GABAergic hypothesis of mood disorders," in *Bipolar Disorders*, pp. 143–165, New York: Marcel Dekker, 2000.

84. Pearce, R. A., "Physiological evidence for two distinct $GABA_A$ responses in rat hippocampus," *Neuron*, Vol. 10, pp. 189-200, 1993.

85. Cobb, S. R., E. H. Buhl, K. Halasy, and O. Paulsen, "Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons," *Nature,* Vol. 378, pp. 75-78, 1995.

86. Cheng, A. T., E. W. Loh, C. Y. Cheng, Y. C. Wang, and Y. P. Hsu, "Polymorphisms and intron sequences flanking the alternatively spliced 8-amino-acid exon of gamma2 subunit gene for GABAA receptors," *Biochem. Biophys. Res. Commun*., Vol. 238, pp. 683-685, Sep 1997.

87. Loh, E.W., and D. Ball, "Role of the GABAAb2, GABAAa6, GABAAa1 and GABAAg2 receptor subunit genes cluster in drug responses and the development of alcohol dependence," *Neurochemistry International*, Vol. 37, pp. 413-423, 2000.

88. Chou, I. C., C. T. Peng, C. C. Huang, J. J. P., Tsai, F. J. Tsai, and C. H. Tsai, "Association analysis of gamma 2 Subunit of gamma-aminobutyric acid type A receptor polymorphisms with febrile seizures," *Pediatric Research*, Vol. 54, pp. 26-29, 2003.

89. Harris, R. A., W. R. Proctor, S. J. McQuilkin, R. L. Klein, M. P. Mascia, V. Whatley, P. J. Whiting, and T. V. Dunwiddie, *Alcohol. Clin. Exp. Res.*, Vol. 19, pp. 226–232, 1995.

90. Winterer, G., M. Smolka, J. Samochowiec, C. Mulert, M. Ziller, R. Mahlberg, Y. Wuebben, J. Gallinat, H. Rommelspacher, W. M. Herrman, and T. Sander, "Association analysis of GABAA beta2 and gamma2 gene polymorphisms with event-related prefrontal activity in man," *Hum. Genet.*, Vol., 107;5, pp. 513-518, Nov 2000.

91. Lee, T., P. Seeman, A. Rajput, I. J. Farley, and O. Hornykiewicz, *Nature (London),* Vol. 273, pp. 59-61, 1978.

92. Seeman, P., C. Ulpian, C. Bergeron, P. Riederer, K. Jellinger, E. Gabriel, G. P. Reynolds, and W. W. Tourtellotte, "Bimodal distribution of dopamine receptor densities in brains of schizophrenics," *Science* , Vol. 225, pp. 728-730, 1984.

93. Arinami T, M. Gao, H. Hamaguchi, M. Toru, "A functional polymorphism in the promoter region of the dopamine D2 receptor gene is associated with schizophrenia," *Hum Mol Genet*, Vol. 6, pp. 577-582, Apr 1997.

94. Noble, E. P., "The gene that rewards alcoholism," *Sci. Am. Sci. Med.*, Vol. 3, pp. 52–61, 1996.

95. Noble, E. P., "D2 Dopamine receptor gene in psychiatric and neurologic disorders and its phenotypes," *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics)*, Vol. 116B, pp. 103–125, 2003.

96. Tanda, G., F. E. Pontieri, and G. Di Chiara, "Cannabionoid and heroinactivation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism," *Science*, Vol. 27;276, pp. 2048-2050, 1997.

97. Wong, A. H., C. E. Buckle, H. H. Van Tol, "Polymorphisms in dopamine receptors: What do they tell us?," *Eur J. Pharmacol.*, Vol. 410, pp. 183-203, 2000.

98. Chen, W. J., C. H. Chen, J. Huang, Y. P. P. Hsu, S. V. Seow, C. C. Chen, and A. T. A Cheng, "Genetic polymorphisms of the promoter region of dopamine D2 receptor and dopamine

transporter genes and alcoholism among four aboriginal groups and Han Chinese in Taiwan," *Psychiatric Genetics*, Vol. 11, pp. 187-195, 2001.

99. Li, T., X. Liu, J. Zhao, X. Hu, D. M. Ball, E. W. Loh, P. C. Sham, and D. A. Collier, "Allelic association analysis of the dopamine D2, D3, 5-HT2A and GABAAg2 receptors and serotonin transporter genes with heroin abuse in Chinese subjects," *Am. J. Med. Genet.*, Vol. 114, pp. 329–335, 2002.

100. Sohn, Y. H., G. W. Kim, K. Huh, and J. S. Kim, "Dopaminergic influences on the P300 abnormality in Parkinson's disease," *J. Neurol. Sci.*, Vol. 158, pp. 83-87, 1998.

101. Lin, C. H., Y. W. Yu, T. J. Chen, S. J. Tsa, and C. J. Hong, "Association analysis for dopamine D2 receptor Taq1 polymorphism with P300 event-related potential for normal young females," *Psychiatr. Genet.*, Vol. 11, pp. 165-168, Sep 2001.

102. Chen, T. J., Y. W. Yu, J. Y. Chen, Y. C. Wang, M. C. Chen, C. J. Hong, and S. J. Tsai, "Association analysis of two dopamine D2 receptor gene polymorphisms and P300 event-related potential in depressive patients," *Neuropsychobiology*, Vol. 46, pp. 141-144, 2002.

103. Mullis, K., F. Faloona, S. Scharf, R. Saiki, G. Horn, H. Erlich, "Specific enzymatic amplification of DNA in vitro: The polymerase chain reaction," *Cold Spring. Harb. Symp. Quant. Biol*., Vol. 51, pp. 263–273, 1986.

104. Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, H. A. Erlich, "Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase," *Science*, Vol. 239, pp. 487–491, Jan 1988.

105. Sanger, F., S. Nicklen, A. R. Coulson, "DNA sequencing with chain-terminating inhibitors," *Proc. Natl. Acad. Sci. U. S. A.*, Vol. 74, pp. 5463–5467, Dec 1977.

106. Fiorentino, F., M. C. Magli, D. Podini, A. P. Ferraretti, A. Nuccitelli, N. Vitale, M. Baldi and L. Gianaroli, "The minisequencing method: An alternative strategy for preimplantation genetic diagnosis of single gene disorders," *Molecular Human Reproduction,* Vol. 9, pp. 399-410, 2003.

107. Bruin, K. J., A. A. Wijers, "Inhibition, response mode, and stimulus probability: A comparative event-related potential study," *Clin. Neurophysiol.*, Vol. 113, pp. 1172-1182, Jul 2002.

108. Fallgater, A. J., and W. K. Strik, "The NoGo-anteriorization as aneurophysiological standard-index for cognitive response control," *Int. J. Psychophysiol.,* Vol. 32, pp. 233 -238, 1999.

109. Fallgatter, A. J., "Electrophysiology of the prefrontal cortex in healthy controls and schizophrenic patients," *J. Neural Transm.*, Vol. 108, pp. 679-694, 2001.

110. Courchesne, E., B. A. Kilman, and A. Galambos, "Stimulus novelty, task relevance and the visual evoked potential in the man," *Electroencephalogr. Clin. Neurophysiol*., Vol. 59, pp. 238-248, 1984.

111. Walter, W. G., and R. Cooper, "Contingent Negative Variation: An electric sign of sensorimotor association and expectancy in the human brain," *Nature,* Vol. 203, pp. 380-384, Jul 1964.

112. Regan, D., *Human* B*rain Electrophysiology: Evoked potentials and evoked magnetic fields in science and medicine*, pp. 217-218, Amsterdam: Elsevier, 1989.

113. Başar, E., C. Başar-Eroğlu, S. Karakaş, and M. Schürmann, "Oscillatory brain theory: A new trend in neuroscience," *IEEE Engineering in Medicine and Biology*, Vol. 18, pp. 56-66, 1999.

114. Başar-Eroğlu, C., E. Başar, T. Demiralp, M. Schürmann, "P300 response: Posibble psychological correlates in delta, and theta frequency channels," *International Journal of Psychophysiology*, Vol. 24, pp. 101-112, 1992.

115. Klimesch, W., "EEG-alpha rhythms and memory processes," *International Jjournal of Psychophysiology*, Vol. 26, pp. 319-340, 1997.

116. Yordonova, J., V. Kolev, and J. Polich, "P300 and alpha event-related desynchronization (ERD)," *Psychophysiology*, Vol. 38, pp. 143-152, 2001.

117. Llinnas, R., and U. Ribary, "Coherent 40-Hertz oscialltion characterizes dream state in humans," *Proceedings of the National Academy of Sciences (USA)*, Vol. 90, pp. 2078-2081, 1993.

118. Miyamoto, Y., K. Yamada, Y. Noda, H. Mori, M. Mishina, and T. Nabeshima, "Hyperfunction of dopaminergic and serotonergic neuronal systems in mice lacking the NMDA receptor epsilon 1 subunits," *J. Neurosci.*, Vol. 21, pp. 750–757, 2001.