Mismatch Negativity in Subjects at High Risk for Alcoholism

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Background: Evidence from P300 studies in both alcohol-dependent and high-risk (HR) individuals suggests that the reduced P300 amplitudes that often characterize these individuals may reflect a deficit in inhibition (hyperexcitability) in the central nervous system. In this context, the mismatch negativity (MMN) was investigated in the male and female HR offspring of alcohol-dependent fathers and a mixed-sex, low-risk (LR) control group.

Methods: As subjects read popular materials, they received a random sequence of 500 binaurally presented tones of 600 Hz and 1600 Hz. The designation of the rare stimulus (n = 60 trials) and frequent stimulus (n = 440 trials) was alternated across subjects.

Recordings of MMN were made from 61 electrodes; risk group comparisons were restricted to the five frontal midline electrodes: Fpz, Afz, Fz, Fcz, and Cz. The MMN was obtained by calculating the integral of the area under the curve for both the frequent and rare waveforms over an interval from 100 to 190 msec and then subtracting the former from the latter.

Results: The primary observation was that MMN responses in the HR group were significantly larger than those in the LR group. In addition, both LR and HR individuals manifested differential responses to the rare and frequent stimuli, and MMN responses in both groups were largest at Fcz and smallest at Fpz.

Discussion: The results indicate that individuals at high risk for alcoholism differ electrophysiologically from LR controls. These differences were manifested as larger magnitudes of the MMN. The findings suggest the possibility that as measured by the MMN, individuals at high risk for alcoholism may be characterized by a deficit in inhibition (excessive neural excitation). The presence of these preexisting central nervous system states may lead to ethanol use for self-medication, which then may facilitate the development of both tolerance to and dependence on ethanol.

Key Words: Mismatch Negativity, NMDA, High-Risk Alcoholism.
The major excitatory neuronal systems in the central nervous system (CNS) use glutamate or related excitatory amino acids as their neurotransmitters. It seems that one category of glutamate receptors, N-methyl-D-aspartate (NMDA), may be responsible for the diverse effects of ethanol on the CNS. It has been postulated that a potential neurobiochemical basis for human alcoholism derives from the observation that ethanol interferes with glutamatergic neurotransmission by blocking the NMDA receptor (Tsai et al., 1995). Prolonged inhibition of NMDA receptors by ethanol results in the development of supersensitivity, whereas acute removal of ethanol markedly increases postsynaptic neuronal activity. Indeed, neurobiological effects of alcohol, such as intoxication, withdrawal seizures, delirium tremens, and Wernicke-Korsakoff syndrome (associated with thiamine deficiency), all can be understood as a spectrum of consequences of ethanol’s effects on the glutamatergic system. Although there is growing evidence to indicate both neuroelectrical and behavioral correlates of CNS inhibitory deficits in HR subjects, currently there is no experimental evidence for increased glutamatergic activity (which would suggest an inhibitory deficit or hyperexcitability) in nondependent HR individuals. One possible index for glutamatergic activity in the human brain is an ERP component named the mismatch negativity (MMN) that can be elicited by infrequent, physically deviant sounds that occur in a series of unattended standard auditory stimuli (for review, see Näätänen, 1992, 1996). MMN generation involves a neural, sensory-memory representation of the standard stimulus (Kraus et al., 1995; Näätänen et al., 1989; 1993; Sams et al., 1985; Tervaniemi et al., 1994). This sensory-specific mechanism reflects preconscious detection of stimulus deviation that activates frontal mechanisms related both to conscious discrimination of stimulus deviation and to the orienting response (Näätänen and Michie, 1979). It has been suggested that the activation of NMDA receptors amplifies the neuroelectrical response to auditory stimuli and converts a probability of firing code into an intensity of firing code (Daw, 1993). Furthermore, blocking NMDA receptors abolishes both the epidurally recorded MMN (after peripheral injection) and its intracortical correlate (after direct intracortical injection; Javitt et al., 1991, 1992).

In the present investigation we hypothesized that MMN in HR individuals would be augmented as a consequence of either NMDA activation or disturbed homeostasis between excitatory and inhibitory neural transmission systems. MMN responses were compared between a group of HR individuals and a low-risk (LR) control group. An increased MMN in the HR group compared with the controls could provide evidence to suggest a deficit in inhibitory cortical processes (or hyperexcitability) in this population, as well as clues about the genetic contribution to the predisposition to develop alcoholism.

Subjects
Each of the two subject groups contained nonalcoholic young males and females. The HR group (n = 16) contained 11 males and 5 females ranging from 17 to 26 years of age (mean = 22.8, SD = 2.52). The LR control group (n = 22) contained 10 males and 12 females ranging from 19.6 to 30.3 years (mean = 24.3, SD = 3.44). All subjects were right-handed. LR individuals were recruited either through newspaper ads or via notices posted in the Health Science Center. In contrast, HR subjects had fathers who had been diagnosed as alcohol-dependent (DSM-III-R) and who had been hospitalized for alcoholism. Initially, the prospective subjects filled out a questionnaire that described the alcohol and drug use and the medical and psychiatric histories for both themselves and their relatives. Participation in the study depended on the responses to the questionnaire. Inclusion in the HR group required that at least the prospective subject’s father be classified as alcohol dependent (DSM III-R); however, a high incidence of alcoholism in the first- and second-degree relatives of these individuals also was sought. An individual was excluded from the study if his or her mother was alcoholic or had ingested alcohol during pregnancy (Savage et al., 1991). Prospective candidates for the LR group were rejected if any of their first- or second-degree relatives were diagnosed as alcoholic, whereas candidates for either group were rejected if they had major medical problems, were taking medication that affected the central nervous system, or had a history of psychiatric problems and/or drug abuse. On meeting the aforementioned criteria, each subject was invited to the laboratory wherein he or she underwent a detailed psychiatric interview that focused on questions of drug and alcohol use (quantity/ frequency data) and the medical and psychiatric history for both the subject and his or her first- and second-degree relatives. Table 1 presents the characteristics of each group. The only measure on which the groups differed was the number of drinks per drinking occasion (data presented as mean ± SD): LR = 1.43 ± 1.42 vs. HR = 2.3 ± 1.79, p < 0.007. A drink index (the product of the number of drinking days per month by the number of drinks per occasion) also was included. Although the index was larger in the HR group, the difference was not statistically significant because of the large variability in the measure.

Some of the LR and HR subjects were members of entire families that participated in a national project regarding the genetics of alcoholism (Collaborative Study on the Genetics of Alcoholism). Each participating family member was interviewed with the Semi-Structured Assessment for the Genetics of Alcoholism (Bucholz et al., 1994), which uses both DSM-III-R and Feighner criteria to determine alcoholism. Interviews with the additional family members helped to document the family history information. All subjects were asked to abstain from alcohol for 48 hrs before testing. A breathalyzer test was administered to all subjects on their arrival at the lab. Those who registered a positive blood alcohol level (BAL) were excluded from the study. All subjects were paid for their participation.

| Table 1. Characteristics of the Individuals in the Low-Risk and High-Risk Groups |
|-------------------------------------------------|--------------|--------------|
| Age (years) | 24.3 ± 3.44 | 22.8 ± 2.52 |
| Education (years) | 14.6 ± 2.11 | 13.8 ± 2.18 |
| Days per month | 1.82 ± 1.87 | 5.9 ± 7.89 |
| Drinks per occasion* | 1.43 ± 1.42 | 2.9 ± 2.18 |
| Drink index | 4.2 ± 4.92 | 24.2 ± 37.4 |
| (days/month × drinks/occasion) | 1.43 ± 1.42 | 2.9 ± 2.18 |
| Number of alcoholic relatives | | | |
| Individuals in this group could not have any alcoholic relatives | 2.5 ± 1.36 |

Values are mean ± S.D.
* P < 0.007.
Experimental Design

The subject was seated comfortably in a moderately lighted, temperature-regulated, sound-attenuated chamber (Industrial Acoustics Corp., New York). While reading popular materials, the subject was presented with 500 binaural tones delivered through headphones (model ER-3A Tubephone Insert Earphones with 50 Ω impedance; Etymotic Research, Elk Grove Village, IL). The stimuli consisted of a 600 Hz, low-frequency tone and a 1600 Hz, high-frequency tone that were randomly and consecutively presented with an interstimulus interval (ISI) of 624 msec. Each stimulus had a 60 msec duration (10 msec rise and fall time, 40 msec plateau) and an intensity level of 60 dB sound pressure level.

The designation of the rare tone (n = 60 trials) or frequent tone (n = 440 trials) was alternated across subjects. Each subject wore a fitted electrode cap (Electro-Cap International, Inc., Eaton, OH) that contained 61 electrodes. Statistical analyses were performed on MMN responses recorded from five frontal midline electrodes: Fpz, Afz, Fz, Fcz and Cz. The nasion served as reference and the forehead as ground. Two additional bipolar derivations were used to monitor the vertical and horizontal electro-oculogram. Electrode impedance was always kept below 5 kΩ. EEG activity was amplified 10 K (Sensorium EPA-2 Electrophysiology Amplifier, Shelburne, VT; bandpass 0.02–50 Hz). Baseline activity was continuously sampled at a rate of 256 Hz, beginning 93 msec before stimulus onset and continuing for 531 msec after stimulus onset. The ERPs to each stimulus presentation were monitored continuously. Subjects were asked to sit still.

Data Analysis

Figure 1 presents the ERP waveforms elicited by the frequent and rare stimuli. The waveforms were derived by averaging the artifact-free EEG samples. In each group, N100 was greater in amplitude and longer in duration for the rare stimuli than for the frequent stimuli. In the rare condition, the early part of the negativity possibly reflects the contribution from an enhanced N100 component that may have been less refractory for the infrequently occurring rare tones (Lang et al., 1990; Scherg et al., 1989; Woods, 1995), whereas the later negativity mainly reflects the addition of the MMN.

The MMN is more readily observed in difference waves obtained by subtraction of ERPs elicited by the frequent stimuli from those elicited by the rare stimuli. Figure 2 presents the results of this subtraction. The difference between the waveforms was quantified by calculating the integral of the area under each, over the interval from 100 to 190 msec after stimulus onset (Jääskeläinen et al., 1996). In addition, because several studies have documented a polarity inversion in MMN as one moves from anterior to posterior regions, and because frontal regions contribute significantly to MMN generation (Alho et al., 1994; Näätänen, 1992), the analyses were restricted to responses obtained from the five frontal midline electrodes, namely Fpz, Afz, Fz, Fcz, and Cz.

RESULTS

Initially, independent group t tests were used to determine if there were significant sex-related age differences between the individuals in each group. The results indicated that there were no age differences between males and females in either group (data shown as mean ± SD): LR males = 25.8 ± 3.04 vs. LR females = 23.1 ± 3.42 (not significant); HR males = 22.5 ± 2.93 vs. HR females = 23.3 ± 1.33 (not significant).

Next, for each risk group, a 2 (sex) x 5 (electrode) repeated-measures ANOVA (SAS version 6.11; SAS Institute, Cary, NC) was used to assess whether there were any significant sex-related differences in the measures of area derived from the waveform subtractions. Again, neither

Event-related Potentials (ERPs)

Mismatch Negativity (MMN)

Fig. 1. Responses from low-risk control subjects (left) and high-risk subjects (right) to rare (broken line) and frequent (solid line) stimuli at Fpz, Afz, Fz, Fcz, and Cz. The shaded area represents the difference between the two responses.

Fig. 2. Mismatch negativity (MMN) recorded from low-risk control subjects (left) and high-risk subjects (right) at Fpz, Afz, Fz, Fcz, and Cz. The waveform represents the subtraction of the frequent response from the rare response.
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group analysis yielded a significant sex-related difference. Therefore, because none of the aforementioned analyses revealed sex-related differences, the data from both males and females in each risk group were combined for all subsequent within-group and between-groups comparisons.

MMN responses then were analyzed with a 2 (group) × 5 (electrode) repeated-measures ANOVA. The results demonstrated that MMN was significantly larger in HR individuals compared with LR individuals [F(1,36) = 8.35, p < 0.007].

Figure 3 illustrates the fact that the two risk groups manifested a similar pattern of MMN responses across the five midline electrodes [F(4,114) = 11.78, p < 0.0001, Greenhouse-Geisser ε = 0.481] such that MMN magnitude was largest at Fcz and smallest at Fpz.

To determine whether the two groups differed in their responses to the rare and frequent stimuli, a 2 (group) × 2 (stimulus) × 5 (electrode, repeated factor) mixed-model ANOVA (SAS version 6.11) was used to compare the integral of the area under the curve for each stimulus condition. The analyses yielded significant group [F(1,72) = 6.21, p < 0.02] and stimulus effects [F(1,72) = 52.79, p < 0.0001]. The results indicated (1) that within the interval from 100 to 190 msec poststimulus, there were significant risk group differences in response amplitude, and (2) that in each group the frequent and rare stimuli elicited significantly different responses. In addition, the analyses yielded two significant interactions: group × stimulus [F(1,72) = 5.43, p < 0.03] and electrode × stimulus [F(4,228) = 6.38, p < 0.003, Greenhouse-Geisser ε = 0.485]. To identify the variables that contributed to the interactions, additional 2 (group) × 5 (electrode) repeated-measures ANOVAs were performed for each stimulus condition. These analyses revealed that the significant interactions resulted from a significant group effect [F(1,36) = 7.90, p < 0.0079], wherein the HR group had larger responses in the rare stimulus condition, and a significant electrode effect [F(4,114) = 4.57, p < 0.02, Greenhouse-Geisser ε = 0.477], also in the rare stimulus condition, wherein responses from the HR group were larger than those from the LR group at each electrode.

DISCUSSION

The results of the present investigation demonstrate that individuals at high risk for alcoholism differ electrophysiologically from LR controls. These differences are manifested as larger magnitudes of the MMN. Furthermore, it is particularly striking that this augmented ERP component is present in a group of “experimental subjects.” Previously, this laboratory has documented reduced P300 amplitudes in boys at high risk for alcoholism (Begleiter et al., 1984) and in abstinent, chronic alcoholics (Porjesz and Begleiter, 1981, 1983). Moreover, it was demonstrated that the amplitude reduction in alcoholics could not be reversed with prolonged abstinence (Porjesz and Begleiter, 1985). In view of current evidence that P300 may reflect the activation of inhibitory processes (Begleiter and Porjesz, 1999; Desmedt, 1980; Ramachandran et al., 1996; Roberts et al., 1994; Rockstroh et al., 1992; Schupp et al., 1994; Verleger, 1988), the reduced P300 amplitudes suggest a deficit in cortical inhibition (hyperexcitability). In alcoholics, this amplitude reduction may function as a possible trait marker, because it both precedes the alcohol abuse and remains reduced in amplitude regardless of length of abstinence.

MMN in Alcoholics

The present findings offer the possibility that, as measured by MMN, HR individuals may be characterized by a deficit in cortical inhibition (excessive neural excitation). The presence of this preexisting CNS excitatory state may lead to ethanol use for self-medication, which may then facilitate the development of both tolerance and dependence on ethanol. In view of this hypothesis, it is important to note the results of two recent investigations that compared MMN responses from abstinent alcoholics and social drinkers. The first (Ahveninen et al., 1999a) examined the effects of backward masking on MMN. In the baseline condition wherein no masking stimuli were presented, the alcoholics generated larger amplitude, shorter latency, MMN responses. The second (Pekkonen et al., 1998) used magnetoencephalography to record auditory evoked magnetic field responses. The MMN magnetic field response was recorded at 100 to 200 msec after stimulus onset. Although there were no group differences in amplitude, at an ISI of 2.5 sec, MMN magnetic field response latency was significantly faster in the alcoholics. Furthermore, both investigations also reported that N100 responses to the standard stimuli were augmented in the alcoholics. In contrast, Ahveninen et al. (1999a) found no correlation between augmented MMN responses or augmented N100 responses and duration of abstinence; the authors interpreted their results as possibly reflecting CNS hyperexcit-
ability after withdrawal or a “more permanent or premor-
bid CNS change that needed to be confirmed by later
studies.” It is unfortunate that major differences in both
subject demographics and experimental design make it dif-
ficult to compare the results from Pekkonen et al. (1998)
with those from the present investigation. For example, in
Pekkonen et al. (1998), both control and alcoholic subjects
were almost twice the age of the present study sample.
Furthermore, the alcoholics had an average drinking his-
tory of 17 years and a high weekly ethanol intake. With
regard to experimental design differences, Pekkonen et al.
(1998) used ISIs of 0.5 and 2.5 sec and measured MMN
over the hemisphere that gave the largest response; in the
present study we used a single ISI of 624 msec and mea-
sured MMN responses at the five frontal midline

Although the results from Ahveninen et al. (1999a) and
Pekkonen et al. (1998) suggest the possibility that the al-
coholic brain is characterized by a deficit in inhibitory
processes (hyperexcitability), they must be interpreted cau-
tiously. For example, the alcoholic subjects studied by Ah-
veninen et al. (1999a) had been abstinent an average of 20
days (range, 7–45 days) and those studied by Pekkonen et al.
(1998) had been abstinent an average of 27 days (range,
13–43 days). Previous investigations of abstinent alcoholics
in the postwithdrawal period also have reported enhanced
ERP and magnetic responses (Ahveninen et al., 1999b;
Pekkonen et al., 1998; Porjesz and Begleiter, 1985). It was
proposed that the augmented responses may reflect re-
duced inhibitory and increased excitatory neurotransmis-
sion (Buck and Harris, 1991; Tsai et al., 1995), likely me-
diated by changes in benzodiazepine binding that may exist
for up to 3 months after detoxification (Lingford-Hughes et
al., 1998). In this context, Pekkonen et al. (1998) observed
that decreased N100 m latency in the alcoholics reversed as
a function of increased abstinence and ultimately elimi-
nated the group difference in latency.

**MMN and Ethanol Challenge**

Several studies have demonstrated that the MMN is
sensitive to ethanol administration; low-dose ethanol sup-
presses and delays the MMN, and in an ethanol challenge,
the magnitude of MMN attenuation is dose-related (Jääskeläinen et al., 1995a,b). For example, Jääskeläinen et al.
(1995a) reported that low-dose ethanol (5 g/kg) both
decreased MMN amplitude and increased MMN latency
equally in males and females. Another investigation
(Jääskeläinen et al., 1995b) compared the effects of two
doses of ethanol (0.35 and 0.55 g/kg) on MMN responses
elicited by small (5%) and large (10%) differences between
the frequencies (Hz) of the frequent and rare tones. The
study demonstrated that the decrease in MMN amplitude
was greater when the deviation between the frequent and
rare stimuli was small. Last, Jääskeläinen et al. (1996)
examined the effects of two different ISIs (0.8 and 2.4 sec)
on ethanol-induced suppression of MMN and documented
that MMN was reliably attenuated only with the longer ISI.
In general, the results of these studies suggest that rela-
tively low BALs (Jääskeläinen et al., 1996) act on involun-
tary switching mechanisms to increase the threshold for
detecting stimulus change outside of attention (Jääskeläi-
en et al., 1995b). The effect is reflected in both reduced
MMN amplitude and increased MMN latency. In addition,
it seems that the automatic processing of stimulus change
information is more sensitive to ethanol than is attention-
dependent processing (Jääskeläinen et al., 1995b, 1996)
because no changes in P3b responses were recorded at the
same BALs.

To date, there have been no ethanol challenge studies of
MMN in HR individuals. Moreover, it would have been
unethical to conduct such a study in the present subjects,
because several members of each risk group were below the
minimum drinking age of 21 in New York State. In con-
trast, two baseline studies of MMN have been reported in
HR children. In one (Rodriguez Holguin et al., 1998) the
subjects were 8 to 15 years old, and in the other (van der
Stelt et al., 1997) they were 9 to 18 years old. However,
either study observed risk group differences in any re-
ponse measures.

**Ethanol and NMDA Receptors**

Accumulating evidence now suggests that the neurophys-
iological and pathological effects of ethanol on the CNS are
mediated to a considerable extent through the glutamat-
gic system, although it seems that acute ethanol generally
facilitates GABAergic activity, whereas chronic ethanol
would exert the opposite effect. Ethanol is a well-known
NMDA antagonist (Carboni et al., 1993), and the blocking
action of ethanol on NMDA receptors can be reversed in a
time- and concentration-dependent manner (McCown et
al., 1986). Long-term blockade of NMDA receptors that
results from chronic exposure of neurons to ethanol has
been found to both increase NMDA receptor density (Gu-
lya et al., 1991) and sensitize neurons to the excitotoxic
effect of NMDA receptor activation (Chandler et al.,
1993). Therefore, because of the potentially higher baseline
level of glutamatergic synaptic transmission and up-
regulation of NMDA receptors, a chronic alcoholic with a
positive family history of alcoholism may find it difficult to
abstain from alcohol. Thus, the present findings about
MMN suggest the interesting possibility that, as measured
along the ascending blood alcohol curve, elevated levels of
excitatory glutamatergic transmission in HR individuals
make them more sensitive to acute ethanol ingestion.
Moreover, the augmented response to ethanol may provide
a neurobiological basis for the Differentiator Model (New-
lin and Thomson, 1990), which attempts to explain the
apparently differential sensitivity of HR and LR individuals
to acute ethanol ingestion. The phenomenon of enhanced
acute sensitivity and acute tolerance in HR individuals has
been observed in a variety of measures, which include EEG activity (slow alpha; Cohen et al., 1993), ERPs (Porjesz and Begleiter, 1990b), electromyographic activity (Schuckit et al., 1981), psychomotor performance (O’Malley and Maisto, 1985), and autonomic functions (Newlin and Thomson, 1990).

Ethanol and Serotonin

The results of the present investigation seem to provide an index of neural excitation that may differentiate electrophysiologically between LR and HR individuals. Studies suggest that the development of alcoholism likely reflects an interaction between both a genetic predisposition and the presence of neuropathophysiology associated with alcohol abuse (Ball and Murray, 1994; Begleiter et al., 1984; Goldman, 1993; Karp, 1994; O’Connor et al., 1994). Further evidence for a genetic predisposition for alcoholism derives from studies that have examined serotonergic neurotransmission in the CNS. These studies have reported both reduced central serotonergic transmission in nondrinking adult children of alcoholics (Hunt, 1990; Linnoila et al., 1989; Rosenthal et al., 1980) as well as increased platelet serotonin (5-HT) uptake in alcoholics and their offspring (Ernouf et al., 1993). Central 5-HT neurotransmission has been observed to play a role in the mediation of both alcohol intake and subsequent development of alcoholism (for review, see LeMarquand et al., 1994a,b). Furthermore, 5-HT may be involved in the development of tolerance to ethanol because the process may be accelerated by increased 5-HT levels. The 5-HT mechanism that may mediate the development of tolerance seems to involve NMDA receptors (Khanna et al., 1994), because the blocking action of ethanol on NMDA receptors removes the inhibitory effects of the NMDA system on 5-HT release. However, although the aforementioned interactions between glutamatergic and serotonergic neurotransmission in the CNS have been described, they are highly complex and may be site-specific (Tao and Auerbach, 1996); their actual contribution to the development of tolerance has not been identified.

CONCLUSION

Normal CNS function necessarily reflects homeostasis between inhibitory (γ-aminobutyric acid) and excitatory (NMDA) neural transmission. It is possibly that an imbalance between these two systems yielded the increased excitation that was observed in the HR individuals. However, it will be necessary to implement longitudinal studies to investigate the relationship between the present electrophysiological findings and future patterns of ethanol intake. At present, the size of the HR sample continues to be increased to more accurately describe the topographical distribution of MMN and to further investigate whether there are any sex-related differences. We speculate that the past and current findings from this laboratory point to some neurophysiological anomalies that may be of etiological significance in understanding the genetic predisposition to the development of alcoholism in HR individuals.

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REFERENCES


