ELECTROPHYSIOLOGICAL CORRELATES OF RECOGNITION MEMORY IN 3-MONTH-OLD INFANTS: A 3-DAY AUDITORY PARADIGM

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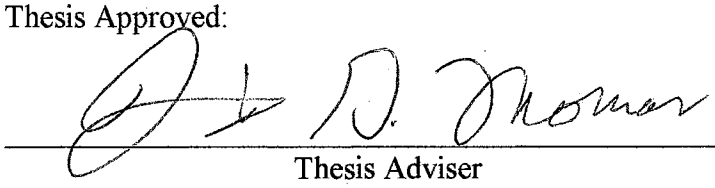

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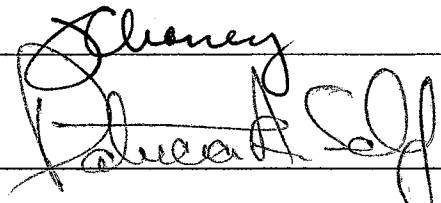

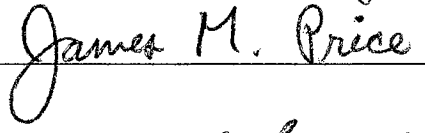

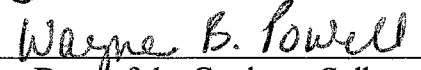

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Statement of the Problem

Recognition memory is an organism's ability to identify a stimulus as previously known or experienced. It is measured as differential responding to novel and familiar stimuli (Fagan, 1973). Researchers have found that infants as young as two months of age will decrease their attention (habituate) when the same visual pattern is repeatedly presented and will attend longer to a novel pattern than to a familiar pattern (Fantz, 1964; Friedman, 1972, Rose, Gottfried, Melloy-Carminer, & Bridger, 1982). Research on infant recognition memory has generally involved behavioral studies utilizing habituation or novelty-preference paradigms. The most well-established area of research of infant memory has been in the visual modality.

More recently, there has been an interest in examining the relationship between brain and cognitive behavior by recording measures of infant brain activity while the subject is performing some task (Nelson & deRegnier, 1992). Much of the electrophysiological work records event-related potentials (ERPs) to examine infant recognition memory. This approach has been very useful for observing ongoing brain function and to explain the neural mechanisms involved in cognitive behavior. Studies in our lab have found evidence for long-term recognition memory using ERPs evoked by familiar and novel auditory stimuli (Thomas & Lykins, 1995, Lykins, 1996).

Much of the research with infant recognition memory has established a pattern of novelty preference (Fantz, 1964; Friedman, 1972; Creighton, 1984) with subjects ranging from four to eight months of age. However, other studies have found that infants younger than two and one-half months indicate a preference for the familiar rather than the novel stimulus (Weizman, Cohen, & Pratt, 1971; Wetherford & Cohen, 1973).

Several hypotheses have been promoted to explain these preferential differences. Hunt (1970) proposed a psychological developmental theory. Dannemiller and Banks (1983) proposed a selective receptor adaptation model to explain early infant habituation. Sokolov (cited in Lewis & Goldberg, 1969) suggested that response differences were

mediated by a central process such as memory or neuronal model formation. Rose et al. (1982) explained the age shift in preference as a change in the speed of information processing that changes across ages. Rose et al. suggest that younger infants require a longer period of stimulus exposure to encode the information.

Previous research in our lab has found that older infants show robust stimulus-specific results (Thomas & Lykins, 1995); however, younger subjects display some degree of generalization to similar stimuli (Lykins, 1996). The present research examined the Rose et al. (1982) hypothesis via ERP measures of long-term infant recognition memory with younger (3-month-old) infants. This project used the identical paradigm from the two previous studies in our laboratory. The only difference was that the subjects received an extra day of exposure to the stimuli. If younger infants do indeed require more experience for stimulus encoding, the results would produce ERP measures that are similar to older (5-month-old) infants.

Summary of Relevant Work Conducted in Our Laboratory

The auditory ERP is recorded from the scalp and is represented as a complex waveform that reflects changes in electrical activity over time (Molfese, 1990). These waveforms are thought to reflect changes in brain activity as indicated by changes in the amplitude or latency of the waveform characteristics at different points in its time course (Callaway, Tueting, & Koslow, 1978). There is a general assumption that a neuronal ensemble becomes synchronously activated when called upon to perform some task (Nelson & deRegneir, 1992). The electrical activity generated by this group of neurons propagates to the scalp via volume conduction and is recorded by metal electrodes, which are placed over a variety of scalp locations. This method permits the researcher to examine the amplitude and latency of the ERP component. Many researchers also examine the scalp topography of the ERP and try to assess neural generator conduction. There has been no general consensus over the results of the latter research because of the way electrical activity conducts throughout the brain. Another major problem is the possible summation at a particular scalp area of activity from more than one underlying brain structure. However, investigators have been able to suggest the sources of a number of ERP components (Andreassi, 1995).

The ERP is differentiated from the more traditional EEG measure because the ERP is time-locked to the onset of some event in the subject's environment (Molfese & Wetzel, 1992). The EEG measures a wide range of neural activity that is involved with the neural and somatic self-regulating systems and the ongoing sensory and cognitive functions in the brain. The time-locked ERP reflects both general and specific aspects of the evoking stimulus and the subject's perceptual response to that stimulus (Molfese, 1983; Nelson & Salapatek, 1986).

Several studies have used ERP measures to study aspects of early infant memory. These studies indicate that infants at different stages of development are sensitive to

familiar versus novel events (Courchesne, Ganz, & Norcia, 1981; Hoffman & Salapatek, 1981; Nelson & Salapatek, 1986; Molfese, 1989; Thomas & Lykins, 1995). Studies of infants ranging in age from 6 weeks to 18 months of age have generally found that reliable differences can be identified in ERPs that indicate novel/familiar differentiation.

One of the earliest auditory ERP studies of memory recorded from frontal, temporal, and parietal scalp locations over the left and right hemispheres of the subjects (Molfese, 1989). Ten infants (14 months of age) were presented with a series of repeated consonant-vowel-consonant-vowel (CVCV) syllables over a period of two days. On the third day, they were tested with 60 presentations of both the familiar stimulus and a novel CVCV. Molfese found differences in the ERPs over the left and right frontal electrode sites at 360 ms following stimulus onset; a large positive peak was noted for only the familiar stimulus.

The studies which our research team has conducted have provided additional knowledge on the neural mechanisms underlying information storage processes in infant memory. The initial study (Thomas, Shucard, Shucard, & Campos, 1989) tested 5- to 6-month old infants over a two-day period to determine the effects of repeated laboratory experience on ERP recordings. One group of infants was given 80 presentations of a single tone on Day 1 of the study; the subjects returned 24 hours later and were given an additional 80 tones. The control group underwent the identical procedure (i.e., electrodes placed on infant) except that no actual tone presentations were heard on the first day. Results showed an increase in amplitude from Day 1 to Day 2 for the experimental group (i.e., received tones on both days). This effect was found for a measurement of amplitude from the second negative peak (N2) to the third positive peak (P3) of the ERP waveform. The amplitude measures for the control group on Day 2 of the study were the same as those found for Day 1 of the experimental group. The results suggest that the increase of amplitude found for the experimental group was the result of the additional experience with the stimulus on Day 1.

A second study using 5-month-old subjects (Thomas & Lykins, 1995, Experiment 1) presented auditory stimuli which differed multidimensionally. Infants heard either 100 presentations of a click or a tone on the first day and 50 random presentations of both on the second day. ERPs were recorded from frontal (Fz), central (Cz), and left temporal (T3) electrodes. The primary measures were of the P2, N2, and P3 peaks which had shown amplitude changes in previous studies. The results were similar to those found in the original study. A significant amplitude increase was found from Day 1 to Day 2 for the familiar stimulus at N2. The data also showed significantly larger ERP amplitudes for the familiar stimulus in comparison to the novel stimulus on the second day of the study. These results support a stimulus specific interpretation, i.e., the memory trace was specific to the stimulus (tone or click) that the infant had experienced on the first day of the study.

A third experiment with 5-month-old infants (Thomas & Lykins, 1995, Experiment 2), was designed identically to the previous study. However, the stimuli consisted of two tones differing only in frequency (400 and 700 Hz). The primary purpose of this study was to determine if the results would still be specific for the familiar stimulus or generalize since the stimuli would be so similar. The subjects were given 100 presentations of a single tone (either 400 or 700 Hz) on Day 1 and a randomly-ordered presentation of both tones on Day 2. The results showed increased amplitudes for the previously-experienced stimulus, indicating a stimulus-specific interpretation.

There are two possible mechanisms underlying an increase in the average amplitude from the Day 1 to Day 2: (a) a true increase in amplitude; and (b) a decrease in variability. The average ERP waveform consists of the mean of many single-trial waveforms. The amplitude of the average ERP is influenced, of course, by the amplitude of the single-trial waveforms, but also by the trial-to-trial consistency in the waveform components: The greater the trial-to-trial latency variability of a peak, the smaller the amplitude of that peak in the average ERP. Analyses of adult ERP data have shown that virtually all of the variance in the amplitude of a given peak in the average ERP can be

accounted for by the two single-trial measures of latency variability and peak amplitude (Thomas, Neer, & Price, 1989).

Further analysis of the data from this third experiment (Thomas & Lykins, 1995) found both significant single-trial increases in amplitude for Day 2-Familiar in comparison to both Day 1-Familiar and Day 2-Novel (Peak P2, electrode Fz) and decreases in variability for Day 2-Familiar in comparison to Day 1-Familiar and Day 2-Novel (Peaks P2 and N2, electrode Fz). From these results, it can be concluded that increases in the amplitude of average ERPs to familiar stimuli are due to both actual amplitude increases, and to decreases in response variability. The increase in amplitude (as found by the single-trial analyses) suggests that more neural elements (e.g., synapses, neurons, or groups of neurons) are recruited into the neural ensemble as a result of familiarization. Furthermore, the decrease in variability suggests that the familiarization phase on the first day of stimulus experience stabilizes the neural ensemble which responds to the familiar stimulus resulting in a more "experienced," less variable response.

A fourth experiment (Lykins et al., 1996) was done with 3-month-olds to examine possible developmental differences of infant memory. These infants were tested under the same conditions as the 5-month-olds in Experiment 3 except that the temporal electrode was replaced by one over the prefrontal area (Fpz). The results showed average ERP amplitude increases from Day1 to Day 2 for both novel and familiar stimuli at peak P2 at all three electrodes (Cz, Fz, and Fpz). For N2, Day 2-Familiar average amplitudes were larger compared to Day 2-Novel at Cz but not significantly larger than Day 1-Familiar. In addition, the single trial analyses found a significant amplitude increase for both Day 2-Familiar and Novel over Day 1-Familiar at Peak P2 (all electrodes). Examination of the latency variability also found significant decreases from Day 1 to Day 2 for both Familiar and Novel at P2 (all electrodes). For N2, however, variability decreased only for Day 2-Familiar as was found for 5-month-olds (Thomas & Lykins, 1995).

The results of this study suggested that there are developmental differences between 3- and 5-month-olds. The data for 3-month-olds (P2 amplitude increases for both novel and familiar stimuli on Day 2) indicated generalization to similar stimuli. However, the 5-month-old data suggested stimulus specificity by the amplitude increase found only to the familiar stimulus. Even though the average amplitude for N2 did not show a Day1 to Day 2 increase, differences found in the single-trial analysis (trial-to-trial variability) suggest that infants as young as 3-months-old show stimulus specificity in ERP response measures but only later in the ERP waveform. The measures for latency variability showed that the younger infants were similar to the 5-month-olds in that there was a decrease in latency variability for Day 2-Familiar compared to both the Day 1-Familiar and Day 2-Novel stimuli. These results were found at all three electrodes for Peak N2.

Much of the previous research in novelty preference has explained age differences (familiarity preference of younger infants vs. novelty preference of older subjects) as developmental change of a qualitative nature. Rose et. al (1982) suggests that these changes are quantitative in that the developmental differences found in infant recognition memory can be accounted for by the amount of exposure to a given stimulus. Based on this theoretical position, we tested the hypothesis that, if the amount of familiarization (or experience with a stimulus) is substantially increased over previous studies, ERP measures of recognition memory for 3-month-olds would be similar to those for 5-month-olds. The following literature review examines the evidence in support of the quantitative change theory of Rose et al.

REVIEW OF THE LITERATURE

The ERP studies done in our laboratory have all been designed using a simple novelty preference paradigm that originated in behavioral studies with visual stimuli. Thomas and Lykins (1995) found that familiarization with one auditory stimulus and the subsequent exposure to both the familiar and a novel stimulus resulted in differential responding (similar to the findings in the extensive behavioral research). We have examined the effects found in our research in infant recognition memory in relation to the well-established literature base of behavioral studies of novelty preference.

A Brief History of Infant Recognition Memory

Recognition memory is an organism's ability to identify a stimulus as previously known or experienced and is operationally defined as differential responding to two stimuli given previous experience with one of those stimuli (Thomas & Lykins, 1995). Interest in infant memory has been documented as far back as Darwin, in 1877 (Cohen, 1973). He described in his infant biography the kinds of stimuli his subject looked at and at what age the child would visually track a moving object. In 1914, Valentine was one of the first to measure infant preference (Cohen, 1973). He used balls of colored thread and assumed that if an infant looked longer at one than the other, the infant could discriminate between them.

Cohen (1973) maintained that research on infant memory remained relatively dormant until 1958. That time Berlyne(1958) and Fantz (1958; both cited in Karmel & Maisel, 1975) independently published their studies on visual discrimination; both studies used a paired-comparison technique to measure visual fixation. Berlyne presented two different visual stimuli to an infant simultaneously and recorded which pattern the infant first fixated. Differential response patterns (i.e., longer fixation times of one stimulus over another), were taken to indicate discrimination and preference for a stimulus. Berlyne and Fantz found that infants fixate longer (prefer) certain visual stimuli over others (Karmel & Maisel, 1975), that infants preferred patterns over nonpatterned stimuli, and that some

patterns were preferred over other patterns. Later research found that infants as young as two months of age decreased their attention (habituated) when the same visual pattern was repeatedly presented and attended longer to a novel pattern than to a familiar pattern (Fantz, 1964).

Infant Memory Paradigms

Studies of infant recognition memory have generally utilized *habituation* and *novelty-preference* paradigms. Infants' preference for looking at novel stimuli can be used to determine what information about a stimulus has been encoded and stored in memory (Schacter & Moscovitch, 1984). A pattern of habituation to a familiar stimulus and dishabituation to a novel stimulus is taken as evidence of memory for the original stimulus. This habituation-dishabituation pattern indicates that the infant has noticed a change in the environment because he/she has stored information about the repeatedly presented stimulus (Fagan, 1973). After the infant has habituated to the original stimulus and dishabituated to a novel stimulus, the familiar stimulus is presented again. Delayed recognition is indicated by an immediate decline in response rate.

Another procedure for demonstrating delayed recognition is the *paired-comparison* approach. The infant is exposed to a stimulus for a certain length of time. The infant is then exposed to the original stimulus and a novel stimulus simultaneously. Delayed recognition is measured by the amount of the infant's visual fixation to either the novel or familiar stimulus.

Fagan(1973) reported that the paired-comparison method has been the more successful method for demonstrating delayed recognition memory. Infants, when tested with this method, typically spend the greater part of their visual fixation to the novel target. To test delayed recognition memory, the researcher need only vary the time that elapses between the end of the familiarization period and the testing trials. Fagan (1970, 1971, 1973) found that 5-month-old infants demonstrated novel/familiar differentiation with one, four, and seven minute delays. He also found both immediate and two-hour

delayed novelty preferences (1970, Experiment I) and some support for retention over days (1970, Experiment II) with infants ranging from 13 to 18 weeks of age.

The paired-comparison approach has generally been used with stimuli that vary multidimensionally. Research utilizing the habituation-dishabituation paradigm generally compared stimuli that differed only on one or two dimensions. Because of the differences noted in stimulus dimensions the salience of the stimulus may have biased the paired-comparison results. The increased salience of multi-dimensional stimuli has been found to have more pronounced novel/familiar differentiation with infants (Saayman, Ames, & Mofett, 1964). Saayman et al. found that infants showed a preference for novel stimuli of high attention value, but there was no differential response to novel stimuli of low attention value over familiar stimuli.

Research on infant memory using the habituation paradigm (Zelazo, Weiss, Randolph, Swain, & Moore, 1987), and conditioning studies (Clifton, Meyers, & Solomon, 1972; Hillier, Hewitt, & Morrongiello, 1992) all provided support for memory retention in infants. Researchers examined short-term memory retention (one hour or less) (Friedman & Carpenter, 1971; Zelazo, Brody, & Chaika, 1984), long-term memory retention (24-hours to 13 days) (Ungerer, Brody, & Zelazo, 1978; Rovee-Collier, 1993) and even recognition memory across the prenatal-postnatal boundary (Moon, Cooper, & Fifer, 1993).

Most of the research on infant memory has focused primarily on visual recognition (Ashmead & Perlmutter, 1980). The results from this area of research have been so pronounced and consistent that the visual novelty preference paradigm has become the basic design for research on infant memory (Rose et al., 1982). Investigation of memory using other sensory modalities (i.e., audition) has not been as well established. Research on memory for auditory stimuli has for the most part adapted the visual paradigm (Columbo & Bundy, 1981; 1983; Mehler, Jusczky, Lambertz, Halstead, Bertoncini, & Amiel-Tison, 1988).

Age Differences (explanations and models)

Much of the behavioral literature reports that younger infants (2 months of age or less) show a preference (longer fixation time, increase in measured behavior) for familiar stimuli; however, older infants show a strong novelty preference (Columbo & Bundy, 1983; Dannemiller & Banks, 1983; Rose et al., 1982). Several models have been proposed to explain this age difference in novelty or familiarity preference.

Dannemiller and Banks (1983) explained the differences found between younger and older infants through a *selective receptor adaptation model*. This model states that habituation reflects decreasing excitability among the neurons that respond to the particular stimulus being presented repeatedly to the subject. Repeated excitation, over time, causes fatigue among this set of neurons and this decreases neural responsiveness. Any overt behavior corresponding to the excitation of the particular set of neurons would also show a decrease over trials. If a sufficiently novel stimulus is presented, a different, unfatigued set of neurons would respond and any overt behavior should return to higher levels. Dannemiller and Banks utilized the sensory adaptation model to explain visual habituation but stated that the model was applicable to other modalities as well. There are three requirements to extend this model to other modalities: a) there must be feature-selective neurons involved, b) those neurons must be fatigable, and c) the infant's behavior or response must depend on the degree of excitation among those neurons. The model could also be applied to paradigms using successive or simultaneous stimulus presentation and with response measures other than fixation time.

The *information-processing* or *schema-comparison model* differs from the selective receptor adaptation model in that it requires memory processes to account for early infant habituation (Tarquion, Zelazo, & Weiss, 1990). Dannemiller and Banks (1983) argued that a cognitive-schema model may be more appropriate for infants over 4 months of age. For younger infants they suggested that a sensory adaptation model was more appropriate for explaining habituation because of the neural limitations of the

younger, cortically-immature infant. These authors argue that the cognitive models require a more complex neurophysiology than do the sensory adaptation models. Dannemiller and Banks suggest that both types of mechanisms, sensory adaptation and cognitive schema formation, are involved in infant habituation. However, they also suggest that early in development, automatic, peripheral mechanisms may be involved in habituation; as the infant matures, more sophisticated mechanisms may be implicated for more complex or abstract stimuli.

Several researchers (Slater & Morrison, 1985; Ackles and Karrer, 1991) disagree with the Dannemiller and Banks two-mechanism model. These authors argue that reviews of studies with young infants (less than 4 months old) were not compatible with the neuronal fatigue model and cannot account for infant habituation. They also cite studies that found long-term habituation effects which cannot be accounted for by neural fatigue. Sokolov (1968)(cited in Lewis & Goldberg, 1969) proposed that response decrement and recovery were mediated by some central process such as *memory or neuronal model formation.*

Sokolov (1968) defined the orienting reflex as a "complex functional system that integrates activities of different brain areas. Its distinguishing characteristic is that it arises in response to novelty (p. 576)." Novelty is defined as the lack of match between the experimental external event and the neuronal model (Lewis, Goldberg, & Rausch, 1967). This novelty (or mismatch) results in attentive behavior. When there is not a match between the external event and the internal (neuronal) model, excitation occurs resulting in orienting responses. When there is a match, inhibition occurs and the result is a decrease in orienting responses (Lewis & Goldberg, 1969).

Lewis et al. (1967) suggested that novelty and familiarity preferences could be defined operationally by the same experimental manipulation as the orienting reflex. Some support for this theory comes from the data on cardiac response from the Lewis and Goldberg (1969) study. Their results found an initial response to a new stimulus was

cardiac deceleration that decreased with successive repetitions of the stimulus and recovered when the stimulus was either altered or replaced by a novel one. Other researchers in the field (Sokolov, 1963; Graham & Clifton, 1966) conclude that heart rate deceleration satisfies the criteria usually associated with an orienting response, namely the indication of autonomic components. Lewis et al. based novel/familiar differential responding on Sokolov's orienting response theory; however, this model does not explain age response differences.

Hunt's (1970) explanation of response differences suggested that infants take pleasure in learning to recognize objects and this explained the younger infants' preference for the familiar stimulus rather than the novel stimulus. As the infant develops, the act of recognition becomes more commonplace and the novel stimulus becomes more attractive to the older infant. Thus, the preference for novel stimuli in the older infant is the result of a *developmental stage or change* (Hunt, 1970). Hunt's developmental stage model gives a description of age response differentiation but his circular explanation for these differences seems inadequate.

Rose et al. (1982) questioned this explanation based on qualitative developmental change. The authors tested infants at different ages (3 1/2, 4 1/2, and 6 1/2 months of age) for visual recognition memory of shapes using the paired comparison method. In the first experiment the subjects were familiarized with a stimulus and then tested with both the familiar and a novel stimulus. The 3 1/2 months-olds showed a preference for the familiar stimulus; however, the 4 1/2 and 6 1/2 month-olds indicated a preference for the novel stimulus. In a second study, the 3 1/2 month olds were allowed either 5, 10, 15, 20, or 30 seconds of familiarization time; the 6 1/2 month-olds were allowed either 5, 10, or 15 seconds of time.

Rose et al. (1982) found that both older and younger infants showed a preference for the familiar stimulus after a short period of exposure to the stimulus (5 and 10 sec. respectively). When infants of either age group were exposed to the familiar stimulus for

longer periods of time (15 and 30 sec.), both groups indicated a preference for the novel stimulus.

Rose et al. (1982) proposed that as infants begin to process a stimulus, they prefer to attend to that which is familiar; once information is sufficiently encoded, processing becomes more advanced and their preference shifts to the novel stimulus. These authors suggested that the increase in novelty preference found with both an increase in age and increasing familiarization time indicated that novelty preference represented a more advanced phase of information processing than simply a preference for the novel. The shift from familiarity to novelty is not a developmental shift (i.e., it is neither age specific nor a qualitative development). Infants of all ages prefer familiar initially; however, with additional exposure, these same infants prefer novel stimuli. Younger infants are simply slower at information processing than are older infants. These shifts in preference reflect a change in the *speed of information processing* that changes across ages (Rose et al., 1982). Rose et al. postulate that the increase in novelty scores found with an increase both in age and familiarization time represents a more advanced phase of processing than do familiarity scores. These findings would support the idea that the presence of reliable novelty responses can be used as an unambiguous index in memory.

Auditory Paradigms

Investigation of memory using sensory modalities other than vision has not been as well established. However, auditory recognition memory studies have found that even newborn infants show a novelty preference over a 24-hour period (Swain et al., 1993). Some auditory preference research has been done using the *non-nutritive sucking paradigm* (Mehler, Jusczky, Larbertz, Halstead, Bertoncini, & Amiel-Tison, 1988). Using this paradigm, Mehler et al. found that infants as young as four days of age displayed higher levels of arousal indicated by increased bursts of sucking activity to their parents' native language than to a foreign language.

Spence (1996) applied this paradigm to 1- to 2-month old infants to examine auditory novel/familiar preferences. Each infant heard a nursery rhyme read by his/her mother during two daily time periods. The subjects were then tested at either 1-, 2-, or 3-day intervals following familiarization. Spence found that infants displayed a consistent novelty preference 1 day after familiarization, no consistent preference after a 2-day interval, and found a familiarity preference with a test delay of 3 days. The author suggested that these findings reflect changes in the accessibility of the representation of the stimulus in long-term memory. Infants prefer and attend to novel stimuli if there is little discrepancy between the memory of the previously experienced and the external stimulus. If the subject indicates a preference for the familiar stimulus, a discrepancy exists between the mental representation and the external stimulus (Hunter, Ames, & Coopman, 1983).

Other investigators have adapted the visual novelty preference paradigm commonly used in vision research (Colombo & Bundy, 1981; 1983). Columbo and Bundy measured novelty preference by presenting two identical visual targets for the subject to look at. While subjects were involved with the familiarization phase, the visual targets were not lit; during the test phase, the targets were illuminated. During the familiarization

phase, the infants (2-5 months of age) received either six presentations or 20 presentations of one auditory stimulus. For the test phase, the infants "worked" for the preferred sound. The infant received the familiar sound or a novel one, depending upon which visual target the subject fixated. The results indicated that there was a novelty preference for both conditions of either six or 20 familiarization trials.

Head-turning responses have also been used to measure recognition in newborn infants (Swain, Zelazo, & Clifton, 1993). Swain et al. (1993) presented two words as auditory stimuli ("beagle" and "tinder"). The sounds were presented laterally from either the left or right; the subjects were familiarized with one or the other sound on day one. On day two the subjects received both the familiar sound and a new sound. Head-turning responses were measured by use of a protractor above the infant's head and only head turns of at least 45 degrees were counted. The results indicated that infants habituated head-turning to familiar auditory stimuli and renewed responding to novel stimuli.

These methods for measuring infant memory have been used with some success but they are limited. The non-nutritive sucking paradigm is limited to subjects who use pacifiers. The visual fixation model is problematic in that conditioning effects can confound the measure of habituation; i.e., the intensity and duration of auditory stimuli can affect head-turning response measures (Muir & Field, 1979). Infants often become disinterested during a behavioral task and can be uncooperative in an experimental situation. Infants become tired, fussy, hungry, or bored despite careful plans and controls. Although there is no trouble-free objective method of measurement that is suitable for all infant subjects for measuring infant memory, some of the problems associated with behavioral measurement can be overcome by the use of *electrophysiological measures* such as event-related potentials (ERPs). ERP procedures are especially useful with subjects who are unable to respond to stimuli behaviorally, e.g., brain-damaged individuals, or infants.

Results from previous research with infants conducted in our laboratory addressed both the psychological phenomenon of memory (ERP responses that indicate recognition memory) and the neural processes which underlie it (a decrease in the variability of the neural response to a familiar stimulus, as well as an increase in the magnitude of that response). The present project measured neural aspects of recognition memory in 3-month-old infants using ERPs to auditory stimuli. The study addressed the issue of increased experience with the stimuli and its role in infant recognition memory. Previous work in our lab found that 5-month-old infants indicated a robust differential response to novel and familiar stimuli. Studies with 3-month-olds, using the identical paradigm, found that the younger infants' neural responses produced more indications of stimulus generalization rather than differentiation. The primary purpose of the present study was to determine if increased experience with an auditory stimulus with 3-month-olds would produce the robust novel/familiar differentiation of the previous 5-month-old study. The first objective was to replicate previous work from our laboratory.

HYPOTHESIS 1:

Day 2 of the study would produce results of stimulus generalization. There would be a general increase in amplitude and a general decrease in latency variability to both familiar and novel stimuli on the second day of the study.

To address the first hypothesis, three sets of planned comparisons were designed. The predicted outcomes of these comparisons are also presented.

1) Day 1-Familiar < Day 2-Familiar
2) Day 2-Familiar = Day 2-Novel
3) Day 1-Familiar < Day 2-Novel

The second inquiry asks whether increased experience with a stimulus produces neural responses in 3-month-old subjects similar to those of 5-month-old subjects?

HYPOTHESIS 2:

After the 3 days of stimuli experience, the Day 3-Familiar ERPs would indicate stimulus specificity similar to that found on Day 2 for the 5-month-olds.

To address this hypothesis, three additional sets of planned comparisons were performed:

1) Day 1-Familiar < Day 3-Familiar
2) Day 1-Familiar = Day 3-New Novel
3) Day 3-Familiar > Day 3 New Novel

The last question addresses the effects of the increased stimulus experience. If the increase in stimulus experience results in more mature neural responses in 3-month-old subjects, which is more important, the number of trials or the time period between sessions?

HYPOTHESIS 3: The data of Rose et al. (1982) suggests that the amount of stimuli is of primary importance in infant memory. Therefore Hypothesis 3 states that conditions representing the greatest amount of stimulus experience will show longer memory effects. The following comparisons were designed to address this question:

1) Day 2-Novel = Day 3-New Novel
2) Day 2-Familiar > Day 3-Old Novel
3) Day 2-Familiar < Day 3-Familiar

Method

Participants

All participants were recruited from birth announcements published in a local newspaper. The final sample consisted of 24 full term, healthy 3-month-old infants (14 males, 10 females) with no known history of neurological or auditory problems. Data from an additional nine infants were discarded for the following reasons: one due to equipment problems, three because of state differences across days, and five unable to complete all three days because of fussiness. The infants were randomly assigned to three groups, eight received only low tones on Day 1, eight received only medium tones on Day 1, and eight received only high tones on Day 1.

On Day 2 and Day 3 (24 hours and 48 hours, respectively, after the Day 1 session) the parent was asked to maintain the same schedule as the previous day. The infant returned to the laboratory at the same time of day for the second and third stage of stimuli presentation. The procedure was identical to that of Day 1 with the exception of the type of stimuli presented. Each participant received $10.00 per day as compensation for their time spent in the laboratory.

Participants received 100 auditory presentations of either low tones (400 Hz, 100 ms, 70 dB), medium tones (700 Hz, 100 ms, 70 dB) or high tones (1000 Hz, 100 ms, 70 dB) on Day 1. On Day 2 all three groups received 50 tones identical to those received on Day 1, and 50 presentations of one of the other two tones. On Day 3 all three groups received 50 presentations of all three stimuli for a total of 150 presentations. The order of stimulus presentation was random on Day 2 and Day 3 with the constraint that no more than four tones of the same frequency could occur consecutively. The minimum interstimulus interval was 4 ms.

Apparatus

The stimuli were presented binaurally using headphones attached to an elastic strip which fit snugly over the electrode cap and the infant's head. The electroencephalogram

(EEG) electrodes were placed over midline center and frontal scalp (Cz, Fz, and Fpz, respectively, of the International 10-20 System, Jasper, 1958), and over the left lateral temporal area (T3). Tin electrodes that were sewn into an elastic cap (Electrocap International) especially designed for infants were used. These scalp electrodes were referenced to linked earlobes with the ground located over the right lateral temporal area (T4). Eye movements (EOG) were monitored by electrodes placed above and to the left of the left eye. Impedances for all electrodes were kept below 10 kOhms.

The EEG was amplified by Grass Model 7p511 amplifiers with bandpasses of 1-100 Hz. EEG and EOG data were collected for 390 ms prior to stimulus onset and for 1200 ms following stimulus onset. The EEG was digitized and stored on computer disk at a rate of one sample every 6 ms.

Procedure

Parents were contacted and asked to bring their child to the laboratory at a time when the infant was most likely to be alert and nurse or take a bottle. Upon arrival, the parent was given detailed information concerning the study and informed consent was obtained. The parent was seated in a comfortable chair and the infant was seated in the parent's lap while the cap, electrodes, and headphones were placed. This process generally took about 15 minutes. The infants were then encouraged to nurse or bottle feed. A motorized mobile (for infant observation) was turned on and the experimenter retired to the control room. Continuous monitoring of the participant's state was done via EEG and EOG recording and visual monitoring over a video camera. The experimenter presented stimuli only when the participant was judged to be awake and not moving. An assistant observed the infant on a video monitor and recorded changes in the infant's state using the following classifications:

1--asleep

2--drowsy/eyes closed

3--drowsy/eyes open

4--quiet alert/eyes closed

5--quiet alert/eyes open

6--active alert

7--drowsy agitated

8--crying

Stimuli were presented only when the participant's state was rated between 2 and 7. The state classifications were used to determine the infant's state across the three days sessions (i.e., the percentage of trials spent in the modal state must be within 20% across all three sessions). The experimenter and the assistant independently charted the infant's behavior and state condition both during preparation and stimulus presentation. At the end of each day's session, the parent was asked questions concerning the baby's state of alertness during stimulus presentation. These factors were all taken into consideration in the final analysis of behavior state over the three day period.

Data Processing

The raw data set consisted of 100 single-trial ERPs from Day 1 (Day 1-Familiar or D1F), 100 single-trial ERPs from Day 2, and 150 single-trial ERPs from Day 3. The Day 2 raw data consisted of 50 ERPs recorded to the familiar stimulus (Day 2-Familiar or D2F), and 50 to the novel stimulus (Day 2-Novel or D2N). The Day 3 raw data consisted of 50 ERPs recorded to the Day 1 familiar stimulus (Day 3-Familiar or D3F), 50 to the Day 2 novel stimulus (Day 3-Old Novel or D3ON), and 50 to the Day 3 new novel stimulus (Day 3-New Novel or N3NN). If any trials were judged to have occurred during an agitated or non-awake state (based on EEG criteria and experimenter report), they were discarded.

Trials that were contaminated by EEG artifact were also discarded. A trial was rejected if the voltage value of any channel exceeded 100 microvolts. In addition, any trial was discarded if, in any 216 ms window, any EEG channel exceeded 60 microvolts and the EOG channel exceeded 60 microvolts. The number of trials for each of the six

conditions was then equated within each subject based on the condition with the smallest number of artifact-free trials. The trial sets for the other conditions were then reduced by randomly discarding the needed number based on a random number table. A criterion of 25 artifact-free trials was set as the minimum number needed in each condition for inclusion of a given participant's data. These single-trial waveforms were averaged and digitally low-pass filtered at 50 Hz. Each participant's data consisted of average ERPs from four electrode locations (Fz, Cz, Fpz, and T3) for each of six conditions (D1F, D2F, D2N, D3F, D3ON, D3NN).

Average ERP Peak Amplitude

The peak amplitudes of P2, N2, and P3 were each measured baseline-to-peak, with the baseline being the mean EEG amplitude for the 390 ms preceding stimulus onset. The peaks was identified in the following way based on the criteria of Ohlrich and Barnet (1972). P2 was designated as the largest positive peak between 150 and 350 ms. N2 was the largest negative peak following P2 within the time window 200-600 ms. P3 was the largest positive peak following N2 within the time window 300-1200 ms.

Single-trial Analyses

Because an amplitude increase was found in the average ERPs, single-trial analyses were carried out to assess whether the increase was due primarily to a decrease in variability from one session to the next (i.e., from Day 1 to Day 2, Day 1 to Day 3, or Day 2 to Day 3), or to a true increase in response amplitude. The method used was based on a cross-correlational technique described by Michaelewski, Prasher, and Starr (1986) and Thomas, Neer, and Price (1989). A template was created for the component of interest in the average ERP (e.g., N2). This template consisted of a 41-data point (246 ms) segment of the average ERP with the peak of the component at the midpoint of the segment. The template was moved across a 300-ms time window in each single-trial waveform, and a Pearson correlation coefficient was calculated between the voltage values of the 41-point template and each successive set of 41 points in the search window (i.e., points 1-41,

2-42, 3-43, etc.). This search window consisted of the 150 ms preceding and following the latency of the peak in the average ERP. The point in the search window at which the maximum correlation was found between the template and the single-trial ERP was identified as the component within that single trial. The amplitude and the latency was then measured. The standard deviation of the latency value was used as the estimate of latency variability, and the mean of the amplitudes was used to estimate single-trial amplitude (Thomas, et al., 1989). Latency variability and single-trial amplitude was calculated for each condition.

Results

Primary analyses were planned comparisons using one-tailed Bonferroni tests. An adjusted alpha-level of .032, based on a modified Bonferroni test (Keppel, 1991) was used for all comparisons to maintain an acceptable familywise error rate. This strategy keeps the familywise error rate near the "natural" limit set by the number of available degrees of freedom and permits the investigation of important questions for this project. Because the present study was complex, certain non-orthogonal questions were necessary to examine the three hypotheses fully. Analyses were performed for peaks P2, N2, and P3 at each electrode (Cz, Fz, Fpz, and T3). The following comparisons were done between Day 1-Familiar (D1F) and Day 2-Familiar (D2F), D2F and Day 2-Novel (D2N), and D1F and D2N to examine the first hypothesis concerning research replication of 3-month-olds' data. Comparisons between D1F and Day 3-Familiar (D3F), D1F and Day 3-New Novel (D3NN) and D3F and D3NN were performed to address the second hypothesis that an increase in stimulus experience results in younger subjects' ERP data resembling those of older subjects (from previous studies). Finally, comparisons were done between D2N and D3NN, D2F and Day 3-Old Novel (D3ON) and D2F and D3F to examine the effects of increased experience. If stimulus experience does make a change in ERP data, which is more influential, the number of trials, or the 24 hour consolidation period between

sessions? Post hoc analyses were performed on the data to examine any effects beyond those covered by the planned comparisons.

Average Peak Amplitude

The three tone groups were combined and analyses were performed separately for the peaks P2, N2, and P3 at each electrode (Cz, Fz, Fpz, and T3). The means, standard deviations, and p values for these data are found in Table 1 (P2 peak), Table 2 (N2 peak) and Table 3 (P3 peak). Significant differences for the average peak amplitude were found between D1F and D2N for the P2 peak at the Fz electrode site, ($t[23]=-2.05$, $p=.025$) and the Fpz electrode site ($t[23]=-2.01$, $p=.028$) (see Figure 1). No significant differences were found between between D1F and D2F for any peak; however, there was a trend for an increase in amplitudes for D2F for all peaks, with electrode Fpz at the P2 peak (see Figure 2) approaching statistical significance ($t[23]=-1.36$, $p=.093$). There were no significant differences found between D2F and D2N but there was a nonsignificant trend for larger amplitudes for D2N all at electrodes (except Fpz at P3) for peaks P2 and P3 (see Figures 3 and 4). The N2 peak indicated the opposite nonsignificant trend with larger amplitudes for D2F (see Figure 5). This trend for increased amplitude for D2F approached conventional statistical significance at peak N2 at electrodes Fz and Fpz ($t[23]=-1.89$, $p=.035$, and $t[23]=-1.85$, $p=.039$, respectively).

Support for Hypothesis 2, that an increase of stimulus experience would result in a significant amplitude increase by the third session, is illustrated by Figure 6. A trend for increased amplitude at peak N2 was found at all electrode sites for D3F over D1F. The comparisons between Day 1 and Day 3 show D3F to be significantly larger than D1F for N2 at the Cz electrode site ($t[23]=2.34$, $p=.015$). A significant amplitude increase for D3F over D3NN was found at peak N2 at the Fz electrode site as well ($t[23]=-2.09$, $p=.024$). This trend for increased amplitude on D3F can be seen in Figure 7 for all electrode sites).

For peak P3, a significant amplitude increase was found for D3ON over D2F at the Cz electrode site ($t[23]=-3.59$, $p=.001$) (see Figure 8). Differences were also found between D1F and D3F (with D3F being larger) at site T3, ($t[23]=-2.87$, $p=.005$) and at the Cz site between D1F and D3NN (D3NN showing larger amplitudes) ($t[23]=-2.39$, $p=.013$) (see Figures 9 and 10, respectively). These findings address the point that increased experience does cause the ERP data of younger subjects to resemble findings with older subjects.

Group(low, medium, or high tones as the familiar stimulus), x Condition (D1F, D2F, D2N, D3F, D3NN, D3ON) with repeated measures x Electrode (Cz, Fz, Fpz, T3) analyses of variance (ANOVA) was also performed separately on the P2, N2, and P3 baseline-to-peak amplitude data. A significant main effect for Electrode, $F(3,63)= 3.46$, $p=.021$ at peak P2 was found and at peak P3, $F(3,60)=3.68$, $p=.017$. Additionally a significant interaction between Group and Condition was found, $F(10, 105)= 2.05$, $p=.035$ at peak N2 and for peak P2, $F(10,105)=2.29$, $p=.018$. The patterns of responding for all groups (low, medium, and high tones as the familiar stimulus) were similar across groups for those paired comparisons that were found to be significantly different as seen in Figure 11 and Figure 12. However, other conditions were dissimilar enough to result in the interaction.

Latency Variability

Planned comparisons found no significant differences for latency variability for peak P2. Means, standard deviations, and p values for latency variability measures for peaks P2 and N2 can be found in Tables 4 and 5, respectively. For the N2 peak, all of the comparisons for D2F and D2N found increased latency variability measures for D2N (see Figure 13); however, significant differences were found between D2F and D2N only at the Fpz electrode, ($t[23]=-2.19$, $p=.02$). This supports previous findings with same-age subjects and for 5-month-olds.

Differences were also found between D1F and D3F at electrodes Cz, (t[23]=4.25, p=.000), Fz (t[23]=2.43, p=.012, and T3 (t[23]=2.82, p=.005) (see Figure 14). These indicated that increased experience with the stimuli did result in a significant decrease in latency variability. All measures of latency variability found a decrease on Day 3 with the familiar stimulus over the new stimulus presented on the third day; however, significant differences, as illustrated in Figure 15, were only found between D3F and D3NN at electrode Fz, (t[23]=-2.21, p=.019). Additionally, significant differences were found between D2F and D3F at electrode Cz, (t[23]=2.52, p=.009) (see Figure 16) which indicated a decrease in latency variability with stimulus experience.

Post-hoc analyses were performed to examine additional effects. A 3 x 4 x 6 analysis of variance with repeated measures over Condition and Electrode was performed on the P2 and N2 latency variability data with Group (low, medium, or high tones as the familiar stimulus), Condition (D1F, D2F, D2N, D3F, D3ON, D3NN), and Electrode (Cz, Fz, Fpz, and T3) as factors. As would be expected from the planned comparisons, a significant main effect for Condition was found at the N2 peak, F(5,105)=3.31, p=.008. A significant interaction for Condition by Electrode at peak N2, F(15, 315)=2.69, p=.001 was found. Additionally a significant interaction for Group by Condition F(10,105)=2.03, p=.037, was found for peak P2 (see Figure 17).

Single-trial Amplitude

Planned comparisons found a significant difference in amplitude between D1F and D2N at peak P2 at electrode Cz (t[23]=-2.33, p=.015)) (see Figure 18) with increases for D2N which supports previous findings with 3 month-old subjects There were also differences found between D1F and D3F (t[23]=1.96, p=.031) and between D1F and D3NN for peak N2 (t[23]=2.73, p=.006 at electrode site T3 (see Figures 19 and 20, respectively). D1F showed lower single-trial amplitude measures than either D3F or D3NN. These findings indicate that increased experience with a stimulus does influence

younger subjects' neural responses and effect a more mature response. The means, standard deviations, and p values for these data are found in Tables 6 and 7 (peaks P2 and N2, respectively). A post-hoc 3 x 4 x 6 analysis of variance with repeated measures over Condition and Electrode was performed on the P2 and N2 single-trial amplitude data with Group (low, medium, or high tones as the familiar stimulus), Condition (D1F, D2F, D2N, D3F, D3ON, D3NN), and Electrode (Cz, Fz, Fpz, and T3) as factors was performed to examine any additional effects. A significant main effect for Electrode was found for peak P2, $F(3,63)=4.21$, $p=.009$ and for peak N2, $F(3,63)=5.72$, $p=.002$. For peak P2, a significant Group by Condition effect was found, $F(10,105)=3.55$, $p=.000$. Figure 21 shows an increase from the first day of experience to the final session for all groups, the exception is a decrease in single-trial amplitude on D3F from D1F with the high tone group. A significant interaction for Condition by Electrode was found for the N2 peak, $F(15,315)=1.72$, $p=.047$. However, Figure 22 indicates that there is a consistent increase from D2F over D1F at all sites, and for D3F over D2F at all sites except for Fpz. There is an overall increase for D3F over D1F at all electrode sites.

Average ERP Latency

Post-hoc analyses (ANOVA) were performed to evaluate the average latency (or time of response from stimulus onset) for peaks P2, N2, and P3, at each electrode site (Cz, Fz, Fpz, and T3). The means and standard deviations for these data are found in Tables 8, 9, and 10). The ANOVA for Condition (D1F, D2F, D2N, D3F, D3ON, D3NN), Electrode (Cz, Fz, Fpz, T3), and Group (low, medium, high tones) (repeated measures) indicated a significant Condition main effect, $F(5, 105)$, $p=.043$ for the N2 peak (see Figure 23). A Tukey HSD was performed based on the significant differences for the Condition main effect. Tukey HSD pairwise differences between the six means revealed significant differences between the D3F condition and both the D2N and D3ON conditions. The D3F group mean indicated significantly smaller latency measures than

either of the other two conditions (see Table 11). This decrease in latency over the three sessions gives further evidence of the effects of increased experience with a stimulus.

Discussion

The first objective of this study was to replicate previous work from our laboratory. The first hypothesis was drawn from this objective, that is, Day 2 of the study would produce results of stimulus generalization and that there would be a general increase in amplitude and a general decrease in latency variability on the second day of the study. Three sets of planned comparisons were conducted to test this hypothesis: a) Day 1-Familiar vs. Day 2-Familiar, b) Day 2-Familiar vs. Day 2-Novel, and c) Day 1-Familiar vs. Day 2-Novel.

The results of the study indicated that the amplitude of the average ERP increased from Day 1 to Day 2 for both the familiar and novel stimulus. However, these differences were significant only for the novel stimulus at the Fz and Fpz electrode sites for peak P2. These findings partially support previous research using this paradigm (Lykins, 1996). This earlier work by Lykins (1996) found much more robust results at all electrode sites for peak P2. Lykins' (1996) study found larger responses for D2F over D1F and larger amplitude measures for D2N over D1F at peak P2. There were no differences between D2F and D2N, indicating a stimulus generalization pattern of neural responses. The Thomas and Lykins (1995) study with 5-month-old subjects indicated larger amplitude responses for D2F over D1F and larger responses for D2F over D2N; however, D2N and D1F responses were equal for peak P2. These results indicate a stimulus specific response pattern in average amplitude measures at peak P2.

For the N2 peak, Lykins' (1996) results showed that 3-month-old subjects responded with larger amplitudes for D2F over D2N and there were no differences between the D2F and D1F responses (stimulus discrimination pattern). However, 5-month-old subjects (Thomas & Lykins, 1995) showed larger responses for D2F over D1F and no differences between D1F and D2N, indicating a stimulus specific response. The present study was unable to replicate previous findings of novel/familiar differentiation at the N2 peak on Day 2 of the study. One possible explanation for the

failure to replicate could be due to a random sampling error; chance alone may have resulted in the measure of a non-representative sample. However, this trend was found for the N2 peak, with all electrode sites exhibiting a larger response (nonsignificant) for the familiar stimulus over the novel and was similar to that found by Lykins (1996) for 3-month-olds.

The second objective of the present study was to examine the results of increased stimulus experience with the neural responses of 3-month-olds. A set of planned comparisons was designed to test the second hypothesis that after 3 days of stimulus experience, the Day 3-Familiar ERPs would indicate stimulus specificity similar to that found on Day 2 for the 5-month-olds. The set of planned comparisons were: a) Day 1-Familiar vs. Day 3-Familiar, b) Day 1-Familiar vs. Day 3-New Novel, and c) Day 3-Familiar vs. Day 3-New Novel. This hypothesis was partially supported by the larger amplitude measures for D3F over D1F for all peaks with significant differences found for N2 at the Cz site. However, the means for the D3NN group indicated a larger amplitude trend over the D1F group with a significant finding at the Cz electrode for peak N2. This trend was found for both peaks P2 and P3 as well, however, there were no significant findings. These findings suggest a pattern of stimulus generalization (larger amplitudes for both novel and familiar stimuli) over the 3-day period. However, the overall trend for larger responses for D3F over D3NN at peak N2 (significant differences found at electrode Fz) provide further support for the hypothesis that increased stimulus experience engenders more mature neural responses in younger subjects.

A third set of planned comparisons was conducted to further examine the effect of increased stimulus experience: a) Day 2-Novel vs. Day 3-New Novel, b) Day 2-Familiar vs. Day 3-Old Novel, and c) Day 2-Familiar vs. Day 3-Familiar. There were no significant differences for peaks P2 and N2; however, P3 found larger responses for D3ON over D2F indicating that the extra trials (150 tones vs. 100 tones) did not add to the amplitude response measures. The subject had two days of experience with both stimuli; the

measurement of D2F involved 150 tones and D3ON measured the results of experience with 100 tones.

The ANOVA conducted with Group, Condition, and Electrode as factors found a significant main effect for Electrode at both P2 and N2 peaks. A Group x Condition x Electrode interaction was also found at both peaks P2 and N2. The data were collapsed across electrodes and the means were plotted to examine the data for possible stimulus specific responses. The results showed that even though the medium level tone group's average amplitude was lower that either the low or high tone group, the trend or pattern of responding from Day 1-Familiar to Day 2-Familiar to Day 3-Familiar was the same, that is, increasing over time.

Previous work with 3-month-olds (Lykins, 1996) found greater latency variability for D1F over both D2F and D2N at peak P2. Those results also showed no differences between D2F and D2N, indicating a stimulus general response pattern at the P2 peak. However peak N2 indicated a specific response pattern with more variable responses for both D1F and D2N compared to D2F. There were no differences between D1F and D2N. Previous research with 5-month-olds (Thomas & Lykins, 1995) found even more robust indications of stimulus specificity for both peaks P2 and N2.

Latency variability measures in the present study proved to be the strongest support for the three hypotheses. All of the latency variability measures between D2F and D2N at peak N2 showed greater variability for the novel stimulus with a significant difference at the Fpz electrode site. These findings support previous research from our laboratory (Lykins, 1995; Letterman, 1996). The greater latency variability measures for D3NN over D3F supports the hypothesis stating that increased experience with a stimulus will result in younger subjects responses being similar to the older (5-month-old) subjects response measures.

The final objective examines the importance of the number of trials (or amount of experience with a stimulus) versus the 24-hour period between sessions. Which is more

important in effecting change in neural response measures? The present results seem to support the consolidation period as having more influence on change in neural response measures. We find a decrease between D2F and D3F latency variability measures which indicates that with more experience with a stimulus, the more consolidated and efficient becomes the response. However, there were no differences found between D2F and D3ON; the subjects had an unequal amount of experience (150 trials vs. 100 trials). There were also no differences between D2N and D3NN (same number of trials over 2 days). The differences found between D1F and D3F might be most heavily influenced by the length of time over the three sessions, since the total number of trials does not support the "amount of experience" hypothesis.

The post-hoc examination of the average latency measures provided some support for the notion that increased experience with a given stimulus should result in a decrease in latency measures. The average latency measures for D3F (the condition with the most experience with a given stimulus) were smaller than all other conditions (with measures collapsed across electrodes) and Tukey's HSD found a significant decrease for D3F in comparison to both D2N and D3ON.

The larger responses for Day 3 in the present study suggested a strengthening of the neural ensembles for responding to familiar auditory stimuli. This increase in response measures for the familiar rather than the novel stimulus indicated differentiation between novel and familiar stimuli in response to amplitude and latency variability. The larger responses in concert with the decrease in latency variability measures for the Day 3 familiar stimulus over the novel support the general hypothesis that an increase in experience with a stimulus produces more mature neural responses in 3-month-old subjects that are quantitatively similar to 5-month-olds.

Previous work with 5-month-old subjects found robust novel/familiar differentiation on the second day of the study (Thomas & Lykins, 1995). Another study utilizing the same paradigm but with younger (3-month-old) subjects found a more robust

pattern of stimulus generalization, with an indication of differentiation at peak N2. The present study suggested that with an increase of experience with a given stimulus, 3-month-old subjects would show patterns of neural responding similar to the 5-month-subjects. The lack of robust findings with the 3-month-old subjects in the present study could indicate that there is more than stimulus experience, or the length of consolidation time, influencing the pattern of neural responding.

The lack of robust findings of novel/familiar differentiation on Day 3 could also be the result of an inadequate sample size. Future investigations might utilize a larger (n=36) sample to make better comparisons of younger infants with older subjects. The use of the statistical measure Omega-squared indicated a small to medium effect size (.017 to.163) for D3F and D3N comparisons in the present study. The study with 5-month-old infants found a large effect size, Omega-squared ranged from .20 to .43 for D2F to D2N comparisons. Omega-squared is unaffected by sample size; however, if an effect size is small it is difficult to ascertain if there are true differences between groups if the sample size is also small, because of the lack of a satisfactory level of statistical power. With a larger sample size and thus a higher level of power, the nonsignificant trends found throughout the data for differential responses for the familiar and novel stimuli might have been enhanced.

The data suggest that the neural responses of 3-month-old infants become stronger and less variable with experience. However, both developmental changes and experience affect the number of dendritic spines, the sizes of the synaptic contact area, dendritic branching and myelin formation in the infant brain. During early human development there is a profusion of brain growth (Kolb & Whishaw, 1990). At birth the human brain weighs about 350 grams; by the end of the child's first year the brain has increased to 1,000 grams. Much of this increase in neural growth is due to dendritic proliferation and myelination. Synaptic density increases until about 2 years of age and then declines until about 50% have disappeared by the age of 16 (Kolb & Whishaw, 1990).

However, the normal course of neural development can be strongly influenced by the organism's experience. There is an established body of research detailing the results of experience and brain development. Experience affects both the structure and the chemistry of the neural system. Studies with non-human subjects have found that a single session of learning was sufficient to alter hippocampal synapses both in number and morphology (Rosenzweig, 1984). Experience engenders an increase in acetycholinesterase (AChE) activity, which in turn affects protein development in the brain (Rosenzweig, 1984). With more protein development, brain size and weight increases. It is not currently known how protein affects this change.

The experience of multiple presentations of an auditory stimulus could strengthen a neural pathway by these types of processes. The second and third sessions with familiar stimuli might provide an environment that increases the "survivability" of that neural pathway. This could account for the increase in neural responding on Day 3 for the familiar stimulus. Rather than a "shedding" of synapses, experience may be more necessary for providing the necessary AChE activity (and other enzymatic processes) needed to "build" stronger neural pathways.

The stimuli used in the present study were simple pure tones (400 Hz, 700 Hz, and 1000 Hz). Animal studies have found that subjects exposed to more complex stimuli showed higher levels of cortical AChE activity and greater changes in brain weight (Rosenzweig, 1984). One study used three conditions: a) a control group of rats that were isolated in cages, b) social control groups (SC) of three animals to a cage, and c) groups of three animals with an enriched environment (EE). There were significant differences between all groups. Not only did the additional experience of an enriched physical environment with toys and running wheels cause differences in brain growth, but the mere presence of others influenced development (Rosenzweig, Krech, & Bennett, 1961). There were larger changes between the EE group and the control group than between the EE group and the SC group. Future auditory ERP research with human

infants might therefore employ more complex auditory stimuli. The more complex stimuli could possibly increase the differential response patterns among the six conditions (D1F, D2F, D2N, D3F, D3ON, D3NN).

The present study indicated larger responses for Day 3 Familiar over Day 3 New Novel; however, these findings were not particularly robust. Our laboratory is now examining this issue by collecting ERP data with 3-month-old subjects and using speech sounds for the auditory stimulus. More complex stimuli (speech sounds) might strengthen the response patterns and more robust differential response patterns might emerge.

This project found a weak pattern of stimulus-specific responses for 3-month-old infants with additional experience with a given stimulus; however, there were even more indicators of stimulus generalization throughout the analyses of the various electrode sites and different peaks. This suggests that the younger (3-month-old) subjects can produce (with additional stimulus experience) neural patterns similar to those of older subjects ; however, there seems to be natural developmental differences as well. The behavior studies (Rose et al., 1982), suggest that younger subjects behave much like older infants, given sufficient stimulus experience. Our study does not find such a cut and dry explanation; younger infants with additional stimulus experience show general neural patterns similar to those of same-age subjects with less experience. Younger subjects can be "pushed" to behave neurologically similar to older subjects, but not completely.

Another matter that must be considered in future ERP studies of infant recognition memory involves the theory of time windows in cognitive development (Rovee-Collier, 1995). Rovee-Collier found that an earlier-experienced stimulus can be integrated with a new stimulus if that new stimulus is sufficiently similar to the prior one. This can occur if the two experiences with the stimuli are close enough in time. Rovee-Collier found that her 3-month-old subjects would integrate two similar stimuli within a 4-day time window. The integration of information about two, sequentially experienced stimuli, is thought to result from the simultaneous activity of their representations in primary or working

memory (Rovee-Collier, 1995). This creates a new memory representation that contains aspects of both stimuli . In other words, it is possible that, in the present study, the experience with the familiar stimulus on Day 1 and on Day 2 was integrated with the novel stimulus presented on Day 2. Perhaps by the third session, neural comparisons were actually being made between the Day 3 New Novel stimulus and the combined experience of the other two stimuli presented on the first and second day of the study. This is an important question that would need to be addressed in any future studies of infant recognition memory.

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Table 1

Means, Standard Deviations, and p Values for the Average Peak Amplitude for P2

	Cz		Fz		Fpz		T3	
	X	S	X	S	X	S	X	S
D1F	4.60	3.32	4.82	2.84	5.10	3.08	4.03	3.80
D2F	5.36	4.28	6.17	3.67	6.63	3.62	4.81	3.96
D2N	5.48	3.55	6.45	2.83	6.72	2.74	5.67	3.56
D3F	4.29	5.66	5.53	5.13	5.56	5.06	5.66	4.66
D3ON	5.08	5.16	5.51	4.30	5.62	3.94	4.97	4.72
D3NN	5.10	4.88	5.92	5.26	5.80	5.37	5.81	4.81

Comparisons	Cz	Fz	Fpz	T3
D1F vs. D2F	NS	NS	NS	NS
D2F vs. D2N	NS	NS	NS	NS
D1F vs. D2N	NS	.025	.028	<.1*
D1F vs. D3F	NS	NS	NS	NS
D1F vs. D3NN	NS	NS	NS	<.1*
D3F vs. D3NN	NS	NS	NS	NS
D2N vs. D3NN	NS	NS	NS	NS
D2F vs. D3ON	NS	NS	NS	NS
D2F vs. D3F	NS	NS	NS	NS

* Statistically marginal significance

Table 2

Means, Standard Deviations, and p Values for the Average Peak Amplitude for N2

	Cz		Fz		Fpz		T3	
	X	S	X	S	X	S	X	S
D1F	-5.06	3.16	-5.78	3.26	-5.74	3.62	-4.89	3.90
D2F	-6.45	3.79	-6.82	3.57	-7.00	3.37	-5.87	2.79
D2N	-5.17	3.99	-5.33	3.79	-5.47	3.79	-5.20	4.25
D3F	-7.27	3.66	-7.92	4.13	-7.63	4.32	-6.63	4.12
D3ON	-6.23	4.10	-7.17	4.32	-7.14	4.54	-6.45	4.04
D3NN	-5.99	3.95	-5.97	3.32	-5.95	3.46	-6.54	4.90

Comparisons	Cz	Fz	Fpz	T3
D1F vs. D2F	NS	NS	NS	NS
D2F vs. D2N	NS	.035*	.039*	NS
D1F vs. D2N	NS	NS	NS	NS
D1F vs. D3F	.015	.039*	<.1*	<.1*
D1F vs. D3NN	NS	NS	NS	<.1*
D3F vs. D3NN	<.1*	.024	.037*	NS
D2N vs. D3NN	NS	NS	NS	NS
D2F vs. D3ON	NS	NS	NS	NS
D2F vs. D3F	NS	NS	NS	NS

* Statistically marginal significance

Table 3

Means, Standard Deviations, and p Values for the Average Peak Amplitude for P3

	Cz		Fz		Fpz		T3	
	X	S	X	S	X	S	X	S
D1F	4.86	1.93	4.25	2.27	4.30	1.95	3.78	2.36
D2F	4.10	2.83	4.42	3.13	4.44	3.29	4.33	2.88
D2N	5.07	2.97	4.52	2.53	4.17	3.23	4.40	2.86
D3F	5.80	3.37	5.37	2.86	5.35	2.93	5.14	2.58
D3ON	5.84	3.12	4.99	2.91	4.88	2.90	4.28	2.09
D3NN	6.24	2.13	5.42	3.11	5.33	3.13	4.56	2.76

Comparisons	Cz	Fz	Fpz	T3
D1F vs. D2F	NS	NS	NS	NS
D2F vs. D2N	NS	NS	NS	NS
D1F vs. D2N	NS	NS	NS	NS
D1F vs. D3F	NS	<.1*	<.1*	.005
D1F vs. D3NN	.013	<.1*	NS	NS
D3F vs. D3NN	NS	NS	NS	NS
D2N vs. D3NN	<.1*	NS	NS	NS
D2F vs. D3ON	.021	NS	NS	NS
D2F vs. D3F	<.1*	NS	NS	NS

* Statistically marginal significance

Table 4

Means, Standard Deviations, and p Values for the Latency Variability for Peak P2

	Cz		Fz		Fpz		T3	
	X	S	X	S	X	S	X	S
D1F	88.09	10.22	87.00	8.66	85.48	8.21	88.44	8.36
D2F	85.18	11.58	84.18	14.53	85.55	15.28	86.98	11.02
D2N	87.87	10.43	85.85	9.21	86.89	10.55	88.07	8.74
D3F	85.44	12.54	84.02	14.24	83.62	15.37	81.54	17.87
D3ON	88.33	10.15	88.61	9.33	89.45	9.45	85.30	9.32
D3NN	87.56	8.31	86.79	8.86	86.04	10.29	84.82	13.15

Comparisons	Cz	Fz	Fpz	T3
D1F vs D2F	NS	NS	NS	NS
D2F vs D2N	NS	NS	NS	NS
D1F vs D2N	NS	NS	NS	NS
D1F vs D3F	NS	NS	NS	<.1*
D1F vs D3NN	NS	NS	NS	NS
D3F vs D3NN	NS	NS	NS	NS
D2N vs D3NN	NS	NS	NS	NS
D2F vs D3ON	NS	<.1*	NS	NS
D2F vs D3F	NS	NS	NS	NS

* Statistically marginal significance

Table 5

Means, Standard Deviations, and p Values for the Latency Variability for Peak N2

	Cz		Fz		Fpz		T3	
	X	S	X	S	X	S	X	S
D1F	89.05	6.60	87.75	8.12	86.37	7.75	90.98	10.53
D2F	88.00	9.20	86.51	9.72	85.19	9.52	86.6	9.31
D2N	89.57	7.63	89.80	6.81	89.74	8.14	88.85	7.63
D3F	81.89	10.70	81.74	13.59	82.42	13.99	79.91	15.84
D3ON	88.33	10.15	88.61	9.33	89.45	9.45	85.30	9.32
D3NN	87.08	8.68	88.82	9.74	86.31	10.59	84.55	12.01

Comparisons	Cz	Fz	Fpz	T3
D1F vs D2F	NS	NS	NS	NS
D2F vs D2N	NS	NS	.019	<.1*
D1F vs D2N	NS	NS	<.1*	NS
D1F vs D3F	.000	.012	<.1*	.005
D1F vs D3NN	NS	NS	NS	<.1*
D3F vs D3NN	<.1*	.019	NS	<.1*
D2N vs D3NN	NS	NS	NS	NS
D2F vs D3ON	NS	NS	NS	<.1*
D2F vs D3F	.009	<.1*	NS	.036*

* Statistically marginal significance

Table 6

Means, Standard Deviations, and p Values for Single-trial Amplitude for Peak P2

	Cz		Fz		Fpz		T3	
	X	S	X	S	X	S	X	S
D1F	11.09	6.79	13.51	4.25	13.35	4.13	10.37	6.04
D2F	12.89	7.20	13.01	6.03	13.33	5.66	11.77	8.12
D2N	15.13	6.64	14.64	8.38	14.73	8.31	12.90	7.80
D3F	11.88	8.29	14.23	5.12	14.24	5.11	12.45	5.51
D3ON	14.00	6.48	14.24	6.30	14.14	6.14	11.74	8.38
D3NN	12.60	10.15	14.03	10.04	13.15	9.90	12.78	8.43

Comparisons	Cz	Fz	Fpz	T3
D1F vs D2F	NS	NS	NS	NS
D2F vs D2N	NS	NS	NS	NS
D1F vs D2N	.015	NS	NS	<.1*
D1F vs D3F	NS	NS	NS	NS
D1F vs D3NN	NS	NS	NS	NS
D3F vs D3NN	NS	NS	NS	NS
D2N vs D3NN	NS	NS	NS	NS
D2F vs D3ON	NS	NS	NS	NS
D2F vs D3F	NS	NS	NS	NS

* Statistically marginal significance

Table 7

Means, Standard Deviations, and p Values for Single-trial Amplitude for Peak N2

	Cz		Fz		Fpz		T3	
	X	S	X	S	X	S	X	S
D1F	-12.50	7.28	-14.09	5.29	-13.52	5.74	-10.23	5.60
D2F	-15.35	8.35	-14.40	9.01	-14.63	7.00	-12.53	6.54
D2N	-14.50	7.36	-13.45	7.19	-13.89	7.22	-11.29	7.46
D3F	-15.95	7.17	-15.11	7.57	-14.46	7.11	-13.33	6.80
D3ON	-14.17	7.55	-15.75	6.82	-15.17	7.35	-15.66	5.43
D3NN	-14.70	8.15	-15.16	6.12	-14.71	6.54	-13.98	6.27

Comparisons	Cz	Fz	Fpz	T3
D1F vs D2F	<.1*	NS	NS	<.1*
D2F vs D2N	NS	NS	NS	NS
D1F vs D2N	NS	NS	NS	NS
D1F vs D3F	<.1*	NS	NS	.031
D1F vs D3NN	NS	NS	NS	.006
D3F vs D3NN	NS	NS	NS	NS
D2N vs D3NN	NS	NS	NS	<.1*
D2F vs D3ON	NS	NS	NS	<.1*
D2F vs D3F	NS	NS	NS	NS

* Statistically marginal significance

Table 8

Means and Standard Deviations for the Average Peak Latency for P2

	Cz		Fz		Fpz		T3	
	X	S	X	S	X	S	X	S
D1F	235	48.94	233	44.23	234	44.51	221	54.60
D2F	223	51.82	225	44.44	226	43.91	226	59.55
D2N	217	43.75	213	33.89	213	31.10	228	56.84
D3F	235	38.87	231	38.19	229	38.74	238	52.48
D3ON	235	48.52	227	55.69	222	54.50	244	60.18
D3NN	242	44.23	230	38.30	227	36.70	232	50.10

Table 9

Means and Standard Deviations for the Average Peak Latency for N2

	Cz		Fz		Fpz		T3	
	X	S	X	S	X	S	X	S
D1F	422	94.8	424	86.83	421	88.41	418	104.53
D2F	409	82.29	426	77.61	439	91.42	438	96.15
D2N	441	88.91	453	78.89	456	78.26	445	98.97
D3F	395	81.12	399	84.35	388	73.90	410	90.75
D3ON	441	88.91	461	87.53	460	86.00	436	84.63
D3NN	407	66.47	416	71.71	414	75.05	411	80.28

Table 10

Means and Standard Deviations for the Average Peak Latency for P3

	Cz		Fz		Fpz		T3	
	X	S	X	S	X	S	X	S
D1F	845	208	880	171	881	198	950	188
D2F	832	215	858	230	862	235	873	208
D2N	781	216	793	201	816	197	759	187
D3F	732	235	759	246	738	251	711	259
D3ON	757	194	804	220	796	215	834	203
D3NN	798	215	831	232	857	214	912	186

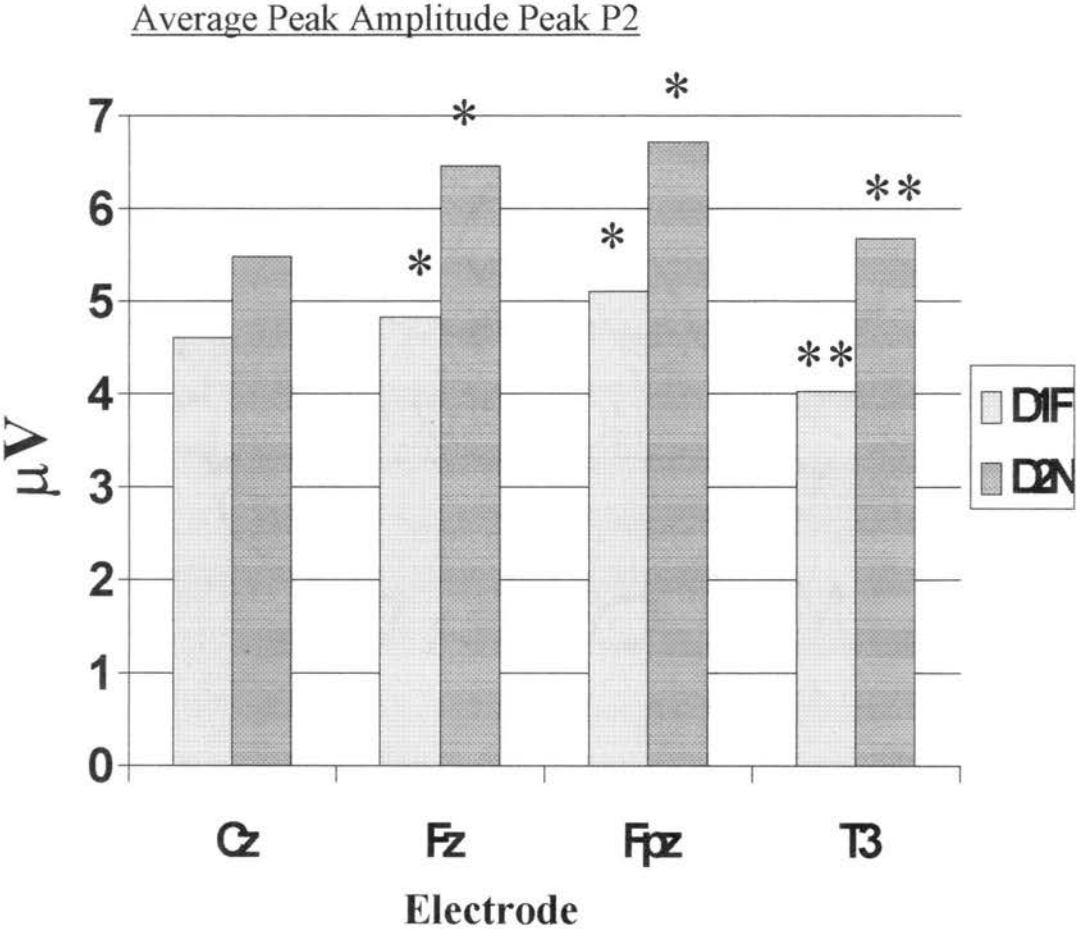
Table 11

Tukey's HSD Test for Condition Mean Differences for Latency at N2 Peak

	Means	D3F	D3NN	D1F	D2F	D2N	D3NN
D3F =	398	----	14	23	30	49*	51*
D3NN =	412	----	----	9	16	35	37
D1F =	421	----	----	----	7	26	28
D2F =	428	----	----	----	----	19	21
D2N=	447	----	----	----	----	----	2
D3N =	449	----	----	----	----	----	----

* Significant Differences Between Means

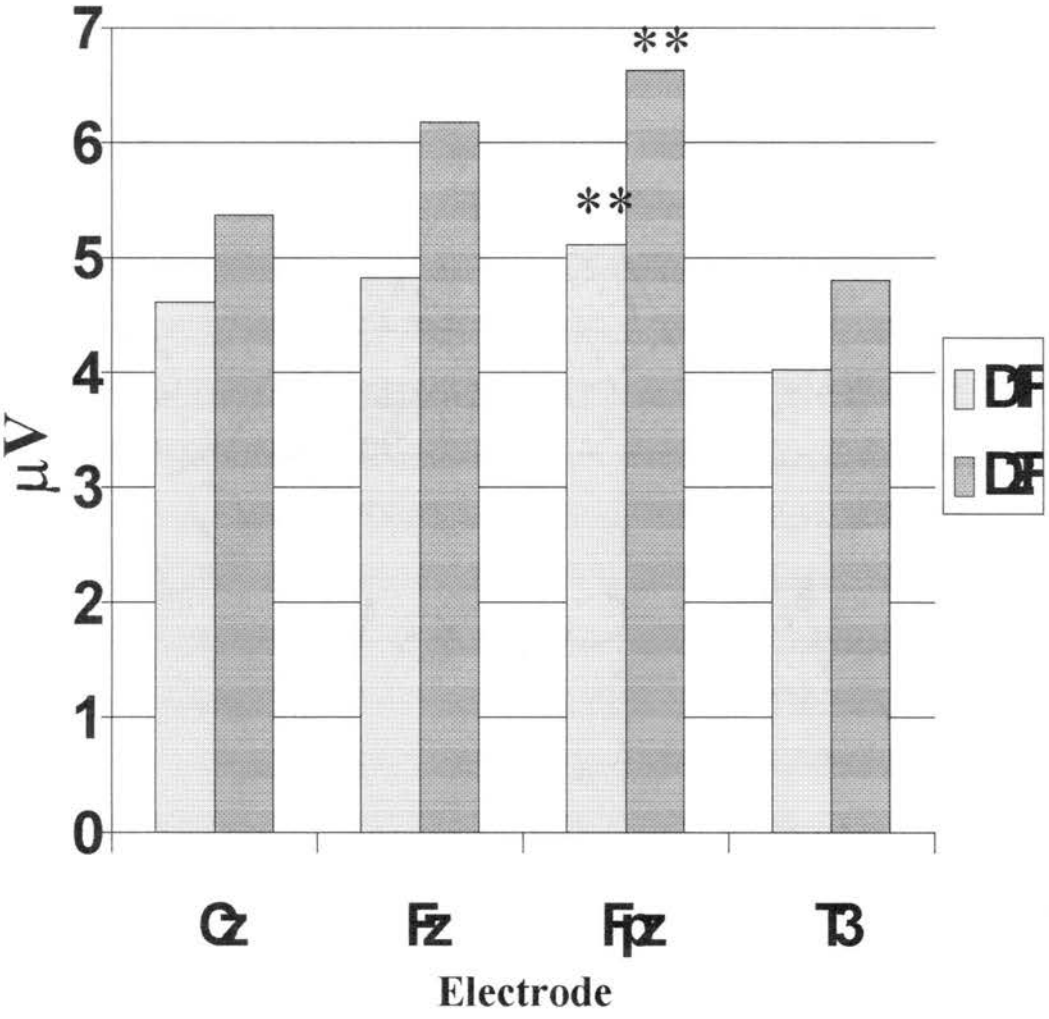
Figure 1



*** p<.032** **** p<.10**

Figure 2

Average PeakAmplitude Peak P2



** p<.10

Figure 3

Average Peak Amplitude Peak P2

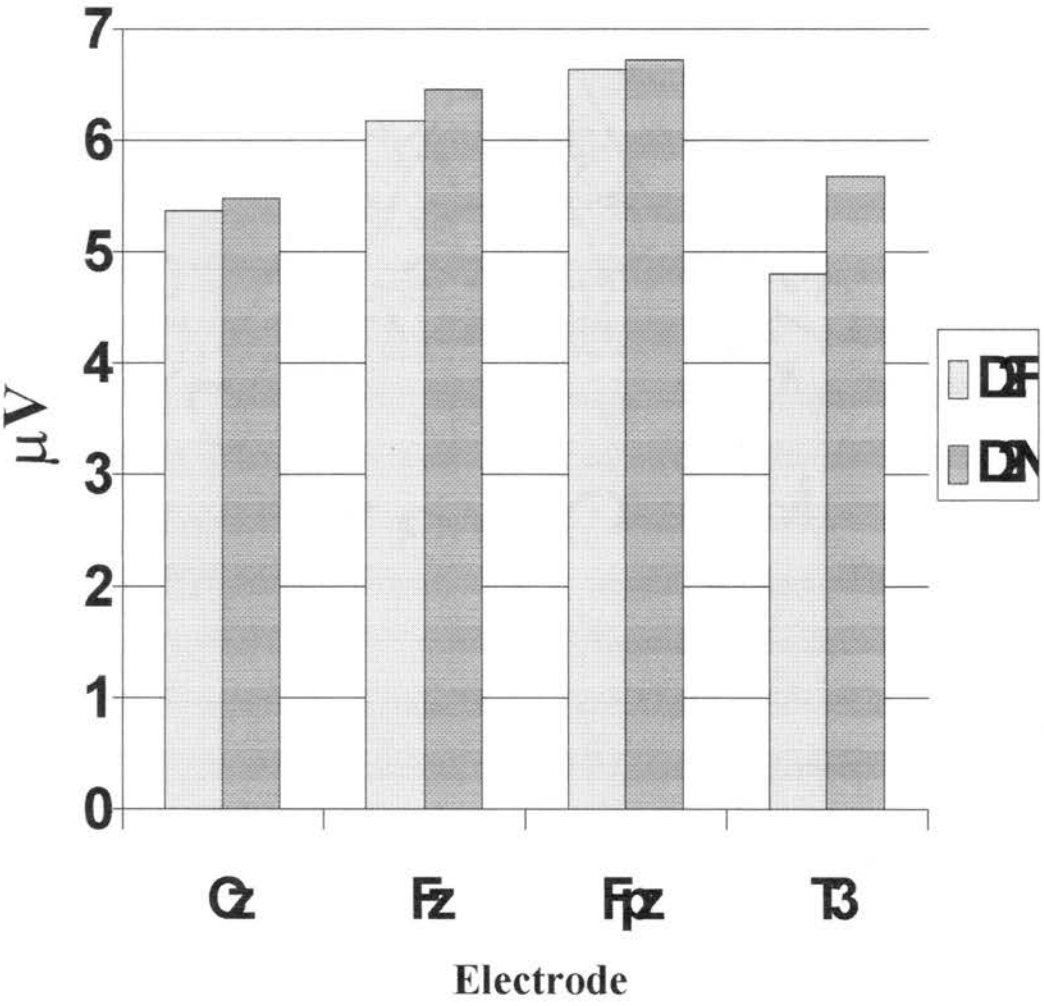


Figure 4

Average Peak Amplitude Peak P3

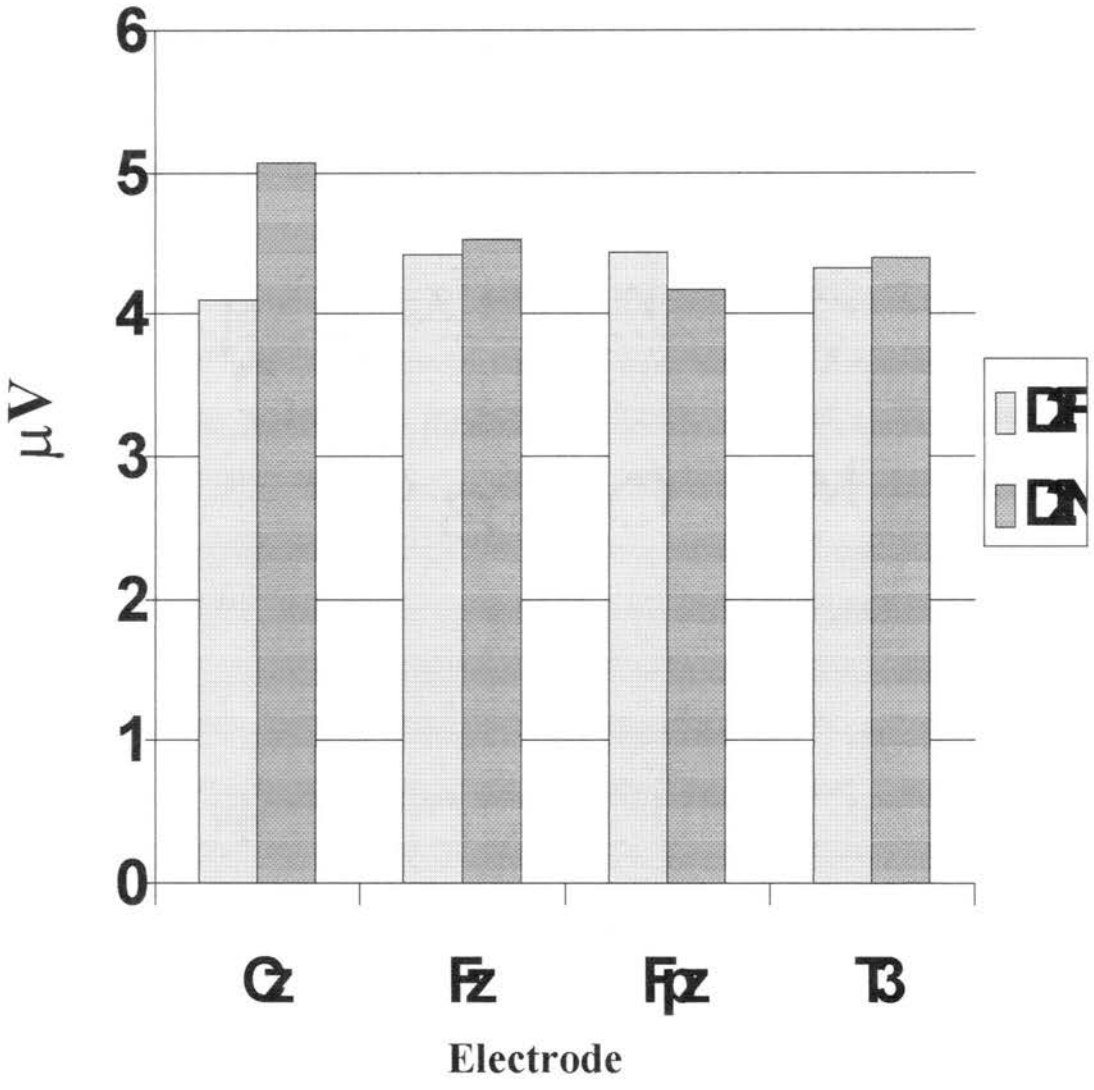
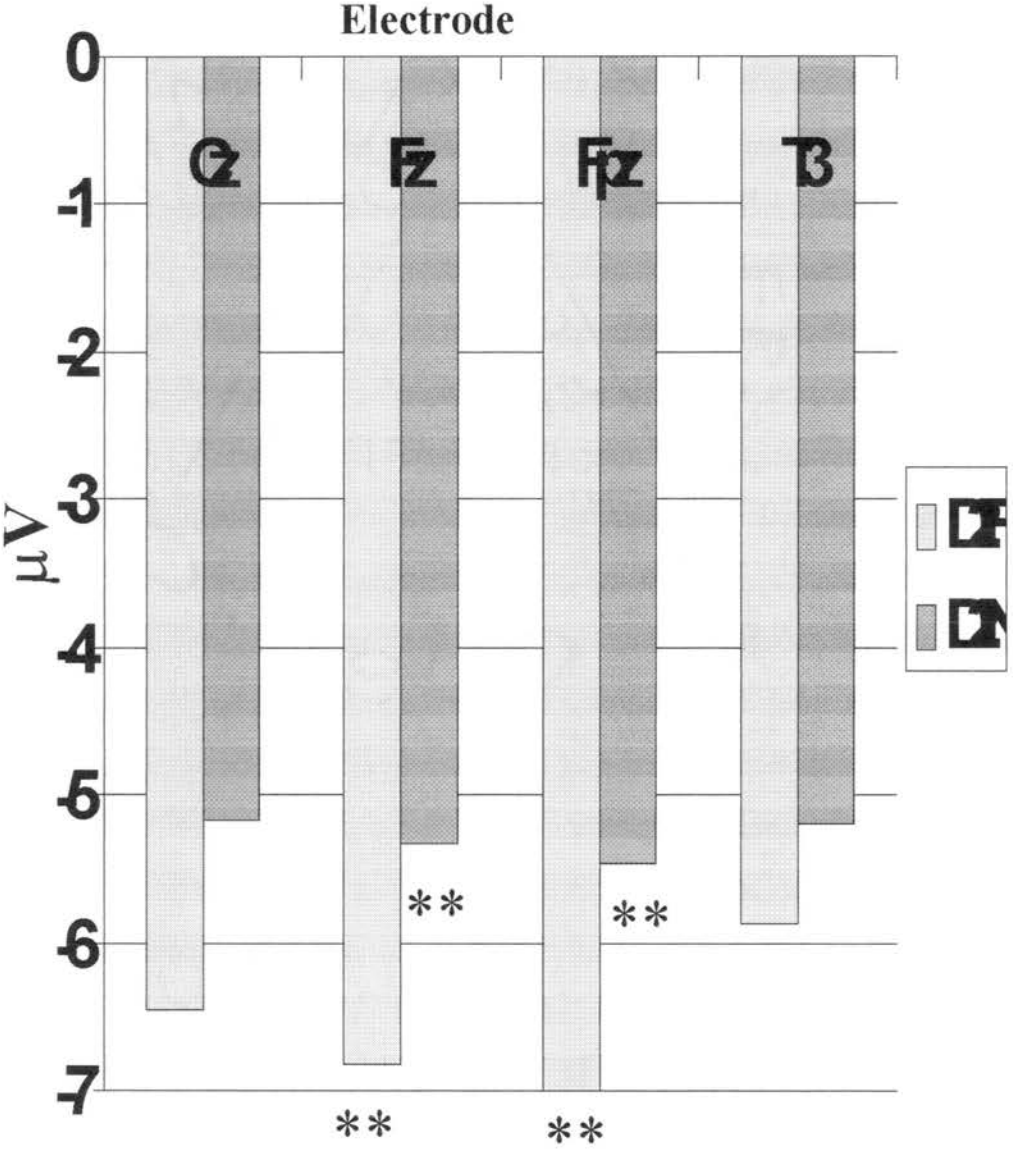


Figure 5

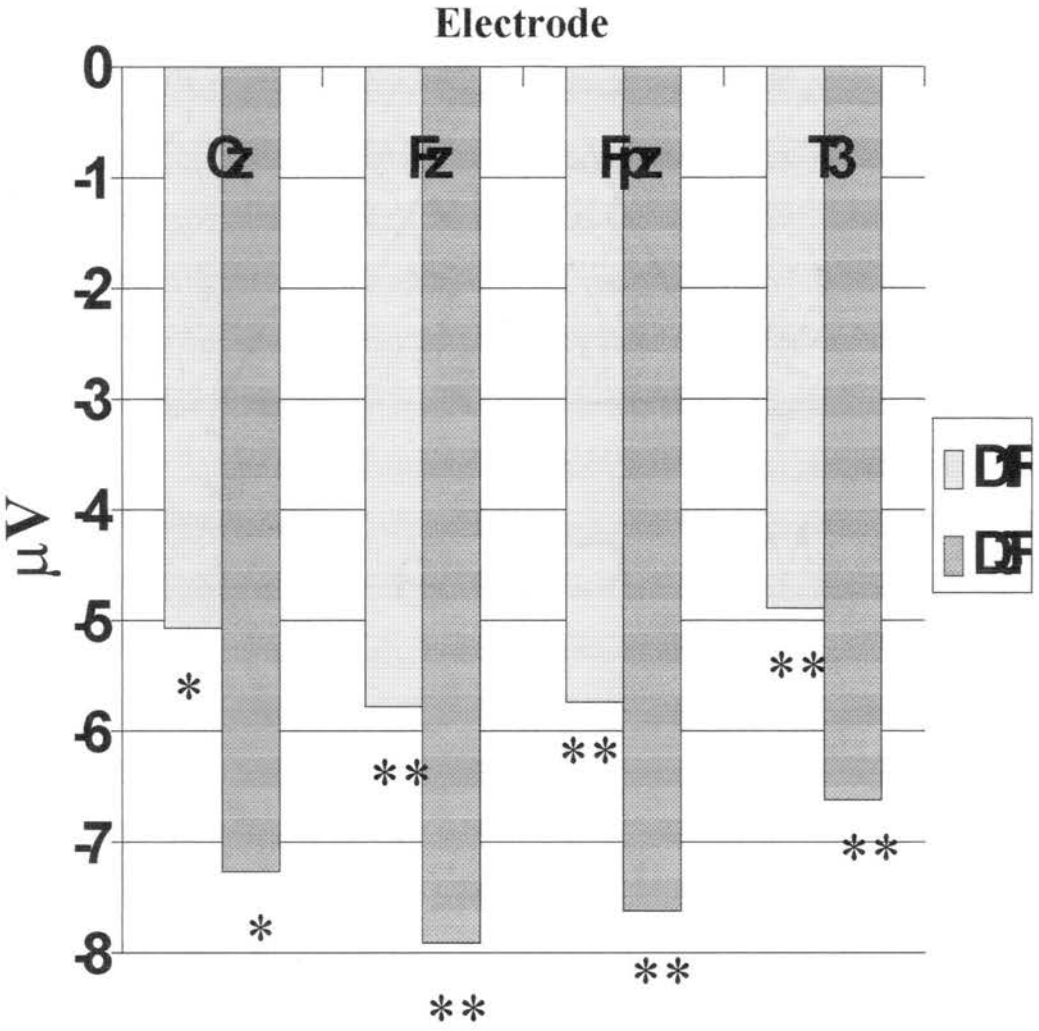
Average Peak Amplitude Peak N2



** p<.10

Figure 6

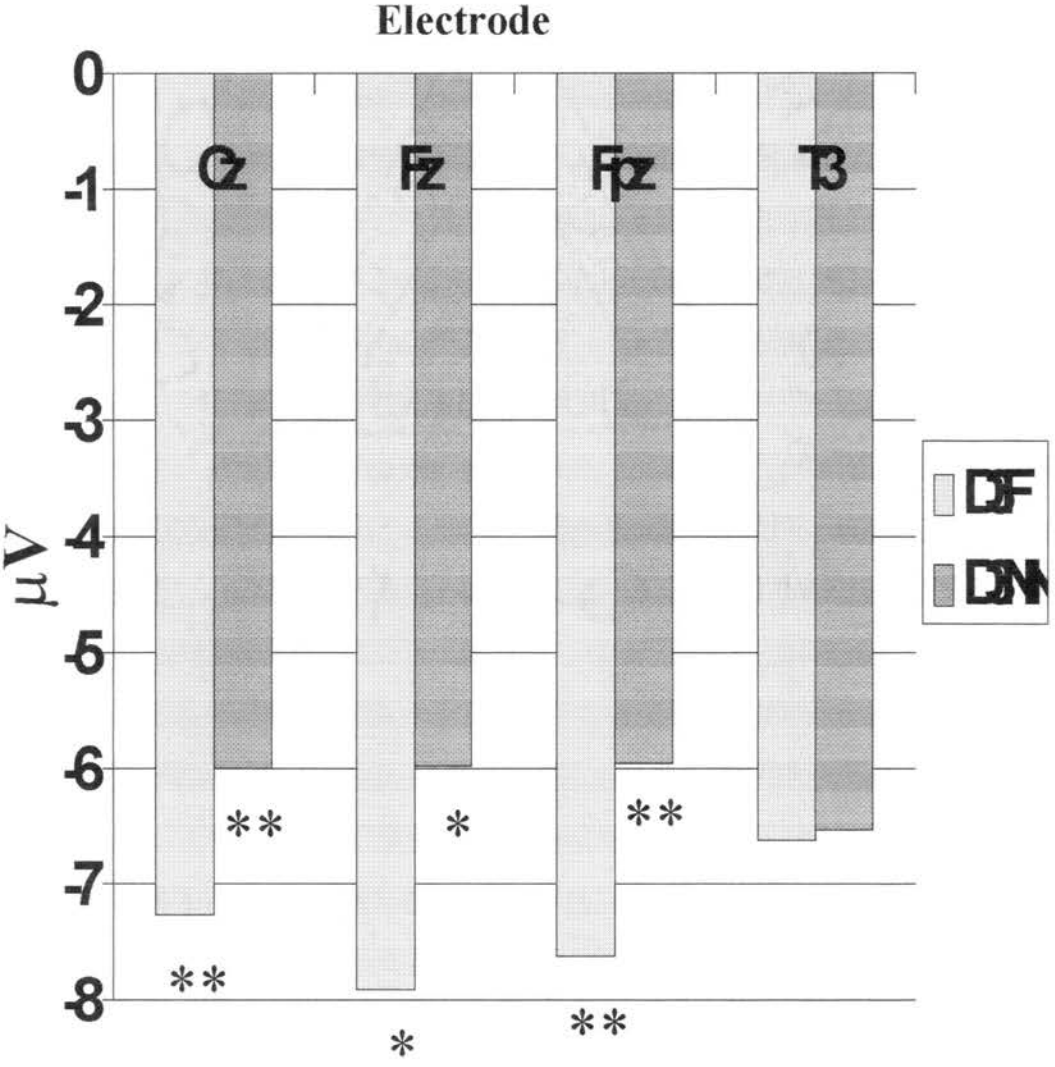
Average Peak Amplitude Peak N2



* p<.032 ** p<.10

Figure 7

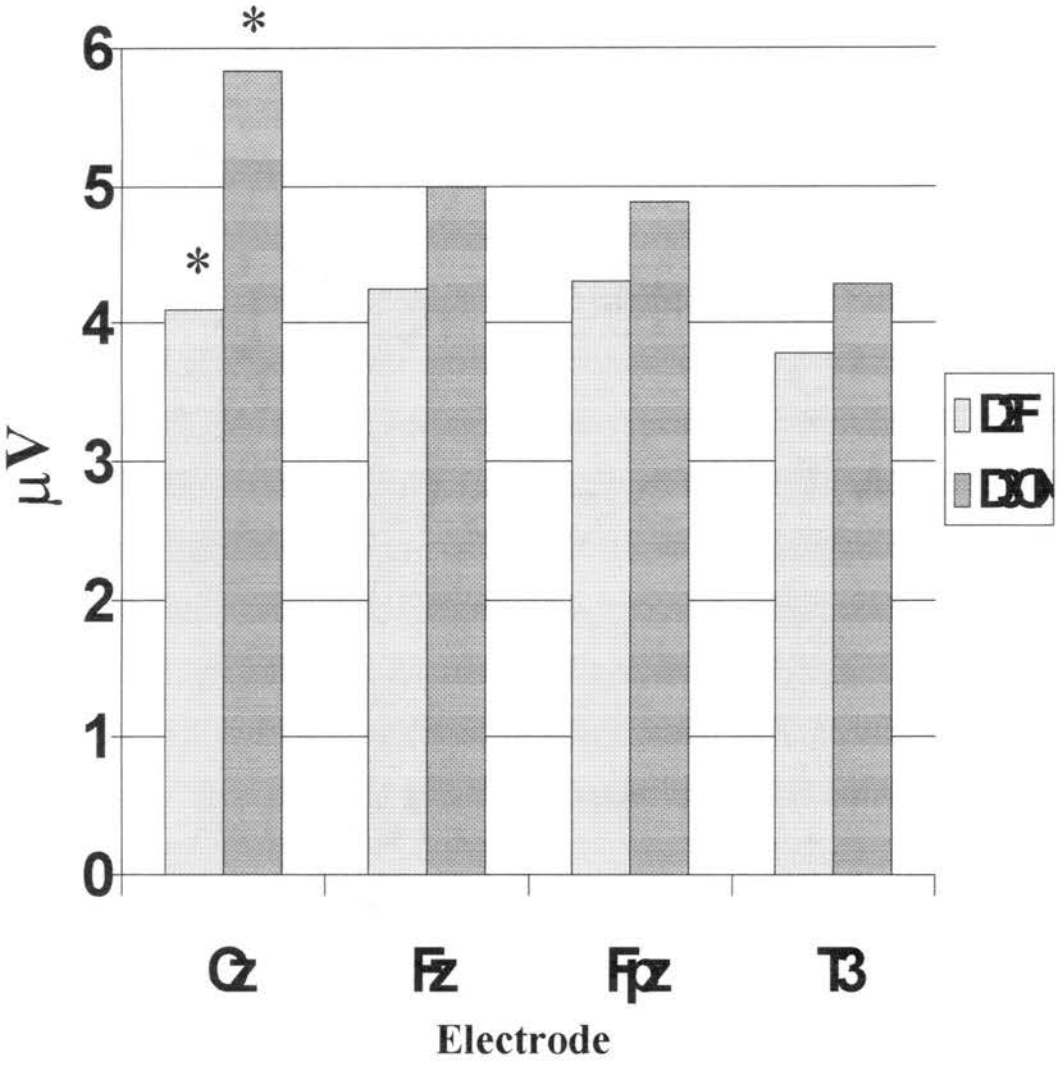
Average Peak Amplitude Peak N2



* p<.032 ** p<.10

Figure 8

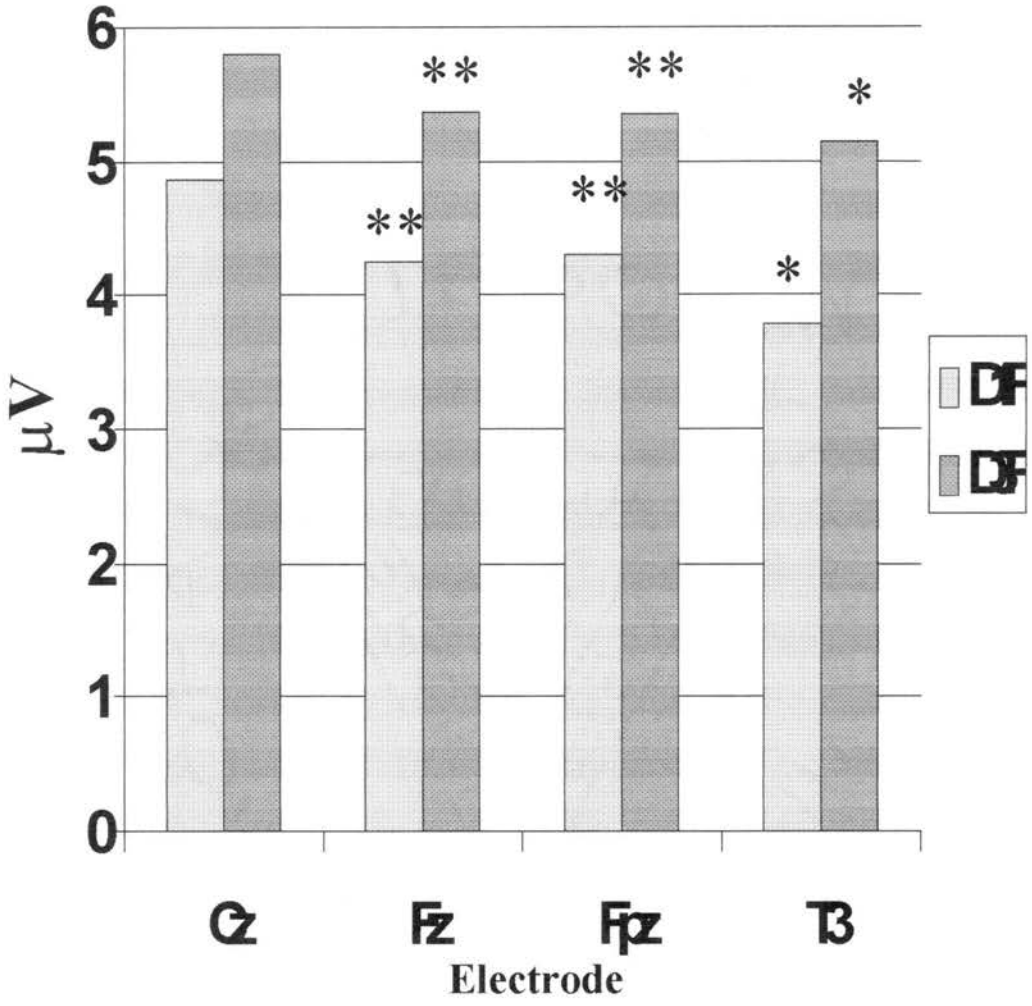
Average Peak Amplitude Peak P3



* p<.032

Figure 9

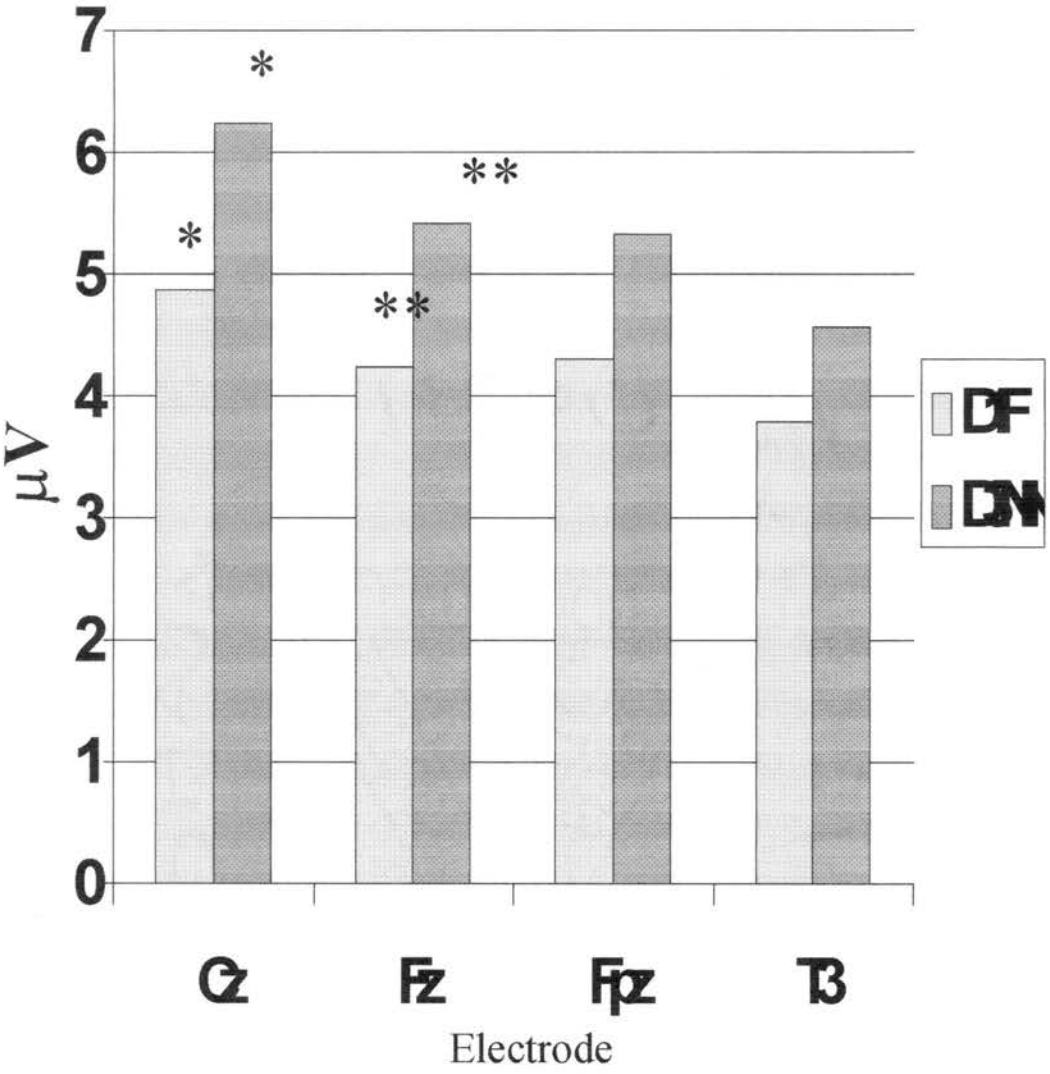
Average Peak Amplitude Peak P3



* p<.032 ** p<.10

Figure 10

Average Peak Amplitude Peak P3



* p<.032 ** p<.10

Figure 11

Peak N2 Single-trial Amplitude Tone by Condition Interaction

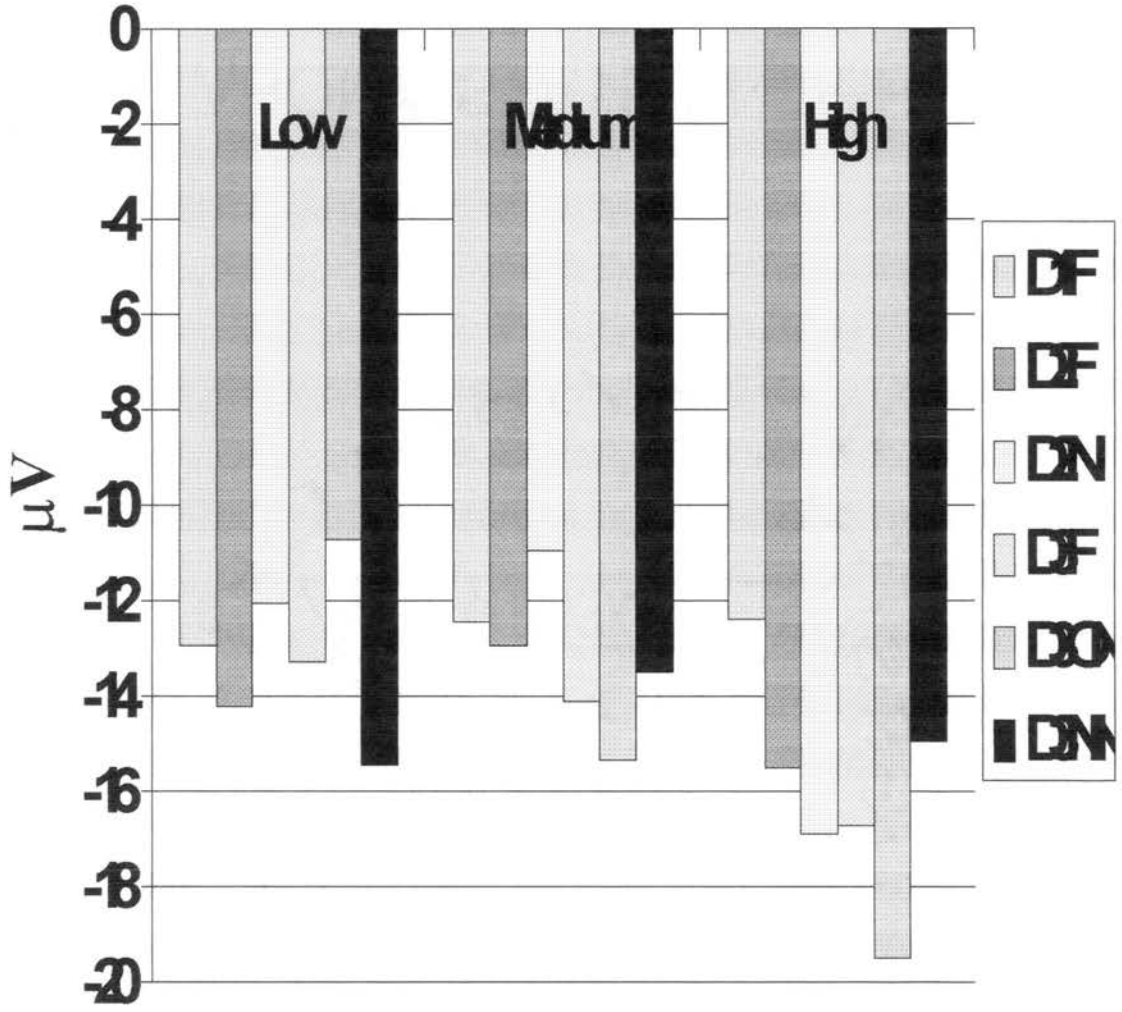


Figure12

Peak P2 Average Amplitude Group by Condition Interaction

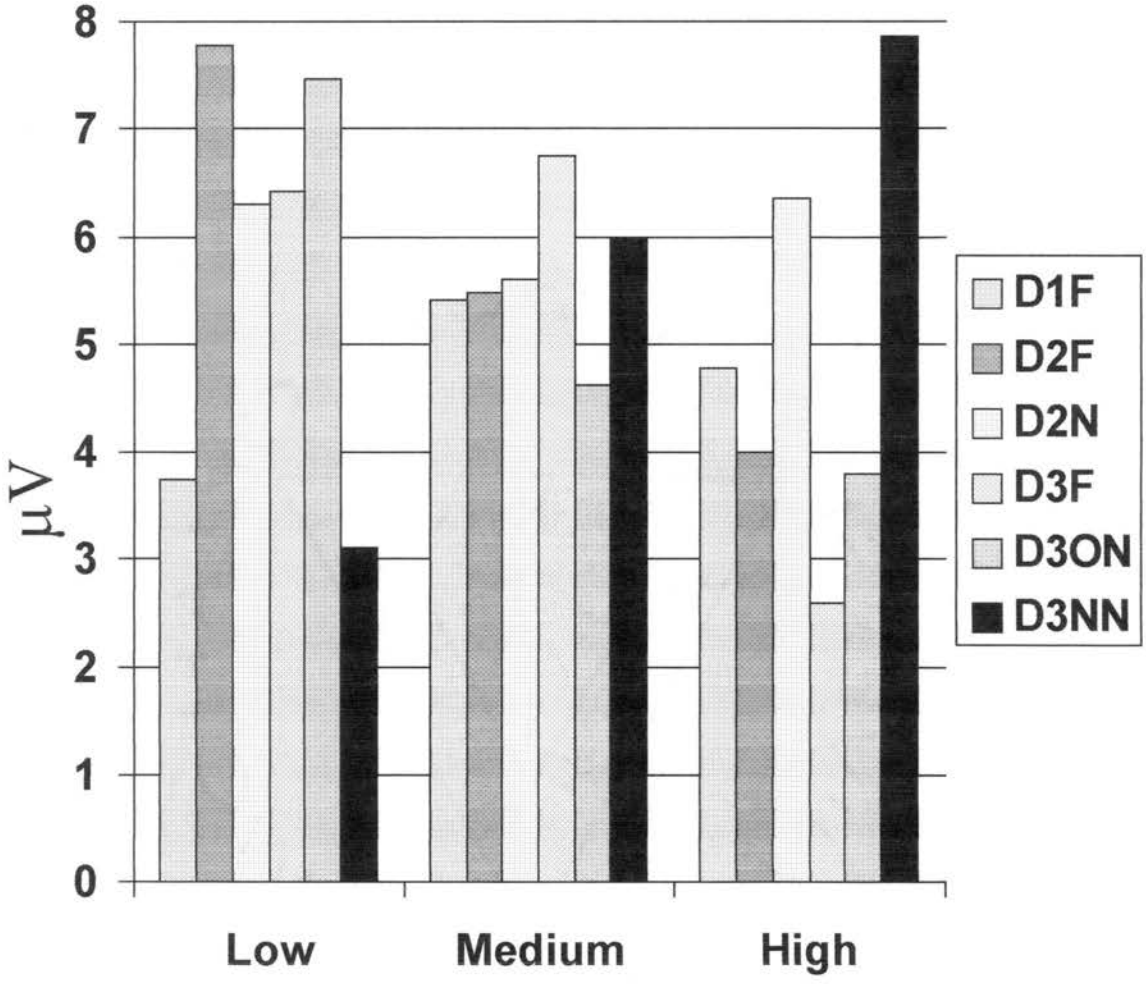
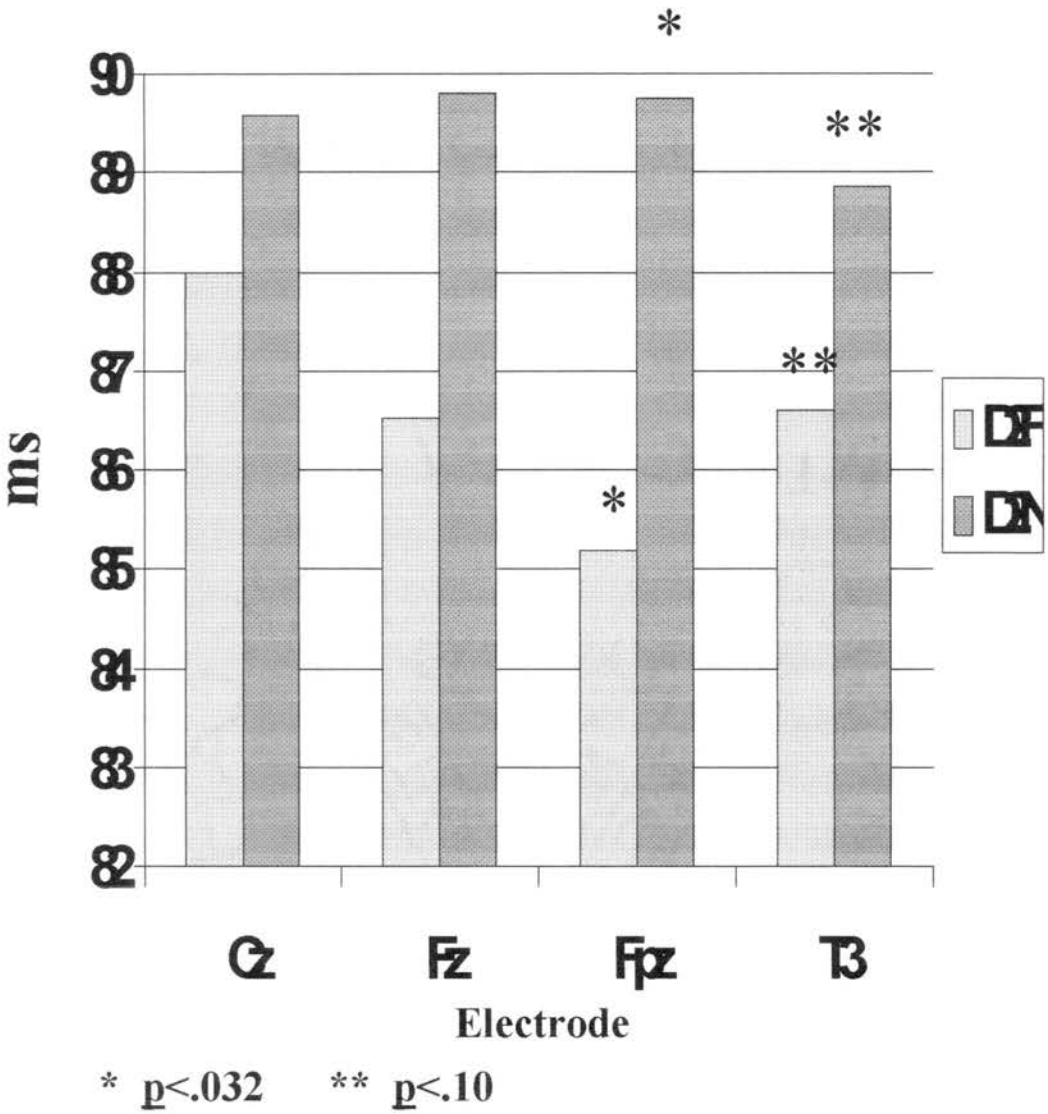
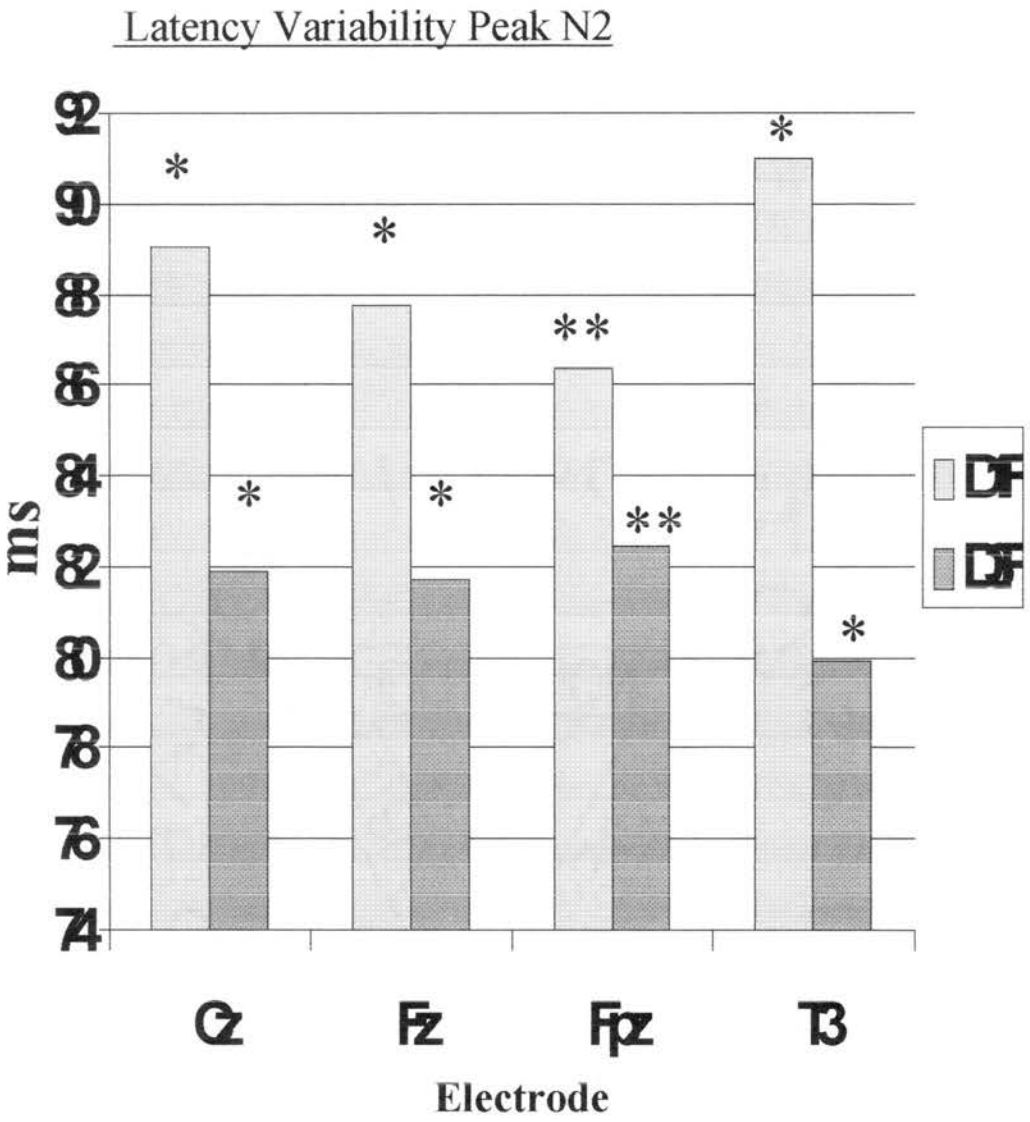


Figure 13



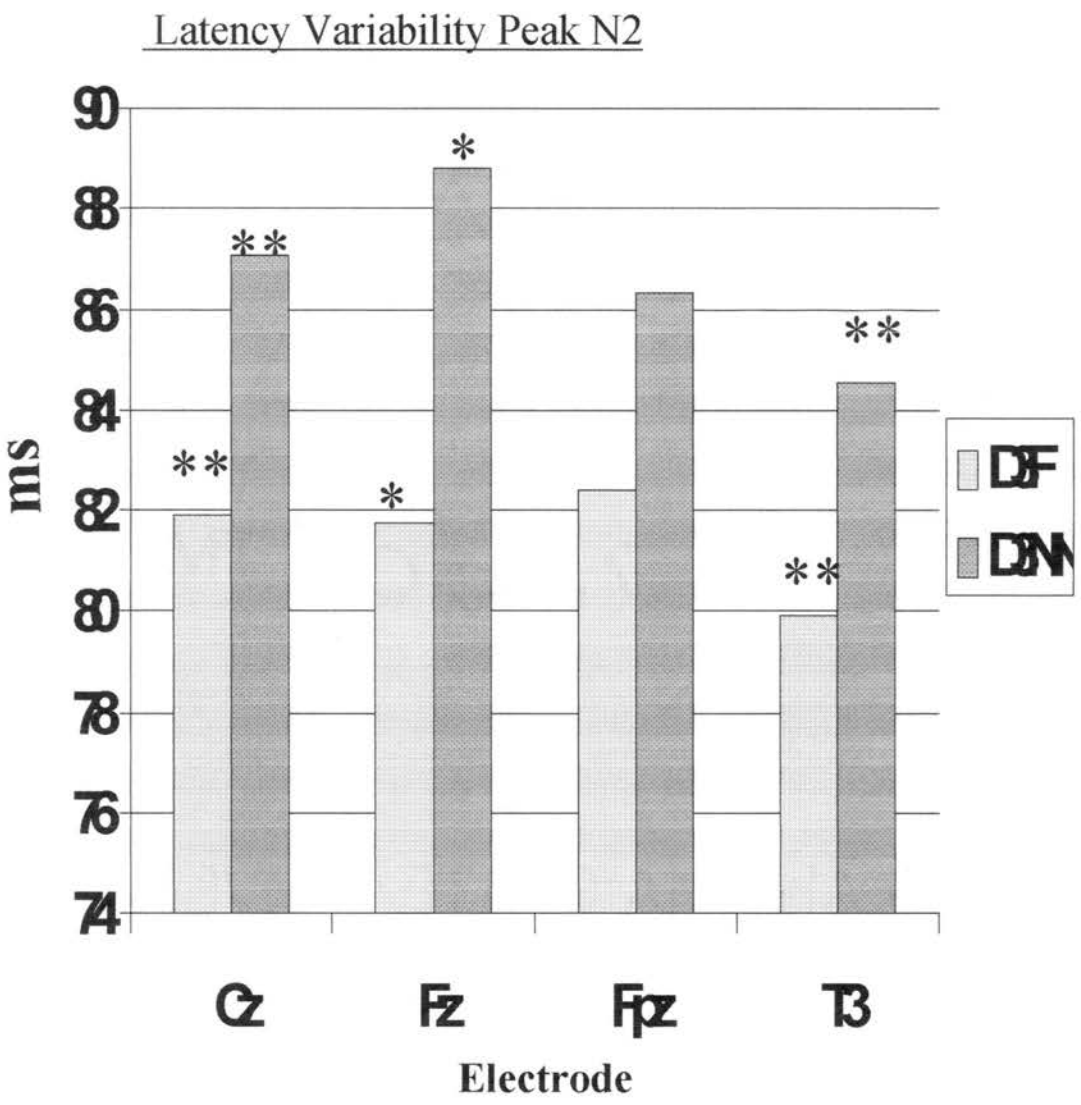
* p<.032 ** p<.10

Figure 14



* p<.032 ** p<.10

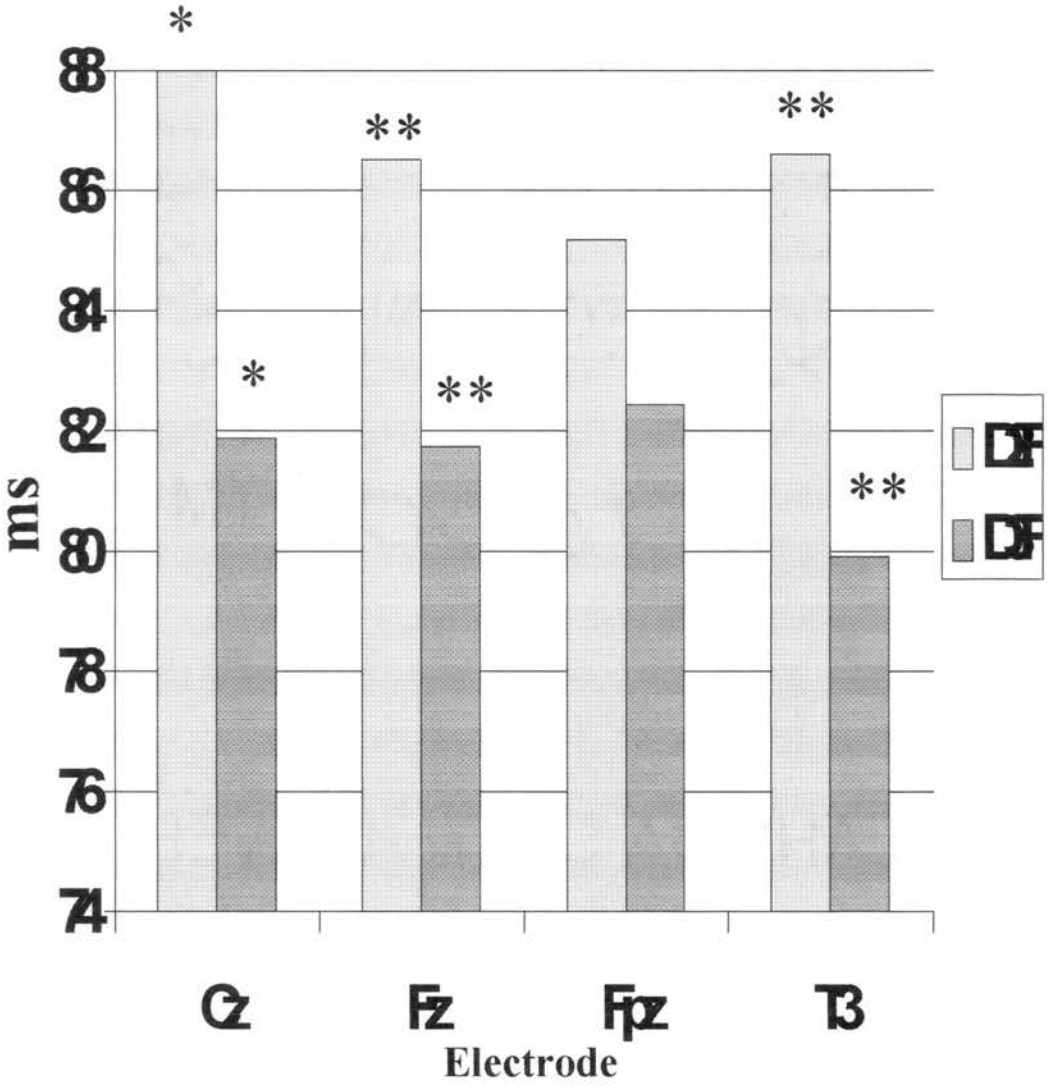
Figure 15



* p<.032 ** p<.10

Figure 16

Average Latency Variability Peak N2



* p<.032 ** p<.10

Figure 17

Peak P2 Latency Variability Group by Condition Interaction

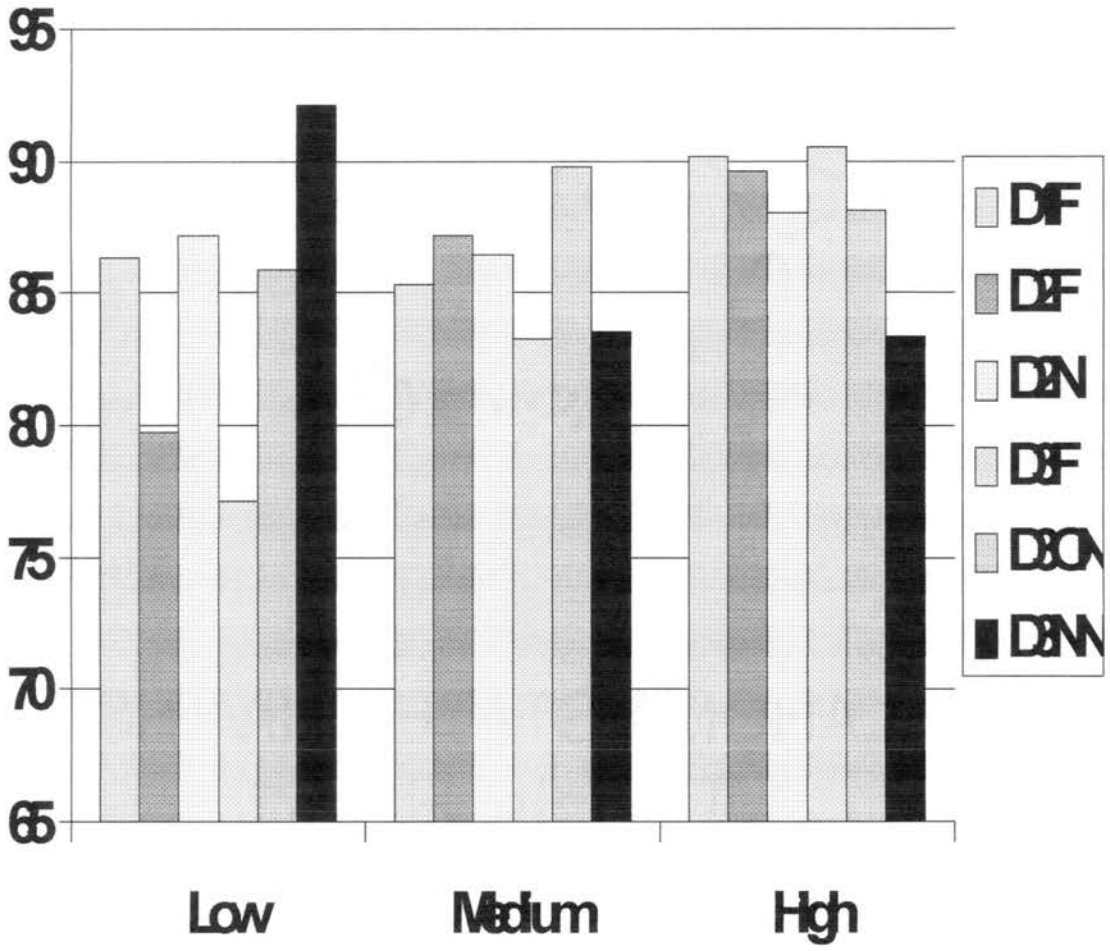
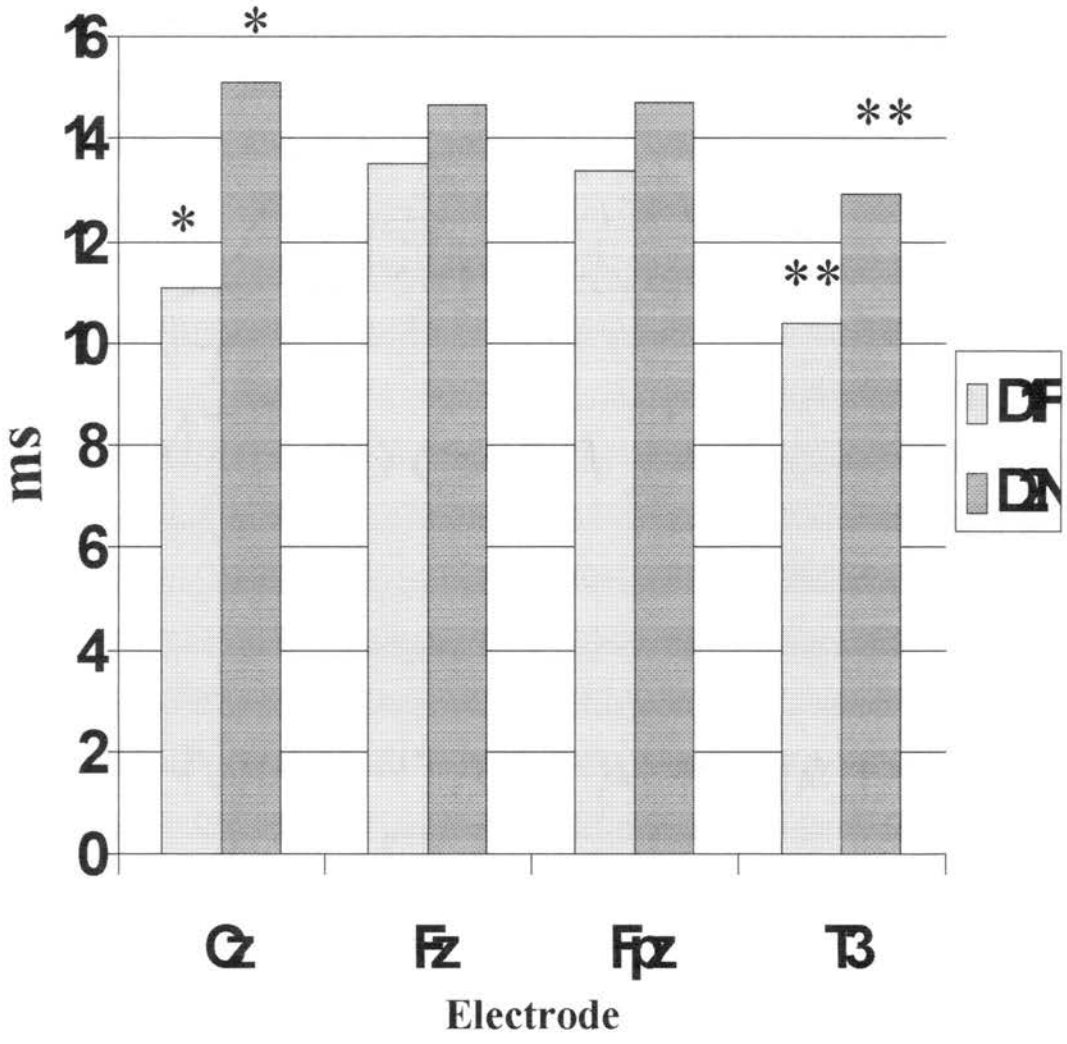


Figure 18

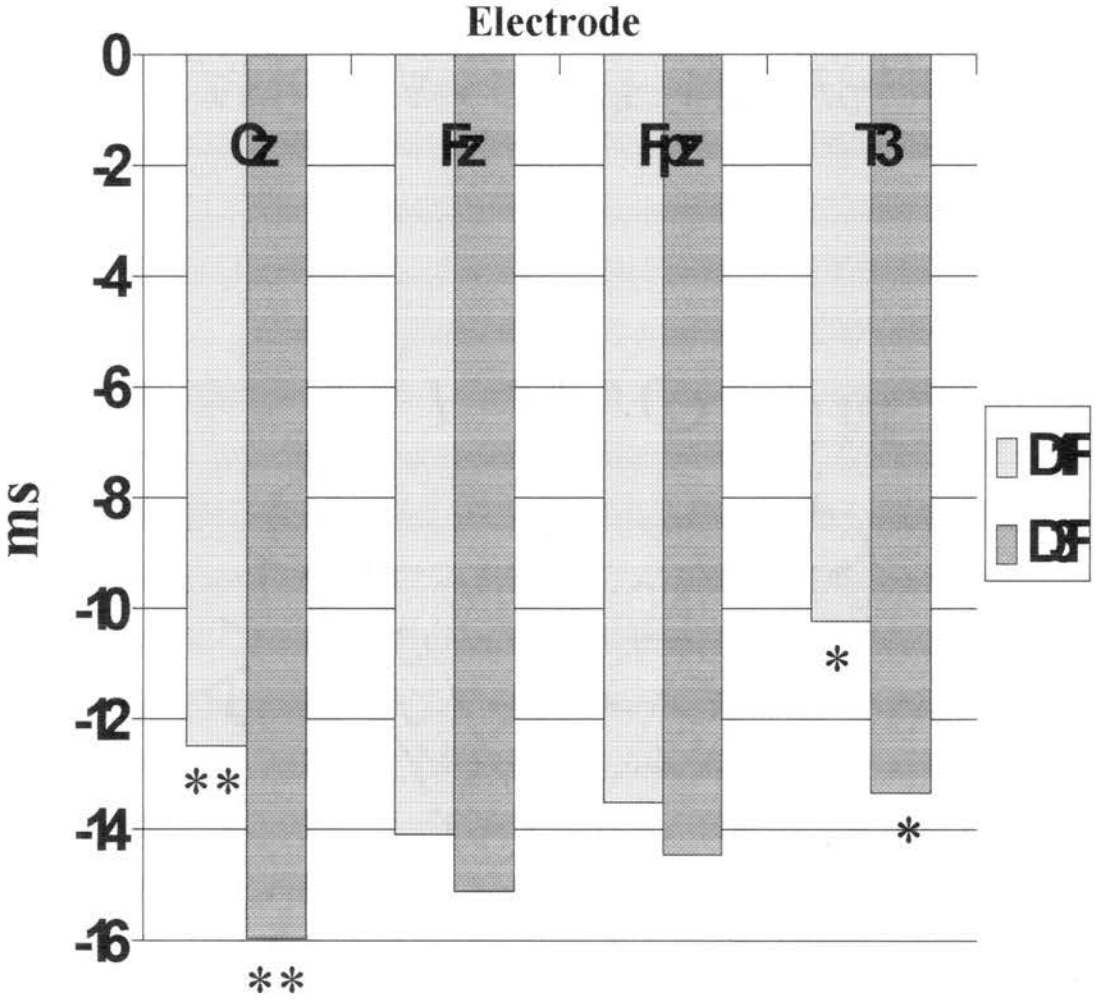
Single-trial Amplitude Peak P2



* p<.032 ** p<.10

Figure 19

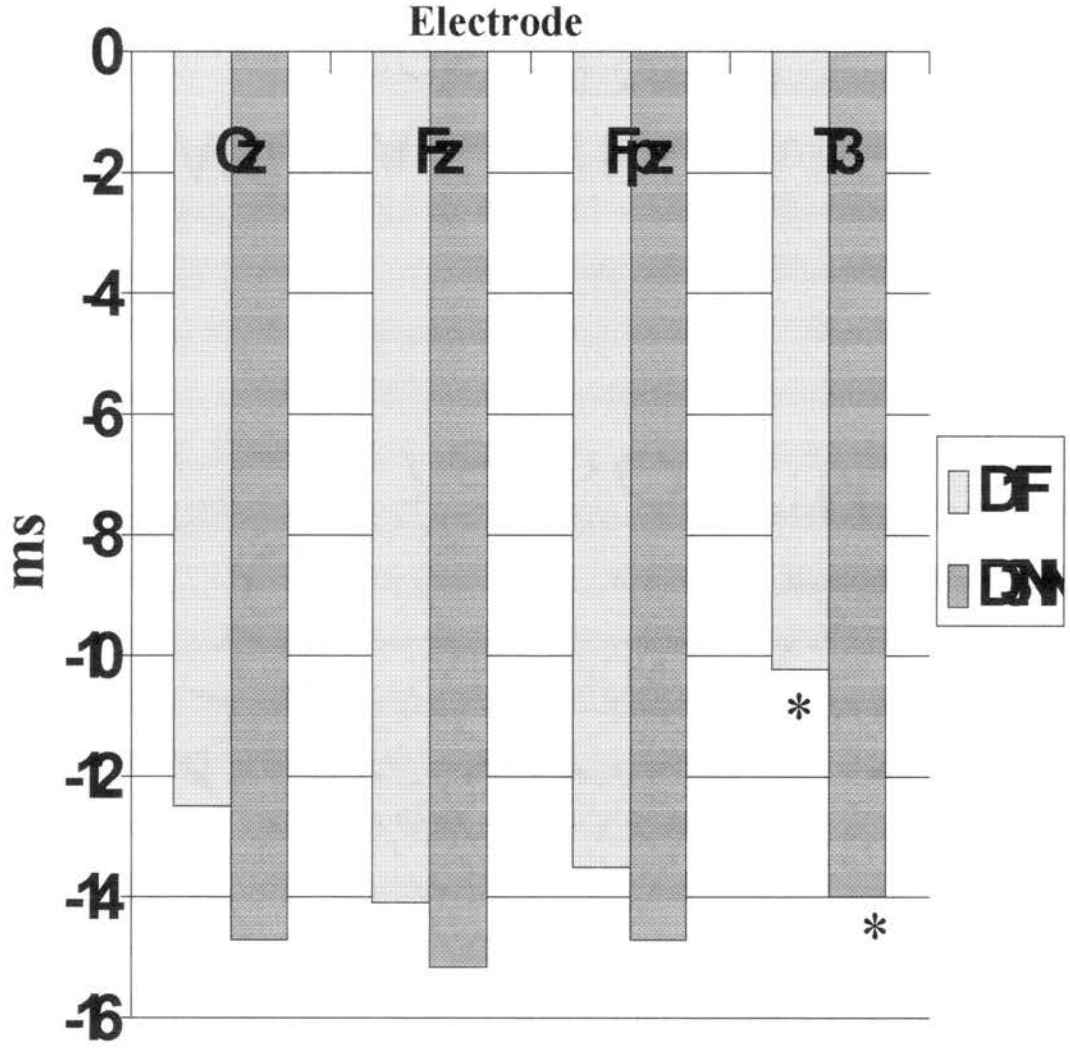
Single-trial Amplitude Peak N2



* p<.032 ** p<.10

Figure 20

Single-trial Amplitude Peak N2



* p<.032

Figure 21

Peak P2 Single-trial Amplitude Group by Condition Interaction

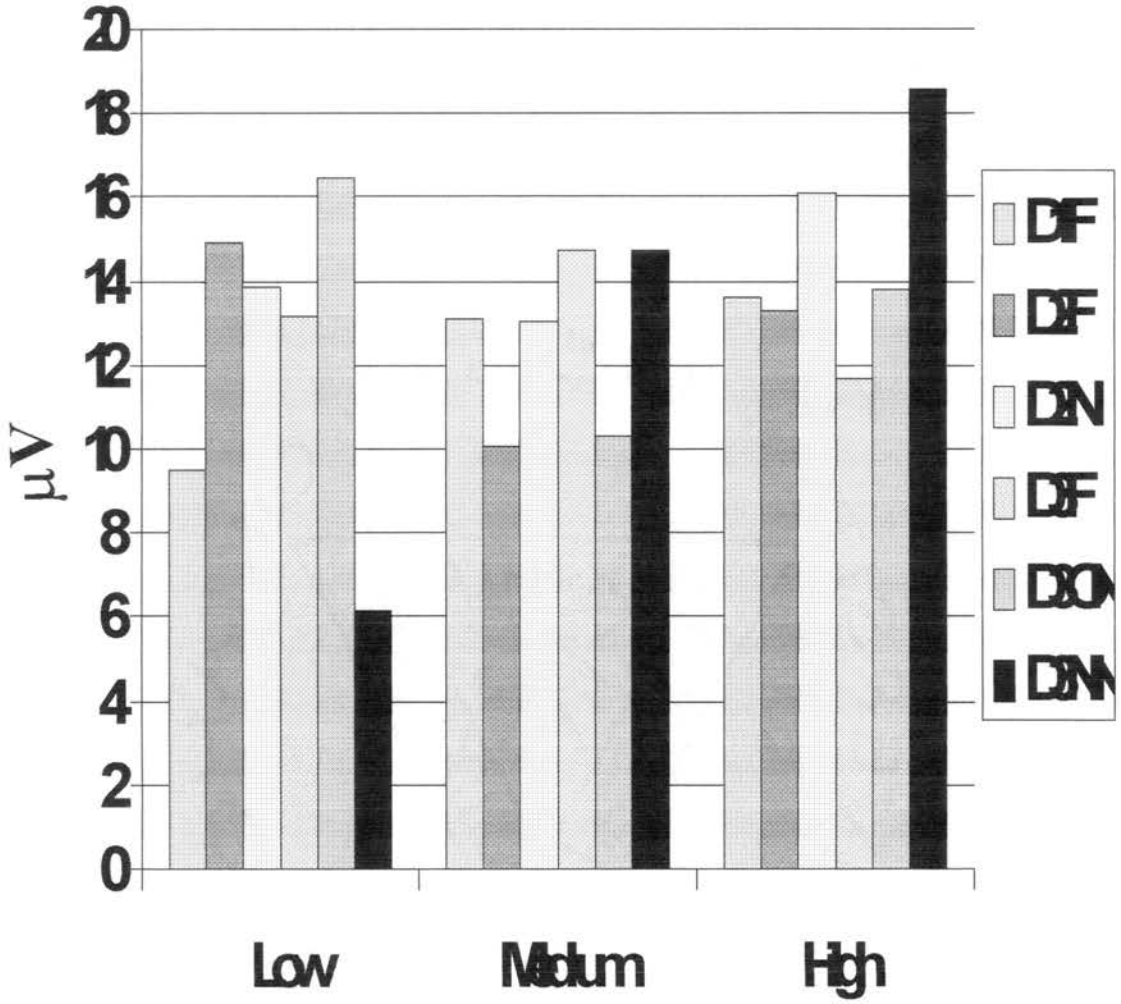


Figure 22

Peak N2 Single-trial Amplitude Condition by Electrode Interaction

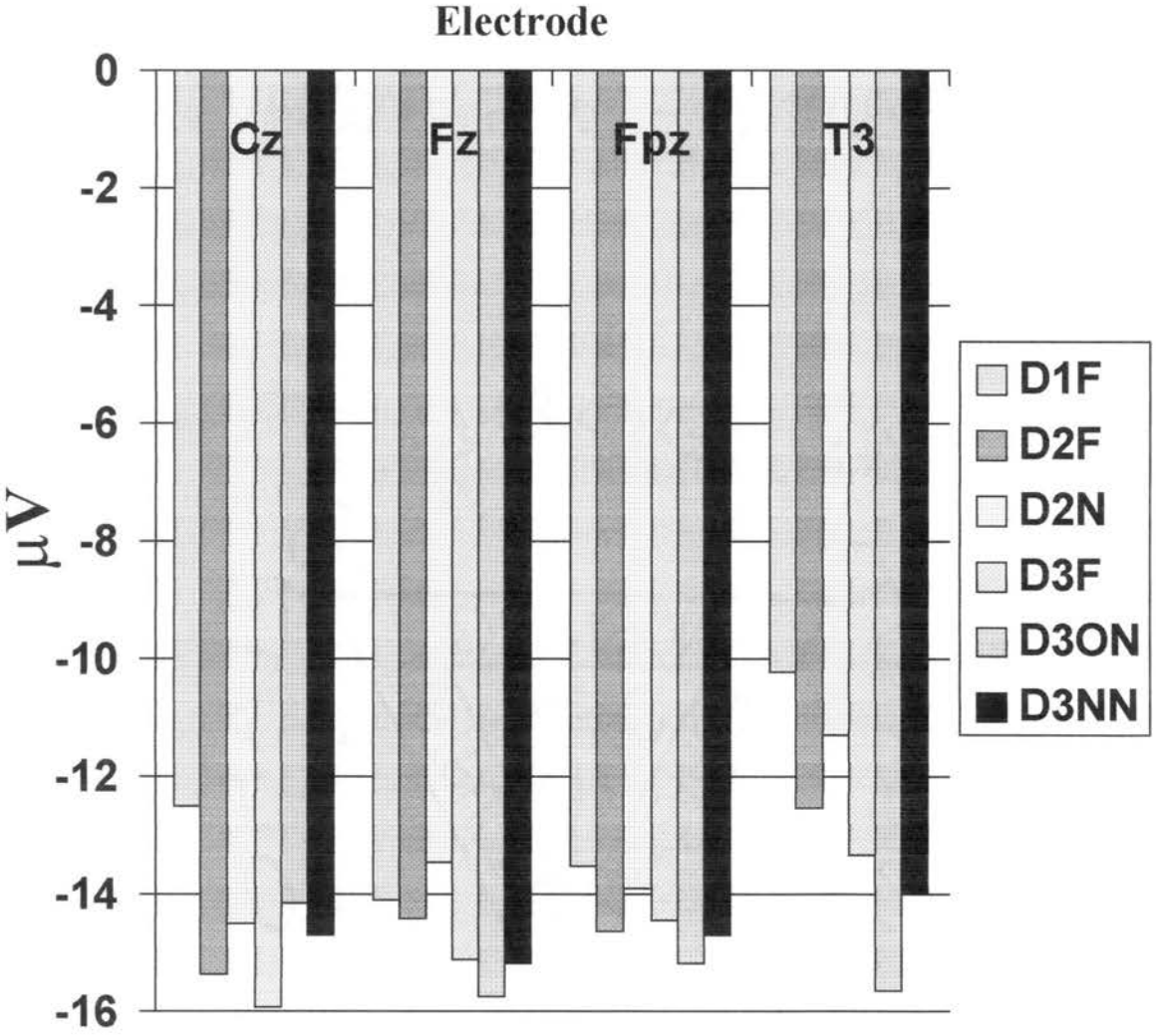
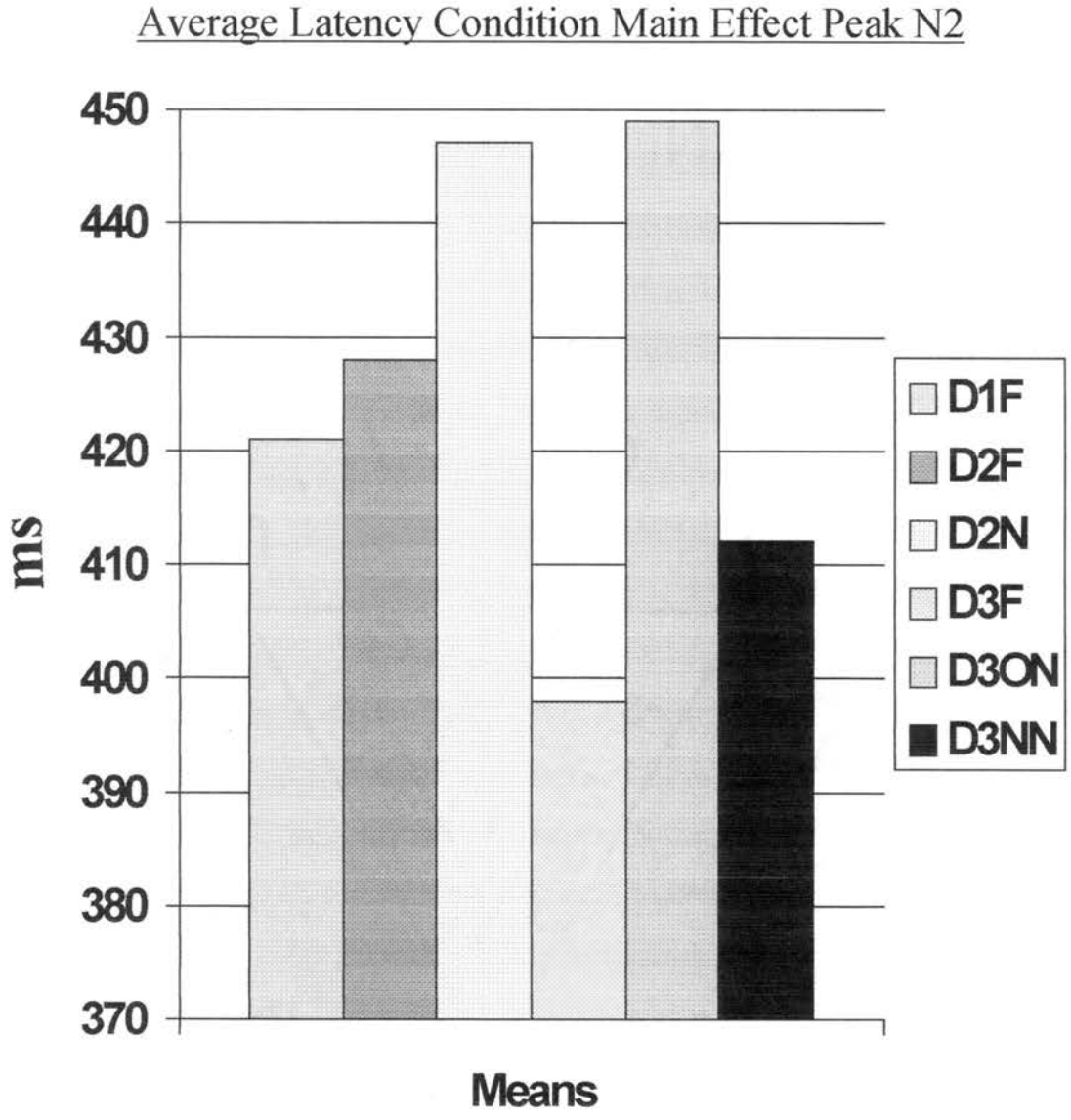


Figure 23


Average Latency Condition Main Effect Peak N2
450
440
430
420
410
400
390
380
370
ms
Means
D1F
D2F
D2N
D3F
D3ON
D3NN

APPENDIX

OKLAHOMA STATE UNIVERSITY
INSTITUTIONAL REVIEW BOARD
HUMAN SUBJECTS REVIEW

Date: 10-17-96 **IRB #: AS-89-043E**

Proposal Title: ELECTROPHYSIOLOGICAL MEASURES OF MEMORY IN INFANTS

Principal Investigator(s): David G. Thomas

Reviewed and Processed as: Modification and Continuation

Approval Status Recommended by Reviewer(s): Approved

ALL APPROVALS MAY BE SUBJECT TO REVIEW BY FULL INSTITUTIONAL REVIEW BOARD AT NEXT MEETING, AS WELL AS ARE SUBJECT TO MONITORING AT ANY TIME DURING THE APPROVAL PERIOD.
APPROVAL STATUS PERIOD VALID FOR DATA COLLECTION FOR A ONE CALENDAR YEAR PERIOD AFTER WHICH A CONTINUATION OR RENEWAL REQUEST IS REQUIRED TO BE SUBMITTED FOR BOARD APPROVAL.
ANY MODIFICATIONS TO APPROVED PROJECT MUST ALSO BE SUBMITTED FOR APPROVAL.

Comments, Modifications/Conditions for Approval or Disapproval are as follows:
The modifications stated in the renewal request pose no additional risks to the subjects of this study. The Principal Investigator is requested to be sure that the informed consent forms reflect the mother also as a subject (they are collecting data from them regarding mothering) and the extent of their involvement in the study.

Signature: _____
Chair of Institutional Review Board

Date: May 6, 1998

Margaret Letterman

1017 E. 56th Street
Stillwater, OK 74074

Home Phone (405) 377-4935
Email mrl6688@okstate.edu

June 26, 1998

Institutional Review Board
Oklahoma State University
Stillwater, OK 74074

To Whom it May Concern:

My adviser and I believe that the title of my research project and dissertation should be changed to be more descriptive and informative. The new title is ELECTROPHYSIOLOGICAL CORRELATES OF RECOGNITION MEMORY IN 3-MONTH-OLD INFANTS: A 3-DAY AUDITORY PARADIGM.

Thank you.

Sincerely,

Margaret Letterman

VITA

Margaret Letterman

Candidate for the Degree of

Doctor of Philosophy

Dissertation: ELECTROPHYSIOLOGICAL CORRELATES OF RECOGNITION MEMORY IN 3-MONTH-OLD INFANTS: A 3-DAY AUDITORY PARADIGM

Major Field: Psychology

Biographical:

Personal Data: Born in Tulare, California, on September 26, 1947, the daughter of Manuel and Ida (Salvador) Borges.

Education: Graduated from Tulare Union High School, Tulare, California in May 1965; received Bachelor of Arts Degree in Psychology from the University of Montana, Missoula, Montana in May 1991; received the Master of Science degree with a major in General Psychology from Fort Hays State University, Hays, Kansas in May 1993; received the Master of Science degree with a major in Psychology from Oklahoma State University, Stillwater, Oklahoma in August 1996. Completed the requirements for the Doctor of Philosophy degree with a major in Biological Psychology at Oklahoma State University in July, 1998.

Experience: Teaching assistant for Introductory Statistics and Experimental Psychology; graduate instructor for Introductory Psychology and the Independent and Correspondence Study Department; proposal reviewer for the Oklahoma State Regents for Higher Education; and graduate student assistant for the Multicultural Development and Assessment Center. Research experience in electrophysiological measures of memory with human infants and study of false memory with adult population.

Professional Memberships: Hispanic Student Association, Minority Graduate student Association, Oklahoma Psychological Society, Psychology Graduate Student Association, Society for Neuroscience, American Psychology-Law Society